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ON THE ROLE OF ENZYME KINETIC PARAMETERS IN DETERMINING THE EFFECTIVENESS WITH WHICH CHANNELLING CAN DECREASE THE SIZE OF A METABOLITE POOL

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ABSTRACT

Recently, it has been argued that the phenomenon of direct transfer of intermediate metabolites between adjacent enzymes, also known as metabolic channelling, would not decrease the concentration of those intermediates in the 'bulk' solution. However, this conclusion has been drawn by extrapolation from the results of simulations with a rather restricted set of parameters. We show that, for a number of kinetic cases, the existence of metabolic channelling can decrease the size of the soluble pool of intermediates. When the enzyme(s) 'downstream' of the channel have a catalytic capacity that is large relative to the enzymes 'upstream' of the channel, the decrease of concentration can be substantial (3 orders of magnitude).

1. INTRODUCTION

Metabolite channelling is a term used to describe the phenomenon in which a product of an enzyme is transferred to the next enzyme in a metabolic pathway without being released to the "bulk" solution. Various authors have addressed the possible advantages of metabolite channelling for cellular metabolism (for comprehensive reviews see Keleti *et al.* 1989 and Ovádi 1991). One of these advantages is widely perceived to be the reduction of the concentration of the channelled intermediate in the bulk solution, sparing the limited solvent capacity of the cell (Atkinson 1971). Recently Cornish-Bowden (1991) presented data for a pathway model with channelling, in which increasing channel flux did not reduce the pool concentration of the intermediate metabolite. He further

extended the interpretation of his results, using a particular set of parameters, to the general case, concluding that "channelling has no effect on the free concentration of a channelled intermediate in a pathway" (Cornish-Bowden 1991).

In this work we reproduced the results of Cornish-Bowden, investigated the generality of his conclusions and extended our analysis to the dynamic behaviour of this model. In contrast to the analysis made by Cornish-Bowden (1991), we show that channelling can decrease the pool concentration of the intermediate *substantially* (to approximately one thousandth of that in the absence of the channel), and describe the parameters that determine the magnitude of this effect.

2. METHODS

All the data displayed were obtained with the simulation program GEPASI (Mendes 1991). This program accepts as inputs the topology of a pathway, the explicit rate equations of each step and a set of initial concentrations. All external metabolites (defined by the user) are kept constant. There is no need for the user to inform the program of the existence of moiety-conserved cycles as the program is able to work this out from the structural properties of the model using numerical methods based on the approach of Reder (1988), more recently extended by Holstein and Greenshaw (1991). The simulator integrates the set of stiff nonlinear ordinary differential equations that describe the temporal evolution of the internal metabolite concentrations. If the integration is taken through a sufficiently large time interval, the result is a steady state, provided that the integration is carried out in a stable region of the phase-space. Alternatively, the program computes steady-state concentrations by setting all differential equations to zero and solving the resultant system of nonlinear homogeneous equations by the damped Newton method. In this way, GEPASI is able to monitor the dynamic behaviour, as well as the steady state, of biochemical pathways. The program also applies control analysis (Kacser & Burns 1973, Heinrich & Rapoport 1974) to the steady-state data. In all models simulated by GEPASI it is assumed that all processes occur in a well-stirred reactor. GEPASI is available from one of the authors (P.M.). For this work, the program was run on an IBM PC-compatible computer with an Intel 80386SX processor and an Intel 80387SX numeric coprocessor running MS-DOS 3.3, although GEPASI also exists for other operating systems (eg. UNIX).

The model studied is the same as that by Cornish-Bowden (1991): a four-enzyme pathway where the two middle enzymes can associate and channel their intermediate metabolite. It should be pointed out that in this model the elementary reactions of enzymes 2 and 3 were detailed explicitly, while enzymes 1 and 4 were described by a single step with Michaelis-Menten-type kinetics. Enzymes 2 and 3, each described by two steps, react using one of the possible mechanisms that generate reversible Michaelis-Menten kinetics. The free form of enzyme 3, E_3, can bind the enzyme complex E_2X and channel the intermediate C. This means that through this branch of the pathway no C is released to the solution; it is directly transferred from E_2 to E_3. This model is represented in the scheme of figure 1 (cf. scheme 3 of reference 3, where the association and dissociation of the two middle enzymes was left implicit).

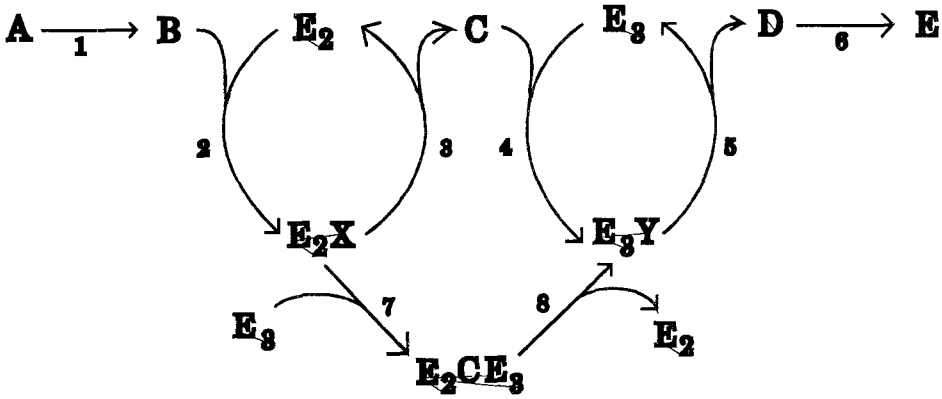

Fig. 1. The pathway model. Reactions are represented by arrows that indicate the forward direction of flow. All steps are considered to be reversible, except step 6 which is irreversible (the data of figure 6 excepted). Both enzymes 2 and 3 were broken down into elementary reactions: steps 2 and 3 for enzyme 2, steps 4 and 5 for enzyme 3. E_2 and E_3 are free forms of enzyme. E_2X, E_3Y are "normal" enzyme complexes and E_2CE_3 is the transient enzyme channel with bound intermediate. Step 1 reacts according to reversible Michaelis-Menten kinetics, step 6 according to irreversible Michaelis-Menten kinetics (unless stated otherwise) and steps 2, 3, 4, 5, 7 and 8 are chemical reaction steps (with two associated kinetic constants each). In all simulations shown [A] = 10 and [E] = 0.

3. RESULTS

3.1. Effect of increasing channel flux when the rest of the system is left unchanged

To study the effect of increasing channel flux on the concentration of C, we compared the steady state in the case where there is no channel flux with steady states for increasing proportions of channel flux. The simplest way of doing this is by changing the rate constants of the two channel reactions leaving all other parameters fixed.

Since the difference in the chemical potentials of E_2X and E_3Y is independent of the route of the reaction, the equilibrium constant of the two branches (diffusion *via* the solvent or direct transfer) must be the same. To reduce the number of parameters we have adopted the same solution as did Cornish-Bowden (1991), that is:

$$k_{+7} = p \cdot K_{eq} \quad k_{-7} = p \quad k_{+8} = p \quad k_{-8} = p$$

By changing p we can effectively make the channel flux go from 0% to almost 100%. We should like to stress that 100% channel flux means that there is no leak and so $[C] \stackrel{def}{=} 0$; it is not further considered here.

Finally, we define a coefficient α which, for a given set of parameters, is the ratio of the steady-state concentration of C at a certain channel flux to the steady-state

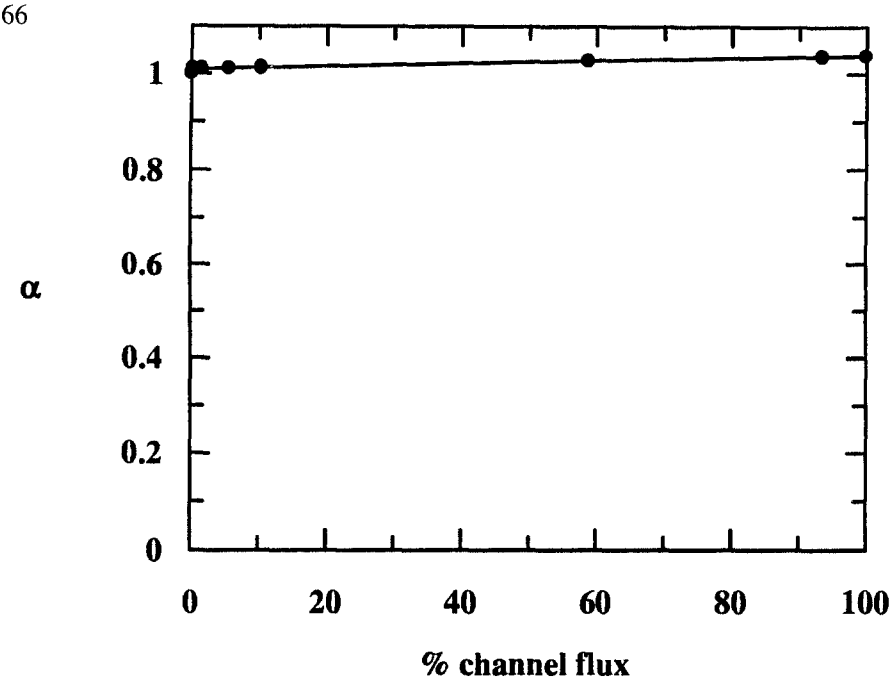

Fig. 2. Dependence of α on the relative channel flux. Parameters as in [3]: step 1 $K_m^s = K_m^p = V_{max}^f = V_{max}^r = 1$; step 6 $K_m = V_{max} = 1$; $k_{+2} = 6$ $k_{-2} = 1$; $k_{+3} = 5$ $k_{-3} = 6$; $k_{+4} = 6$ $k_{-4} = 5$; $k_{+5} = 1$ $k_{-5} = 6$. Steps 7 and 8 as in RESULTS.

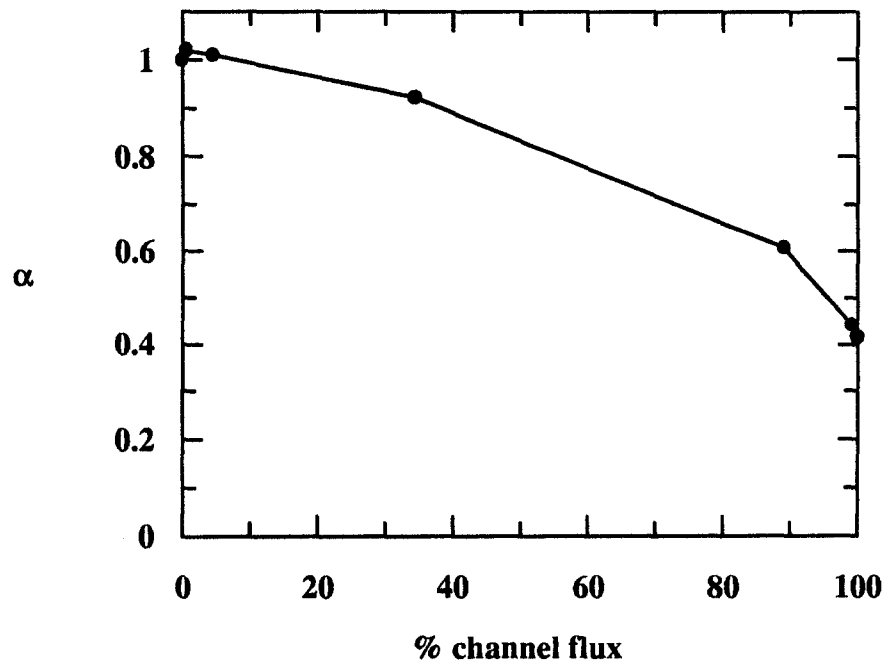

Fig. 3. Dependence of α on the relative channel flux. Parameters: steps 1 and 6 as in figure 2; $k_{+2} = 6$ $k_{-2} = 1$; $k_{+3} = k_{-3} = 10^3$; $k_{+4} = k_{-4} = 0.03$; $k_{+5} = 1$ $k_{-5} = 6$. Steps 7 and 8 as in RESULTS.

concentration of C observed when no channel is present. The lower the value of α, the more effective is the channel in decreasing the concentration of C.

a) K_{eq} = 1 for the conversions of E_2X to E_3Y

The results of Cornish-Bowden (1991) were reproduced successfully using GEPASI. They are shown in figure 2. At nearly 100% channel flux α is 1.03, reflecting an increase in [C] of 3%.

Keeping the equilibrium constant for the conversions of E_2X to E_3Y as 1, we have chosen a different set of values for the constants of steps 3 and 4: the release of C from E_2 and the binding of C to free E_3. The results of simulations using this set of parameters are shown in figure 3. A significant decrease in [C] is reflected by α taking a value of 0.4 at nearly 100% channel flux. It is therefore clear that channelling can in fact significantly decrease the pool concentration of the intermediate.

One thing that can be argued against this set of parameters (and of course those of Cornish-Bowden, 1991) is that in order to have a K_{eq} = 1 in these two parallel branches, the resulting kinetic parameters of the reversible Michaelis-Menten equations of enzymes 2 and 3 are rather strange. Either V_{max}^{r} is larger than V_{max}^{f} for E_3, as in reference 3, or the K_m for the substrate is bigger than that for the product in both E_2 and E_3.

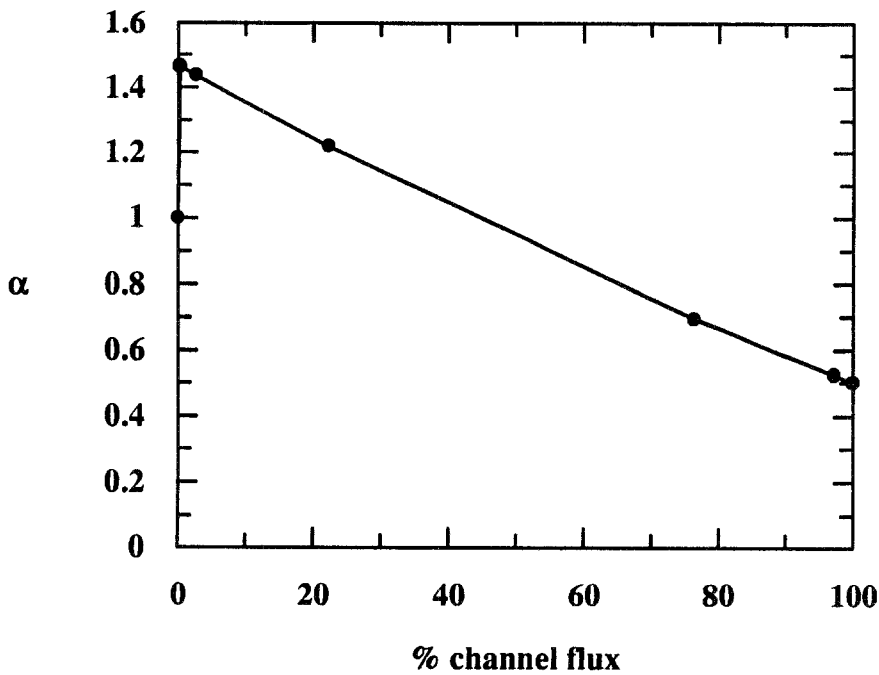

Fig. 4. Dependence of α on the relative channel flux. Parameters: steps 1 and 6 as in figure 2; $k_{+2} = 10^3$ $k_{-2} = 1$; $k_{+3} = k_{-3} = 10^3$; $k_{+4} = 10^3$ $k_{-4} = 1$; $k_{+5} = k_{-5} = 10^3$. Steps 7 and 8 as in RESULTS.

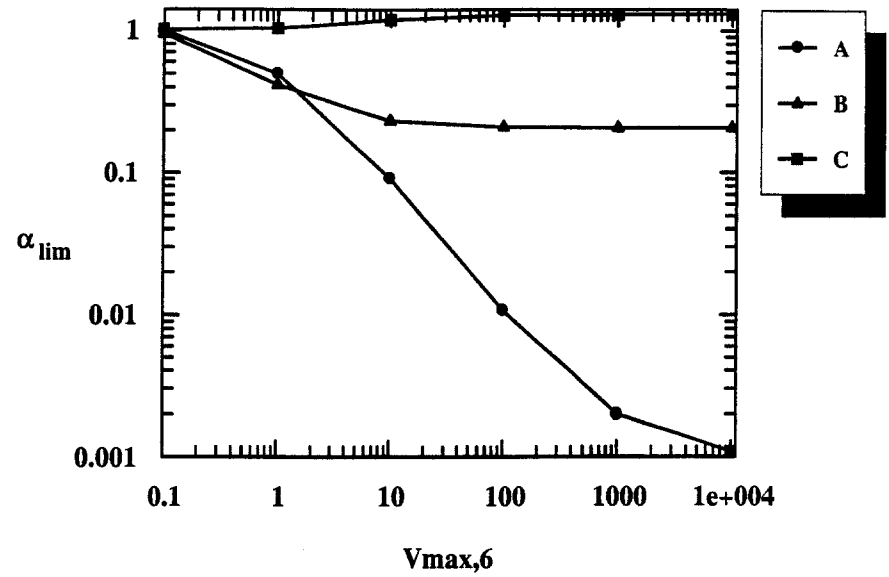

Fig. 5. Dependence of α_{lim} on the Vmax of step 6. Parameters: step 1 as in figure 2; step 6 $\breve{K}_m = 1$, V_{max} variable; steps 2, 3, 4, 5, 7 and 8: A as in figure 4, B as in figure 3, C as in figure 2.

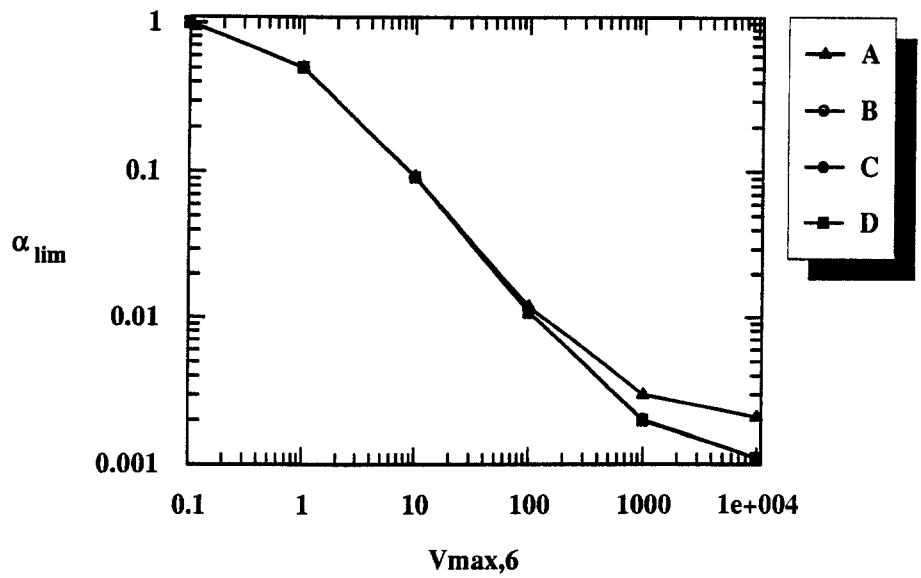

Fig. 6. Dependence of α_{lim} on the $V_{max}{}^f$ of step 6 (reversible except in D). Parameters: steps 1, 2, 3, 4, 5, 7 and 8 as in figure 4. Step 6: $V_{max}{}^f$ variable, $K_m{}^s$, $K_m{}^p$ and $V_{max}r$ with values to make different K_{eq}s according to the Haldane relationship. K_{eq} of step 6: A 1, B 10^3, C 10^8 and D infinity.

b) K_{eq} > 1 for the conversions of E_2X to E_3Y

A different approach is to make enzymes 2 and 3 have kinetic parameters that favour the flux in the forward direction. Accordingly we made V_{max}^{f} equal to 1000, V_{max}^{r} equal to 1 and both K_ms for substrate and product equal to 1 for both E_2 and E_3. This sets K_{eq} = 1000 for the conversion of E_2X to E_3Y, and so k_{+7} = 1000.p in order not to violate the thermodynamic constraint.

As can be seen in figure 4, channelling decreases [C] by a factor of 2. It is interesting that at very low channel flux [C] increases sharply but very quickly starts its monotonic decrease.

3.2. Effect of increasing channel flux at different substrate saturation levels on the last step of the pathway

As previously noticed by Cornish-Bowden (1991), the degree of saturation of the last step could be affecting [C] through high levels of [D] which would leak back and fill up the pool.

To check this hypothesis we changed the V_{max} of step 6 in steps from 0.1 to 100000. This makes [D] range from levels that are almost 100% saturating towards E_4 to values that are very low with respect to its K_m. At each different value for the V_{max} of step 6, simulations were carried out for p=0 (no channelling) and p=100000 (more than 99.7 % flux through the channel in all cases) to find the limiting value of α as the channel flux tends to 100%; we refer to this value of α as α_{lim}.

Figure 5 shows a graph of α_{lim} as a function of the V_{max} of step 6 for the sets of parameters mentioned above. It may be observed that the effect of the channel is *dramatically* to decrease α_{lim}, provided that both V_{max} for reaction 6 and K_{eq} for the conversion E_2X <--> E_3Y are reasonably high.

Since having an absolutely irreversible final step seems very unreal, we also checked that making it reversible does not significantly affect the steady-state behaviour of the pathway. Figure 6 shows how α_{lim} depends on the V_{max}^{f} of step 6 when it was made reversible, at different values for the equilibrium constant of this step.

It is evident that α_{lim} is a function of the V_{max}^{f} of the step(s) following the channel, and that if both (i) the K_{eq} for the reaction involving the direct transfer of intermediate between enzymes and (ii) the maximum velocity of the steps following the channel are high then the effect of the channel will be to decrease [C] *by very large factors.* This is also true for simulations using a "static" channel (data not shown).

3.3. Dynamic behaviour

We also studied the dynamic behaviour of this model. We show here the trajectories of [C] and [E_2CE_3] for an "empty-reactor" type of experiment. Setting the concentrations of all the internal metabolites (obviously excepting the enzymes E_2 and E_3) to zero, we apply a fixed concentration of A, the substrate. This is only one of several initial conditions for the study of the dynamics of the pathway. Mathematically, it may be looked upon as a perturbation of the virtual steady state, evolving to a new, non-virtual, steady state.

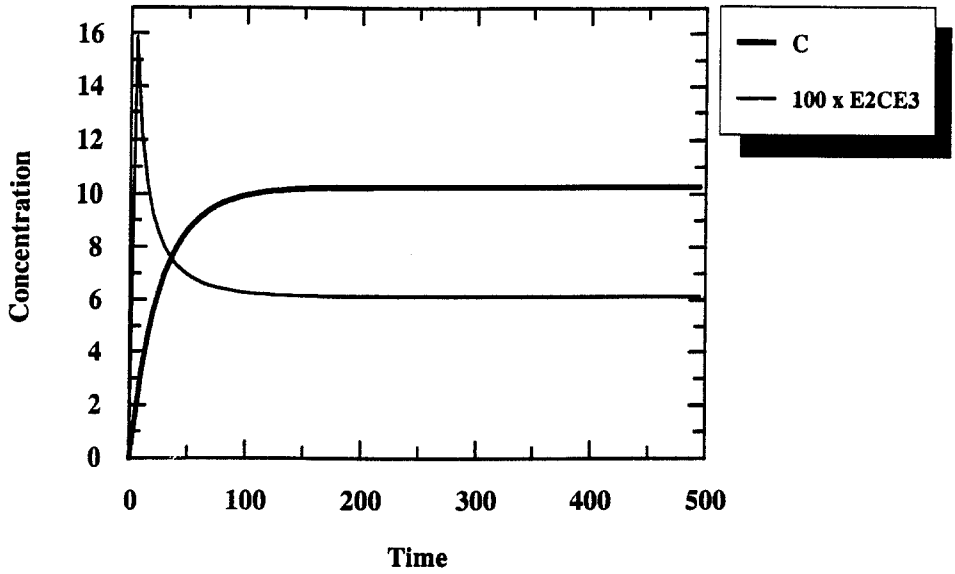

Fig. 7. Time evolution of [C] and [E_2CE_3] after a perturbation of [A]. Parameters as in figure 2 with $k_{+7} = k_{-7} = k_{+8} = k_{-8} = 10^3$ which results in 93.37% channel flux at the steady state.

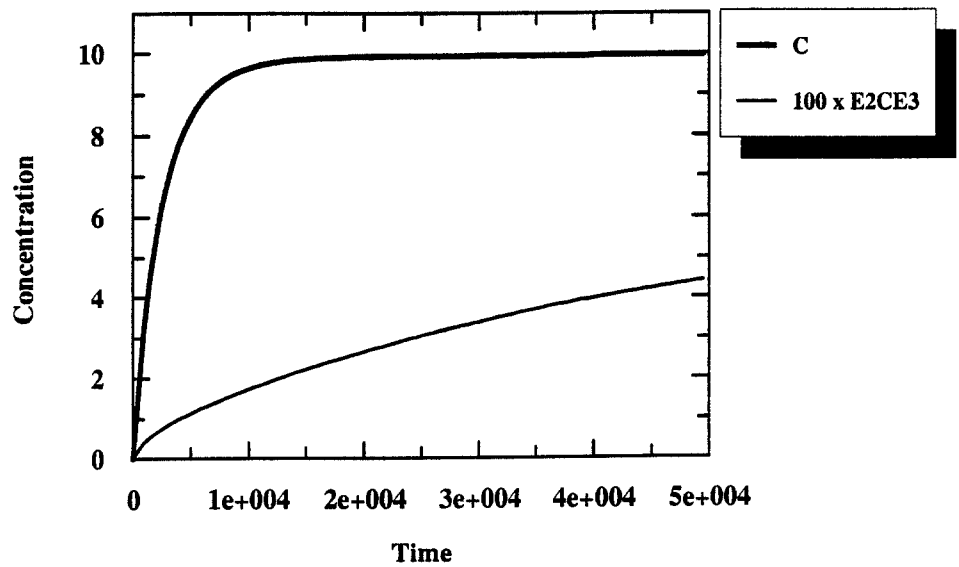

Fig. 8. Time evolution of [C] and [E_2CE_3] after a perturbation in [A]. Parameters as in figure 2 with $k_{+7} = k_{-7} = k_{+8} = k_{-8} = 10^{-3}$ which results in less than 0.01% channel flux at the steady state.

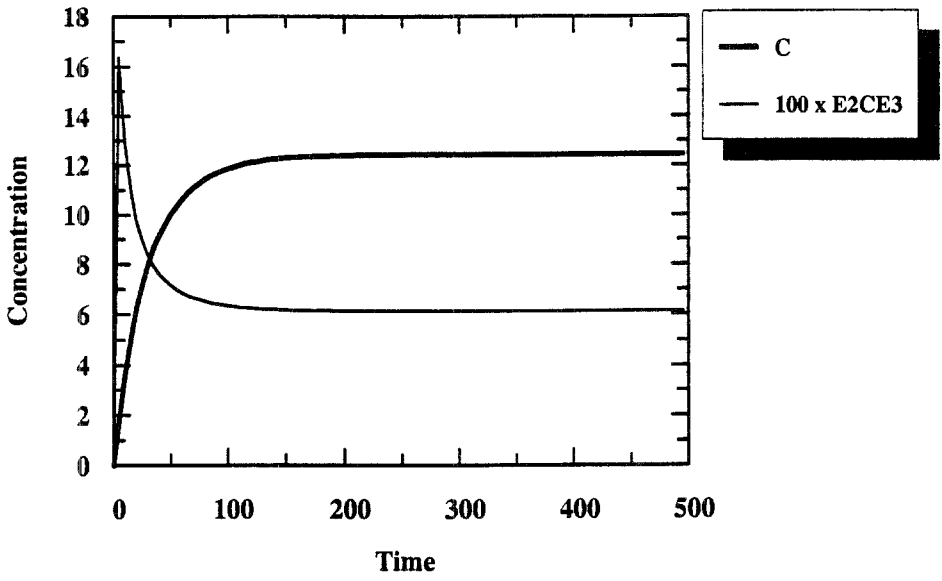

Fig. 9. Time evolution of [C] and [E_2CE_3] after a perturbation in [A]. Parameters as in figure 3 with $k_{+7} = k_{-7} = k_{+8} = k_{-8} = 10^3$ which results in 99.91% channel flux at the steady state.

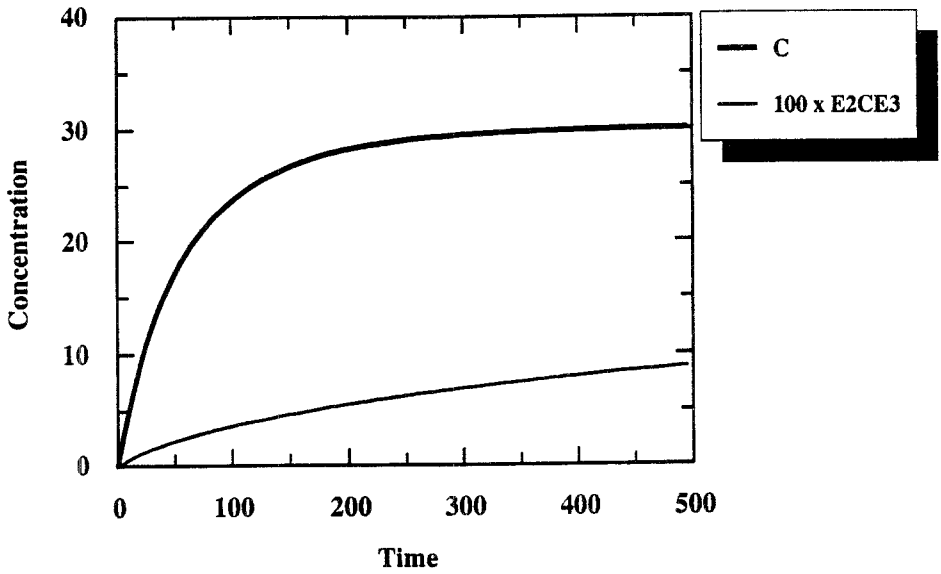

Fig. 10. Time evolution of [C] and [E_2CE_3] after a perturbation in [A]. Parameters as in figure 3 with $k_{+7} = k_{-7} = k_{+8} = k_{-8} = 10^{-3}$ which results in 0.05% channel flux at the steady state.

Figures 7 and 8 show the trajectories for the pathway with parameters as in Cornish-Bowden (1991), figure 7 at low channel flux and figure 8 at high channel flux. Figures 9 and 10 show the trajectories of the pathway with our best parameters for decrease of [C] with K_{eq} of the channelled process =1 and with high channel flux (as in figure 3).

Comparing all these trajectories one can see that there is little difference between the two sets of parameters when the channel flux is high. By contrast, at low channel flux, the system with parameters as described by Cornish-Bowden (1991) is much slower in adjusting to a new steady state. When the channel flux is high the concentration of the channel intermediate rises sharply after the perturbation and then decays to its steady-state value.

4. DISCUSSION

The idea that the channelling of an intermediate metabolite in a metabolic pathway can reduce its pool size has long been put forward (Atkinson 1971). However, only recently have attempts been made to study this with specific numeric values (Cornish-Bowden 1991, Westerhoff *et al.* 1984).

In order to establish the possible effects of metabolite channelling, the model of Cornish-Bowden (1991, our Fig.1) was studied by computer simulation. The mathematical model, representing a so-called dynamic channel, consisted of six simultaneous ordinary differential equations that describe the dynamic behaviour of the intermediate metabolite concentrations. Comparison of the dynamic behaviour and the steady-state properties of a channelled pathway with those of an unchannelled one was carried out with the aid of the simulation program GEPASI, described elsewhere (Mendes 1991).

Cornish-Bowden (1991) showed, in contrast to what might have been expected by some, that not only did the presence of a channel not decrease the concentration of the relevant metabolic intermediate but could actually increase it. We observed identical behaviour using GEPASI (Fig 2).

Notwithstanding, we found that even using the same equilibrium constant for the "channelled" reactions it was possible to establish sets of enzyme kinetic parameters that could decrease the pool size to less than one half of that observed in the absence of the channel (Figs 3,4).

However, the model studied by Cornish-Bowden possessed two important constraints: (a) by using symmetrical rate and Michaelis constants it maintained the equilibrium constant for the channel reaction at a value of 1, and (b) the enzymatic step following the channel step had a V_{max} that was not high with respect to at least one of the enzymes upstream in the pathway.

By relaxing these constraints (Figs 5, 6), we found that incorporating a channel into a pathway of identical structure to that described could in fact decrease the concentration of the pool intermediate to values less than one thousandth of those observed in the absence of the channel. It would therefore seem that channelling can indeed have a substantial effect on the free concentration of a channelled intermediate in a pathway (Westerhoff *et al.* 1984).

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