BIOCHEMICAL LETTERS

Control analysis provides a simple means of understanding the control structure of a metabolic pathway

In a recent paper, Crabtree & Newsholme (1987) present a method for deriving control coefficients which is based on a somewhat different approach from that proposed by Kacser & Burns (1973) and Heinrich & Rapoport (1974). We wish to comment on several important points raised by Crabtree & Newsholme (1987).

Crabtree & Newsholme (1987) conclude that knowledge of the values of flux control coefficients of enzymes in a specific steady state does not provide much information about the control structure of a metabolic pathway. We agree completely. It is hazardous to conclude that external control of pathway flux must be exerted via a step with a high flux control coefficient. An example of an erroneous interpretation of this nature can be found in a recent paper of Pryor *et al.* (1987). They conclude that flux control of the gluconeogenic pathway by the respiratory chain must be exerted via pyruvate carboxylase because this is the step with the highest flux control coefficient. This could be the case in the presence of glucagon, when pyruvate kinase is inhibited. However, in the absence of the hormone the gluconeogenic pathway is branched and flux is very sensitively controlled via pyruvate kinase (Groen *et al.*, 1986). Although this enzyme has a relatively low flux control coefficient it plays an important role in controlling gluconeogenic flux via its very high elasticity coefficient towards phosphoenolpyruvate, i.e. a small change in the phosphoenolpyruvate concentration has a large effect on pyruvate kinase flux and hence gluconeogenic flux. Therefore all steps that have a significant concentration control coefficient on cytosolic phosphoenolpyruvate (for instance the respiratory chain) also control gluconeogenic flux [see Groen *et al.* (1986) for a discussion of how the respiratory chain can control the phosphoenolpyruvate concentration]. This demonstrates nicely that not only flux or concentration control coefficients but also elasticity coefficients are important for understanding the control structure of a metabolic pathway. Indeed the elasticity coefficients generate the control coefficients (for a discussion see Kacser & Burns, 1979).

We have shown that the elasticity coefficients of most enzymes can be calculated relatively simply from enzyme kinetic data and the value of the ratio between the mass action ratio and the equilibrium constant (Γ/K_{eq}) (Groen *et al.*, 1982). In the absence of enzyme kinetic data, elasticity coefficients can be determined experimentally (Kacser & Burns, 1973, 1979; Groen *et al.*, 1982; Salter *et al.*, 1986). Calculation of flux control coefficients and concentration control coefficients from the elasticity coefficients and the flux distribution through

the pathway has become very simple since the development of the matrix method (Fell & Sauro, 1985; Sauro *et al.*, 1987; Westerhoff & Kell, 1987). We agree with Crabtree & Newsholme (1987) that the use of the summation theorem in this method imposes the risk of missing as yet unknown steps. Therefore the flux control coefficient of at least one step in the pathway should be determined directly, for instance by using a well-characterized effector of enzyme activity (Groen *et al.*, 1982). Crabtree & Newsholme (1987) repeatedly claim that we have failed to do so in our study on control of gluconeogenesis. This must be a misunderstanding. In our paper we explicitly discussed the danger of using the summation theorem (Groen *et al.*, 1986). In order to check the calculated values of the flux control coefficients of the gluconeogenic enzymes we measured the combined flux control coefficient of the enzymes located between pyruvate and phosphoenolpyruvate directly by varying the external substrate pyruvate. Excellent agreement was observed between calculated and measured values. In an earlier study we determined the flux control coefficient of phosphoenolpyruvate carboxykinase using published data on the effect of mercaptopicolinic acid on gluconeogenic flux (Groen *et al.*, 1982). Also in this case there was good agreement between measured and calculated values.

Crabtree & Newsholme (1987) suggest that quantification of control does not provide much information about the control structure of a metabolic pathway, since the values obtained apply only under one particular set of conditions. This is, of course, the case. We also agree with Crabtree & Newsholme (1987) that the absolute values of control coefficients are not important because they hold only for one steady state. However, Crabtree & Newsholme (1987) are in error in suggesting that determination of the control distribution in one steady state does not allow one to predict what will happen under other conditions. If the values for the elasticity coefficients of the enzymes in a pathway under certain conditions are known, it is in general not difficult to predict what will happen to these values under other conditions. This also applies to the effect of external effectors. For instance, when flux through a pathway increases, the elasticity coefficients of most non-equilibrium enzymes to their substrate(s) will decrease. This can have a profound but predictable influence on the distribution of control. Since it is now very simple to calculate values for flux control coefficients and concentration control coefficients from the elasticity coefficients, it has also become easy to simulate the influence of changes in the values of elasticity coefficients on the distribution of control in a pathway.

In conclusion, control analysis, if applied in the correct way does, indeed, provide a simple and convenient means of establishing the control structure of a metabolic pathway.

Albert K. GROEN* and Joseph M. TAGER†

Departments of *Gastroenterology and †Biochemistry,
University of Amsterdam, Academic Medical Centre,
Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands

- Crabtree, B. & Newsholme, E. A. (1987) *Biochem. J.* **247**, 113–120
- Fell, D. A. & Sauro, H. M. (1985) *Eur. J. Biochem.* **148**, 555–561
- Groen, A. K., Van der Meer, R., Westerhoff, H. V., Wanders, R. J. A., Akerboom, T. P. M. & Tager, J. M. (1982) in *Metabolic Compartmentation* (Sies, H., ed.), pp. 9–37, Academic Press, New York
- Groen, A. K., van Roermund, C. W. T., Vervoorn, R. C. & Tager, J. M. (1986) *Biochem. J.* **237**, 379–389
- Heinrich, R. & Rapoport, T. A. (1974) *Eur. J. Biochem.* **42**, 89–95
- Kacser, H. & Burns, J. A. (1973) *Symp. Soc. Exp. Biol.* **27**, 65–104
- Kacser, H. & Burns, J. A. (1979) *Biochem. Soc. Trans.* **7**, 1149–1161
- Pryor, H. J., Smyth, J. E., Quinlan, P. T. & Halestrap, A. P. (1987) *Biochem. J.* **247**, 449–457
- Salter, M., Knowles, R. G. & Pogson, C. I. (1986) *Biochem. J.* **234**, 635–647
- Sauro, H. M., Small, J. R. & Fell, D. A. (1987) *Eur. J. Biochem.* **165**, 215–221
- Westerhoff, H. V. & Kell, D. B. (1987) *Biotechnol. Bioeng.* **30**, 1101–1107

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Control analysis based on unvalidated models cannot be used as a short cut to understanding metabolic control

We are pleased to note a wide measure of agreement with many of the points made in our paper (Crabtree & Newsholme, 1987a) and we, in turn, have great respect for the experimental techniques developed in Professor Tager's laboratory. However, since our criticisms of the paper by Groen *et al.* (1986) were made in passing, and seem to have been misunderstood, we will explain them in more detail; as will be seen, neither criticism is directly concerned with misapplications of the 'summation' theorem.

In our paper (Crabtree & Newsholme, 1987a) we outlined a simple and systematic method for deriving algebraic expressions for complex sensitivities (including control coefficients). Our method requires no theorems and is more economical than those which do (e.g. those referred to by Groen & Tager). However, no matrix method can be used without first assuming a 'control model'. In Groen *et al.* (1986) this model is their relatively simple 'Scheme 1' and the resulting quantitative modelling assumptions are contained in the connectivity relationships given on p. 380 of their paper. This scheme and the resulting connectivity equations can only be conjectural, for two reasons. First, it is impossible to know whether all the connectivities *in situ* have been included (for example, their Scheme 1 omits interactions between fructose biphosphate and pyruvate kinase, and between acetyl-CoA and pyruvate carboxylase). Second, it is impossible to be certain that their simplistic calculations of what they term elasticity coefficients give the appropriate values *in situ*, especially since they could

check only a few of them by using alternative methods. These two problems mean that the values they calculated for the gluconeogenic control coefficients apply only to their assumed model, which they must then establish to be a satisfactory representation of the quantitative control of gluconeogenesis *in situ*. We do not believe that they have done this; hence our criticism (p. 119 of Crabtree & Newsholme, 1987a) that the values of these coefficients are not necessarily relevant to the pathway *in situ*. In our opinion these authors need to validate their model by measuring the values of as many control coefficients as possible (not just one or two), using techniques which they have pioneered, and see if these agree with their calculated values.

Our other criticism (p. 120 of Crabtree & Newsholme, 1987a) refers to the lack of distinction made by Groen *et al.* (1986) between different types of regulators. We have recently discussed this subject (Crabtree & Newsholme, 1987b) and have found it necessary to distinguish between 'totally-external' and 'partially-external' regulators and their corresponding 'open' and 'closed' regulatory sequences. Groen *et al.* (1986) have treated gluconeogenesis as if it were a completely 'open' structure and have consequently presented all the control coefficients as if they were of the same type. Unfortunately, this is too simplistic. Thus, when they calculated the control coefficient for pyruvate carboxylase from the response to pyruvate (p. 386 of their paper) they obtained values that apply only to this specific interaction of pyruvate: they do not apply to the interaction of any other regulator of pyruvate carboxylase. This is because pyruvate is not a totally-external regulator (since its concentration is a function of the rate of gluconeogenesis) and its effect on the enzyme will be included in the response to all other regulators of pyruvate carboxylase (see Crabtree & Newsholme, 1987a). It is therefore an example of a partially-external regulator operating via a specific 'closed' regulatory sequence. In contrast, all the other control coefficients calculated by Groen *et al.* (1986) are genuine 'open' sensitivities and apply without qualification to the interactions of any totally-external regulators. Although this distinction is less important for qualitative discussions, for quantitative analyses it is essential (Crabtree & Newsholme, 1987a) and the (pseudo) control coefficient for pyruvate carboxylase should not have been divorced from the interaction of the specific regulator, pyruvate.

This structure also applies to the effects of phosphoenolpyruvate on pyruvate kinase (referred to by Groen & Tager in their letter), since phosphoenolpyruvate is a partially-external regulator of gluconeogenesis via a closed regulatory interaction with pyruvate kinase. Any other system (e.g. the respiratory chain) which communicates with phosphoenolpyruvate will thereby communicate with and hence control gluconeogenesis. Interestingly, here Groen & Tager actually distinguish between the very low (i.e. 'open') control coefficient of pyruvate kinase and the much more sensitive (i.e. 'closed') interaction with phosphoenolpyruvate. Moreover, they include the interaction between phosphoenolpyruvate and the enzyme in their qualitative summary. In our opinion they should have treated pyruvate carboxylase in a similar way and should not have expressed its closed interaction with pyruvate in terms of a confusing (pseudo) control coefficient.

Groen & Tager say that we are in error to suggest that