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Channelling can decrease pool size

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It is widely considered that a possible advantage of metabolite channelling, in which a product of an enzyme is transferred to the next enzyme in a metabolic pathway without being released to the 'bulk' solution, is that channelling can decrease the steady-state concentrations of 'pool' intermediates. This then spares the limited solvent capacity of the cell, and reduces the loss of pathway flux due to leakage or instability of the free intermediate. Recently, however, based on simulations of a particular model of a 'dynamic' channel, Cornish-Bowden ["Failure of channelling to maintain low concentrations of metabolic intermediates" (1991) *Eur. J. Biochem. 195*, 103–108] has argued that this is not in fact the case; his simulations indicated that the channel was rather ineffective at decreasing the concentration of the pool intermediate, and in some cases actually increased it. However, although his simulations were restricted to very specific thermodynamic and kinetic parameters, he generalised his conclusions, arguing that "channelling has no effect on the free concentration of a channelled intermediate in a pathway".

By showing that, for a number of kinetic cases, the concentration of the pool intermediate did decrease substantially with increased channelling, we demonstrate here that the conclusion of Cornish-Bowden is not correct. In particular, if the reaction catalysed by the enzymes forming the channel has an equilibrium constant K higher than 1, and if the enzyme removing the product of the channel reaction is kinetically competent, channelling in the model system studied by Cornish-Bowden (1991) can decrease the steady-state concentration of the pool by a factor of 1000, independently of the mechanism of the terminal reaction and under conditions of essentially constant overall flux. If the channel is a 'static' channel, the decrease in the pool can be to arbitrarily low levels. This conclusion also holds for a system in which other reactions may consume the pool intermediate. Thus, channelling can maintain metabolite concentrations at low levels.

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 (e.g. Srere and Mosbach, 1974; Welch, 1977; Kell, 1979; Srere, 1987; Keleti et al., 1989; Srere and Ovádi 1990; Ovádi, 1991). In addition to possible kinetic advantages, one of the advantages of channelling is widely perceived to be the reduction (via flux through the channel) of the concentration of the channelled intermediate in the bulk solution, thus sparing the limited solvent capacity of the cell (Atkinson, 1969; Clegg, 1984; Friedrich, 1984; Srivastava and Bernhard, 1986; Heinrich et al., 1991). However, very little work has actually been carried out in an attempt to test this view quantitatively, so as to examine the extent to which channels can in fact decrease pool sizes during the steady state of a metabolic pathway.

Westerhoff et al. (1984) analysed a model of membrane energy coupling in which a 'localised' (channel) mechanism existed in parallel with a pool, and showed that the channel could indeed decrease the concentration of the pool intermediate in the steady state. More recently, however, 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 (and in some cases actually increased it). 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). It was thus of interest to resolve this apparent discrepancy and to enquire more closely into the kinetic and other parameters which might determine the effectiveness with which a simple model channel can decrease the concentration of a pool intermediate.

In this work we extended the results of Cornish-Bowden (1991) and investigated the generality of his conclusions. In addition, we studied both static and dynamic channels. In contrast to the analysis of Cornish-Bowden (1991), we show that channelling can decrease the pool concentration of the intermediate substantially (in appropriate cases to less than one thousandth of that in the absence of the channel), and describe the parameters that determine the magnitude of this effect.

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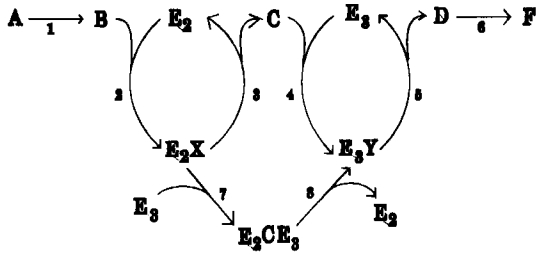

Fig. 1. The primary pathway model. Reactions are represented by arrows that indicate the forward direction of flow. All steps are considered to be reversible, except step 6 which is irreversible (the data of Fig. 6 excepted). Both enzymes 2 and 3 were broken down into elementary reactions: steps 2 and 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. The total concentrations of enzymes E_2 and E_3 are 1. 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, described as k_2, k_{-2}, k_3, k_{-3}, etc). In all simulations shown (except Fig. 8) [A] = 10 and [F] = 0.

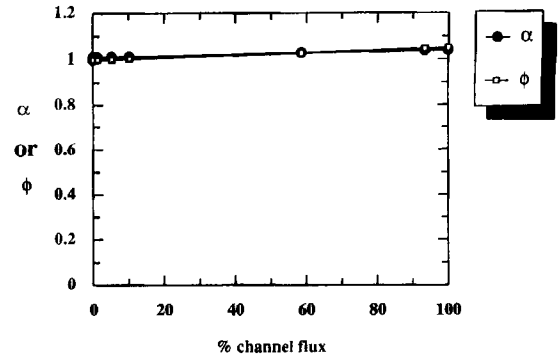

Fig. 2. Dependence of α and ϕ on the relative channel flux. Parameters as in Cornish-Bowden (1991): step 1 $K_m^s = K_m^p = V_{max}^f = V_{max}^r = 1$; step 6 $K_m^s = V_{max}^f = 1$; $V_{max}^r = 0, k_{+2} = 6\,k_{-2} = 1; k_{+3} = 5\,k_{-3} = 6; k_{+4} = 6; k_{-4} = 5; k_{+5} = 1; k_{-5} = 6$. For steps 7 and 8, p was varied from 0 to 10^5.

METHODS

The model which we have studied in most detail in this work is the same as that described by Cornish-Bowden (1991): a four-enzyme pathway in which 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 Fig. 1 (cf. Scheme 3 of Cornish-Bowden, 1991, where the association and dissociation of the two middle enzymes was left implicit).

In addition, Cornish-Bowden (1991) studied a similar channel to which was added a 'leak' reaction (i.e. a metabolic branchpoint) from the bulk metabolite C. We have also considered this (see later).

All the data displayed herein were obtained with the simulation program GEPASI (Letellier et al., 1990; Mendes, 1991), which follows the methodology described by Mendes et al. (1990). 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 (Reich and Sel'kov, 1981; Hofmeyr et al., 1986), since 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), as 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 (Stoer and Bulirsch, 1980). 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 (see Kacser and Burns 1973; Heinrich and Rapoport, 1974; Kell and Westerhoff, 1986; Westerhoff and van Dam, 1987; Kell et al., 1989; Cornish-Bowden and Cárdenas, 1990) to the steady-state data. In all models simulated by GEPASI it is assumed that all processes occur in a well-stirred reactor. GEPASI (version 2) is available from one of the authors (P.M.). For this work, the program was run on an IBM-compatible computer with an Intel 80386SX processor and an Intel 80387SX numeric coprocessor running MS-DOS 3.3.

GEPASI uses a routine for the solution of the nonlinear equations somewhat different from that of the program MetaModel (Cornish-Bowden and Hofmeyr, 1991) used by Cornish-Bowden (1991). To ensure that any effects that we observed were not due simply to differences in the programs, we first repeated the simulations of Cornish-Bowden (1991) in which the equlibrium constant for the conversions of E_2X to E_3Y was 1 whilst the percentage of flux through the channel was varied from 0 to nearly 100%. The results of Cornish-Bowden (1991) were reproduced successfully using GEPASI, and are shown in Fig. 2.

A note concerning units. In the simulations which we present here, it is not in fact important what the scale of the units is (although of course the dimensions are important); for convenience, however, we consider concentrations to be in mM, time to be in s and, therefore, fluxes to be in $mM \cdot s^{-1}$.

RESULTS

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 studied the pathway depicted in Fig. 1 (which is the same as that studied by Cornish-Bowden) and compared the steady state in the case where there is no channel flux with steady states for increasing proportions of channel flux. A number of different variations of the parameters of Fig. 1 will lead to a variation of the channel flux. However, these variations are subject to two types of constraint. The

Table 1. Effect of the rate constants of steps 3 and 4 on the fluxes and the concentrations of the channelled and pool intermediates Simulations were performed as described in the legend to Fig. 2 except that $V_{max,6}$ was 10^5 and p was adjusted to values around 100 so as to give a constant net flux.

k_{+3}	k_{-3}	k_{+4}	k_{-4}	[C]	[E_2CE_3]	Net flux	Pool flux	Channel flux
5	6	6	5	3.339	0.1230	0.6957	0.1545	0.5412
0.5	0.6	6	5	3.223	0.1230	0.6957	0.02720	0.6685
0.05	0.06	6	5	3.200	0.1230	0.6957	0.002957	0.6927
5	6	6	5	3.339	0.1230	0.6957	0.1545	0.5412
5	6	0.6	0.5	3.462	0.1230	0.6957	0.02874	0.6670
5	6	0.06	0.05	3.488	0.1230	0.6957	0.003167	0.6925
5	6	6	5	3.339	0.1230	0.6957	0.1545	0.5412
0.5	0.6	0.6	0.5	3.339	0.1230	0.6957	0.01541	0.6803
0.05	0.06	0.06	0.05	3.339	0.1230	0.6957	0.001548	0.6941

first, the requirement for microscopic reversibility, recognises that 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. In terms of the rate constants

$$\frac{k_3 \cdot k_4}{k_{-3} \cdot k_{-4}} = \frac{k_7 \cdot k_8}{k_{-7} \cdot k_{-8}} = \mathrm{K}.$$

The second restriction is that, for a given metabolic pathway, alterations in the enzymes' properties leading to more or less channelling cannot lead to a change in the equilibrium constants for the interconversion of the metabolites. For the case of Fig. 1, this translates to:

$$\frac{k_1}{k_{-1}} = K_{AB}$$

$$\frac{k_2 \cdot k_3}{k_{-2} \cdot k_{-3}} = K_{BC}$$

$$\frac{k_4 \cdot k_5}{k_{-4} \cdot k_{-5}} = K_{CD}$$

$$\frac{k_6}{k_{-6}} = K_{DF}$$

where the K's are constant. This still leaves quite a number of possibilities with which one may vary the kinetic parameters to modulate the extent of channelling in a given metabolic pathway. In line with Cornish-Bowden (1991) we shall mainly vary channelling by simultaneously varying k_7, k_{-7}, k_8 and k_{-8} by the same factor, p.

In addition to variations which one might make in the parameters, one must also consider the choice of a suitable reference state. We take a reference state in which $K = a$; k_7, k_{-7}, k_8 and k_{-8} have magnitudes such that: $k_{+7} = p.a$, $k_{-7} = p$, $k_{+8} = p$ and $k_{-8} = p$. The values of all other parameters will be given for each specific case. Thus 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, 'a perfect channel', means that there is no leak and so [C] $\stackrel{\text{def}}{=}$ 0; this situation is not considered further here.

Finally, we define three normalised coefficients: α, 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 concentration of C observed when no channel is present (i.e. $[C]/[C]_0$). The lower the value of α, the more effective is the channel in decreasing the concentration of C. ϕ, for a given

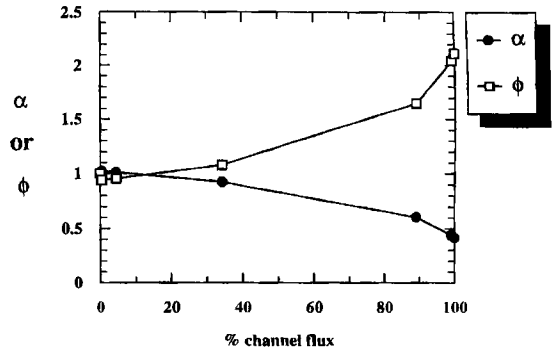

Fig. 3. Dependence of α and ϕ on the relative channel flux. Parameters: steps 1 and 6 as in Fig. 2; $k_{+2} = 6$; $k_{-2} = 1$; $k_{+3} = k_{-3} = 10^3$; $k_{+4} = k_{-4} = 0.03$; $k_{+5} = k_{-5} = 6$. For steps 7 and 8, p was varied from 0 to 10^5

set of parameters, is the productive flux (towards metabolite F of Fig. 1) when a channel is present divided by that when it is absent (i.e. J/J_0). When the leak reaction (metabolic branch) is present, we also define β, for a given set of parameters, as the ratio of the flux through the branch when a channel is present to that when it is absent (i.e. $J_{branch}/J_{branch(0)}$).

K = *1 for the conversions of E_2X to E_3Y*

Under the stated conditons, Cornish-Bowden (1991) showed that at nearly 100% channel flux, $\alpha = 1.03$, reflecting an increase in [C] in the steady state of 3% (and see Fig. 2). The effect on ϕ, the normalised productive flux, is virtually identical (Fig. 2).

Whilst the overall equilibrium constant for the conversion of B to D may be constrained to take a value of 1, this may be accomplished in a variety of ways, since $K_{BD} = K_{BC} \cdot K_{CD}$. Provided that the activity of step 3 is lowered to enhance channelling, the pool size decreases (as may be expected); similarly, if one enhances channelling by limiting reaction 4 then the pool size increases (Table 1). However, these effects are rather modest.

Choosing 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, which changes K_{BC} and K_{CD} in a reciprocal manner, we obtained the data shown in Fig. 3. An increase in channelling was accompanied by a significant decrease in [C], 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

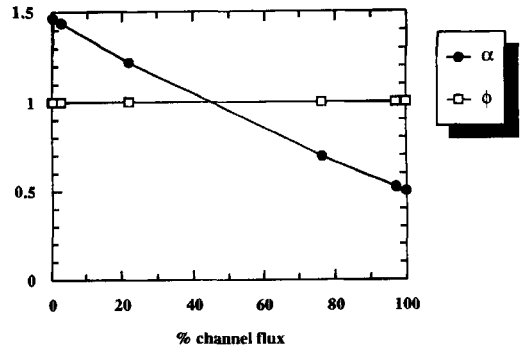

Fig. 4. Dependence of α and ϕ on the relative channel flux. Parameters: steps 1 and 6 as in Fig. 2; $k_{+2} = 10^3$; $k_{-2} = 1$; $k_{+3} = k_{-3} = 10^3$; $k_{+4} = 10^3$ $k_{-4} = 1$; $k_{+5} = k_{-5} = 10^3$. For steps 7 and 8, p was varied from 0 to 10^5

concentration of the intermediate. Under these conditions (Fig. 3), the total flux is actually increased by a factor of approximately 2.

K > 1 *for the conversions of* E_2X *to* E_3Y

In all of the simulations performed by Cornish-Bowden (1991), and in our Fig. 3, the equilibrium constant for the channelled reaction was 1, i.e. the reaction was highly reversible. This in itself might be expected to inhibit the ability of any channel to decrease a pool, since, under these circumstances, every time a molecule passed through the channel it could still return rapidly to the pool from the product side. Another argument against this set of parameters is that in order to have a $K = 1$ 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^r_{max} is larger than V^f_{max} for E_3, as in Cornish-Bowden (1991), or the K_m for the substrate is bigger than that for the product in both E_2 and E_3. We therefore considered a scheme in which the equilibrium constant for the channelled reaction was greater than 1, i.e. one which would favour flux in the forward direction.

In order to make enzymes 2 and 3 have kinetic parameters that favour the flux in the forward direction, we made V^f_{max} equal to 1000, V^r_{max} equal to 1 and both K_m values for substrate and product equal to 1 for both E_2 and E_3. This sets $K = 1000$ for the conversion of E_2X to E_3Y, and so $k_{+7} = 1000 \cdot p$ in order not to violate the constraint of microscopic reversibility.

As can be seen in Fig. 4, channelling decreases [C] by a factor of as much as 2, as judged with respect to its value in the absence of the channel. It is interesting that at very low channel flux [C] increases sharply but very quickly starts its monotonic decrease, so that the effect of the channel on [C] relative to the highest [C] observed is almost threefold. Importantly, these effects of the channel were observed under conditions of constant flux (i.e. ϕ remains at 1 throughout, Fig. 4).

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

As previously mentioned by Cornish-Bowden (1991), the (high) degree of saturation of the last step of the pathway of Fig. 1 could be affecting [C] via high levels of [D], which would

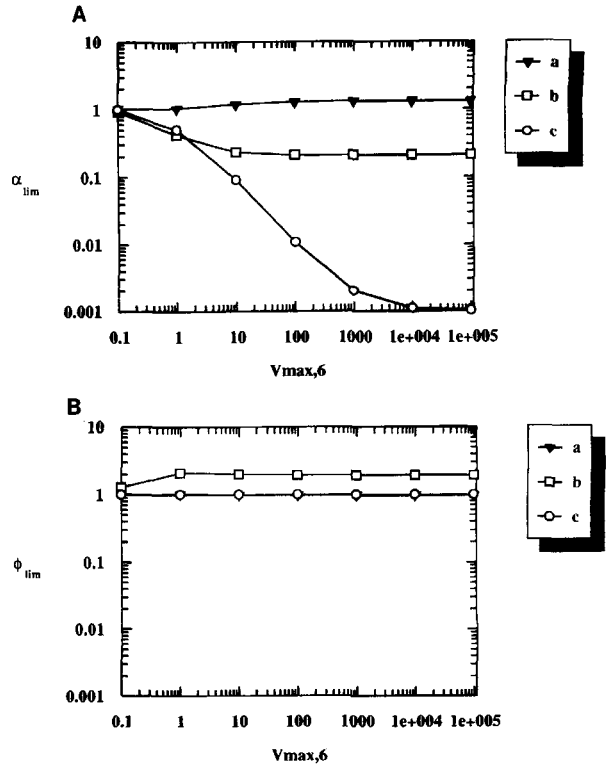

Fig. 5. Dependence of α_{lim} (A) and ϕ_{lim} (B) on the V_{max} of step 6. Parameters: step 1 as in Fig. 2; step 6 $K_m = 1$, V_{max} varied as indicated; steps 2, 3, 4, 5, 7 and 8: conditions (a) as in Fig. 2, (b) as in Fig. 3, (c) as in Fig. 4.

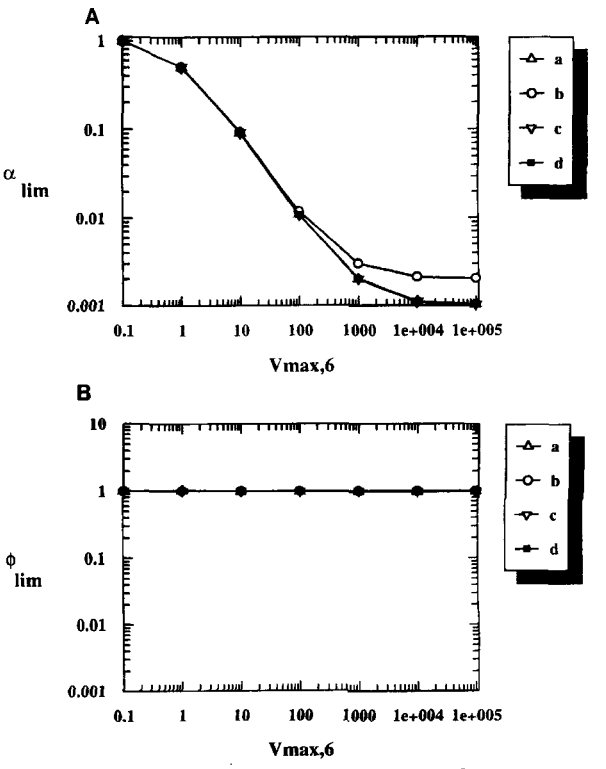

Fig. 6. Dependence of α_{lim} (A) and ϕ_{lim} (B) on the V^f_{max} of step 6 (which is reversible except in parameter set d). Parameters: steps 1, 2, 3, 4, 5, 7 and 8 as in Fig. 4. Step 6: V^f_{max} varied as indicated, K^s_m, K^p_{max} and V^r_{max} with values to make different values of K, according to the Haldane relationship. K of step 6: (a) infinity, (b) 1, (c) 10^3, (d) 10^8. [F] was equal to 0.1.

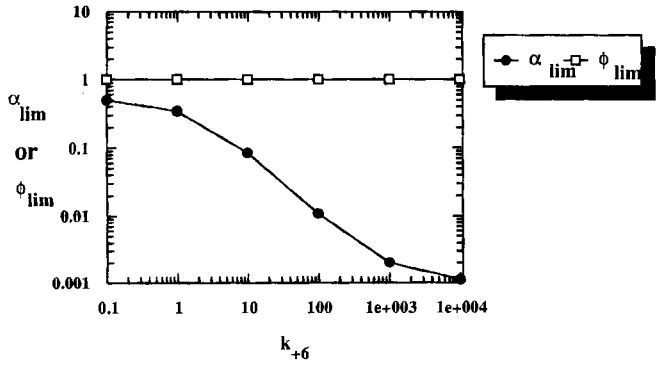

Fig. 7. Dependence of α_{lim} and ϕ_{lim} on the forward rate constant of step 6, when this is made unsaturable. Other parameters as in Fig. 5, conditions (c).

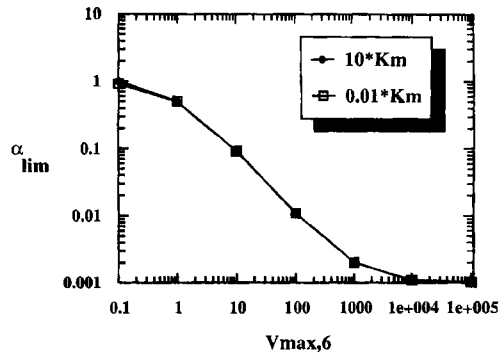

Fig. 8. Effect of the concentration of metabolite A on the effectiveness of the V_{max} of step 6 in decreasing the concentration of C. Other parameters as in Fig. 5, conditions (c).

Table 2. Effect of the rate constants of steps 3 and 4 on the fluxes and the concentrations of the channelled and pool intermediates. Simulations were performed as described in the legend to Fig. 4 except that $V_{max,6}$ was 10^5 and p was adjusted to values around 100 so as to give a constant net flux

k_{+3}	k_{-3}	k_{+4}	k_{-4}	$10^6 \times$[C]	$10^3 \times$[E_2CE_3]	Net flux	Pool flux	Channel flux
100000	100000	100000	100	9.281	9.101	0.9089	0.8270	0.08191
1000	1000	100000	100	2.732	75.69	0.9089	0.1605	0.7484
10	10	100000	100	1.033	91.52	0.9089	0.001998	0.9069
100000	100000	100000	100	9.281	9.101	0.9089	0.8270	0.08191
100000	100000	1000	1	174.7	75.70	0.9089	0.1604	0.7485
100000	100000	10	0.1	221.0	91.53	0.9089	0.001997	0.9069
100000	100000	100000	100	9.281	9.101	0.9089	0.8270	0.008191
1000	1000	1000	1	98.57	82.79	0.9089	0.08940	0.8195
10	10	10	0.01	111.2	91.62	0.9089	0.001000	0.9079

leak back and fill up the pool, thus inhibiting the ability of the channel to decrease the pool. It was therefore of interest to see whether the converse might therefore be true: would decreasing [D], and/or affecting the degree of saturation of step 6, stop the channel from leaking back into the pool?

To decrease [D], we changed the V_{max} of step 6 in decadic steps from 0.1 to 10^5. At each different value for the V_{max} of step 6, simulations were carried out for $p = 0$ (no channelling) and $p = 10^5$ (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}. Similarly, we define ϕ_{lim} as the value of ϕ as the percentage of channelling tends towards 100%.

Fig. 5 shows graphs of α_{lim} and ϕ_{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 now dramatically to decrease α_{lim}, provided that both the V^f_{max} for reaction 6 and the K for the conversion $E_2X \rightarrow E_3Y$ are reasonably high. It is worth stressing again that, particularly for values of $V_{max,6} > 1$, the effect on the total flux was negligible.

Since it constitutes a boundary condition, having an absolutely irreversible final step seems rather unreal; thus we also checked that making it reversible does not significantly affect the steady-state behaviour of the pathway. Fig. 6 shows how α_{lim} and ϕ_{lim} depend on the V^f_{max} of step 6 when it was made reversible, at different values for the equilibrium constant of this step. It is evident (Figs 5 and 6) that α_{lim} is a function of the V^f_{max} of the step(s) following the channel, and that if both (a) the K for the reaction involving the direct transfer of intermediate between enzymes and (b) 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.

Although changing the maximum velocity of the terminal reaction will change its degree of saturation, this does not mean that the changing saturation is in fact the cause of the dramatic ability of the channel to lower the pool concentration (Figs 5 and 6). We therefore changed step 6 into a simple first-order reaction (which cannot therefore be saturated), with rate constant k_{+6}. Were the earlier effects due to changes in the saturation of step 6, as intimated by Cornish-Bowden (1991), increasing the value of k_{+6} would be expected to have only a marginal effect on the effectiveness of the channel in decreasing [C]. Fig. 7 shows the actual effect of k_{+6} on α_{lim} and ϕ_{lim}. It may be observed, again, that the effect of the channel is substantially to decrease the concentration of the bulk metabolite C, under conditions of constant total flux, provided that the value of k_{+6} is sufficiently high. It may be concluded that decreasing the ability of metabolite D to fill up the pool does indeed increase the effectiveness of the channel in reducing [C] (Figs 5–7), but that this is not due to saturation of the terminal step (Fig. 7).

Finally, we maintained K at a high value (1000) and varied the channel flux not by modulating the rate constants of steps 7 and 8 but by varying those of steps 3 and 4 (i.e. varying the channel flux indirectly by varying the pool flux). In this case (Table 2), increasing the channel flux could be associated with either a decrease or an increase in the concentration of the pool intermediate, depending upon whether the rate constants for step 3 or for 4 are changed from their reference values. The general behaviour is similar to that observed in Table 1, although the relative amplitudes are somewhat greater. However, these modulations represent the effect of a pool on a channel, and not that of a channel on a pool.

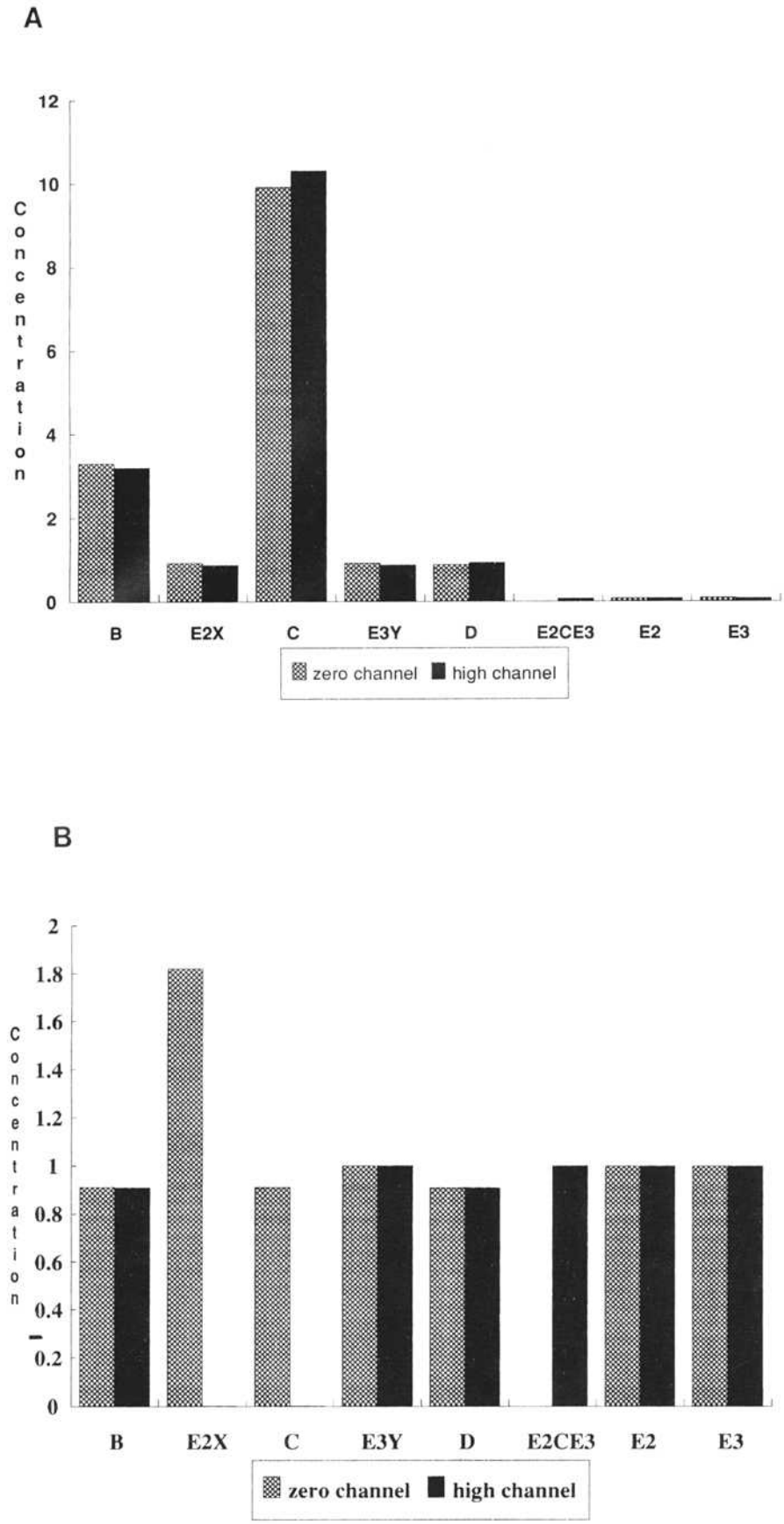

Fig. 9. Effect of channel flux on the concentration of all internal metabolites in the primary model. (A) Parameters as in Fig. 2 (and in Cornish-Bowden, 1991). The ordinate gives the concentrations in millimolar. (B) Parameters are as in Fig. 5, conditions c. The ordinate gives the concentration in millimolar except that, to improve clarity, the true numbers have been multiplied by the following factors: [B], [E_2X], [C], [E_3Y] and [E_2CE_3], 1000; [D] 10^4; [E_2] and [E_3], 1

Effect of the initial substrate concentration

Since the kinetic parameters of step 6 were so important in determining the effectiveness of the channel in decreasing the pool concentration, it was of interest to study whether the initial (clamped, external) substrate concentration might also be significant. Fig. 8 shows, however, that α_{lim} is essentially independent of the initial substrate concentration, at all values of the V_{max} of step 6. Under these conditions, the total flux did not change by more than 0.001% of its starting value (not shown).

Effect of channelling on other substrate concentrations

Since we had found that channelling could indeed dramatically decrease the concentrations of metabolite C, it was of interest to study whether channelling might also affect the steady-state concentrations of other components of the system. To this end, Fig. 9 displays the steady-state concentrations of each of the relevant components at zero and high (99.92% of the maximum achievable) flux through the channel, using (a) the parameters of Cornish-Bowden (1991) (our Fig. 2), where the channel is rather ineffective at decreasing the pool of C, and (b) those of our Fig. 5 with condition c (in which V_{max} of step 6 was 10^5 and the channel flux >99.99% of the maximum achievable). In the former case, channelling has little effect upon any of the metabolite concentrations. However, Fig. 9B shows that $[E_2X]$ is strongly decreased as channelling is enhanced. Given that $[E_2]$ is unchanged, $[E_2CE_3]$ is raised since the total of the enzyme-bound forms of the relevant metabolites must be constant.

The effect of using a 'static' channel

The above simulations were performed using what is termed (Friedrich, 1985; Keleti et al., 1989) a dynamic channel. A dynamic channel (as in Fig. 1) is one in which a necessary condition for the enzymes to form a complex is that one of them has already bound the common intermediate. In principle, however, one may also have a so-called 'static' channel, in which this condition is absent, and in which (in our terminology) an E_2E_3 complex exists in the absence of the common intermediate. For simplicity, we shall assume that the affinity of E_2 for E_3 does not depend upon whether either has a ligand (X, Y or C) bound. Therefore the $[E_2E_3]_{total}$ (i.e. the total concentration of the E_2E_3 moiety) is now a parameter, which may be varied (as a function of the total $[E_2]$ and $[E_3]$). This pathway is illustrated in Fig. 10.

To summarise a great deal of data, Fig. 11 shows the effect on α_{lim} and ϕ_{lim} of the V_{max} of step 6 under conditions in which the $[E_2E_3]_{total}$ is 25%, 50% and 75% of the total amount of E_2 or E_3. Comparison of Fig. 11A with curve c of Fig. 5A shows that, particularly at high values of $V_{max,6}$, the static channel can be even more effective than the dynamic channel in decreasing the concentration of the pool metabolite, apparently to arbitrarily low levels. It is noteworthy that as the V_{max} of step 6 continues to increase, the dramatic decrease in the pool induced by enhancing the channel (α_{lim} of Fig. 11A) is not accompanied by any change in flux (ϕ_{lim} in Fig. 11B).

Effect of a metabolic branch on channelling

Cornish-Bowden argued that one 'purpose' of the channel in having the (potential) ability to decrease [C] would be manifest if C could participate in another metabolic pathway (summarised by reaction 9 in Fig. 12) in which it was converted to a cytotoxic product. We therefore modelled this process, using the dynamic channel, to see if the channel would also be effective at decreasing the pool in this situation. Fig. 13 shows the effect on α_{lim}, ϕ_{lim} and β_{lim} of the V_{max} of step 6 for various sets of parameters. It may be observed that the presence of the leak reaction has a negligible effect (with respect

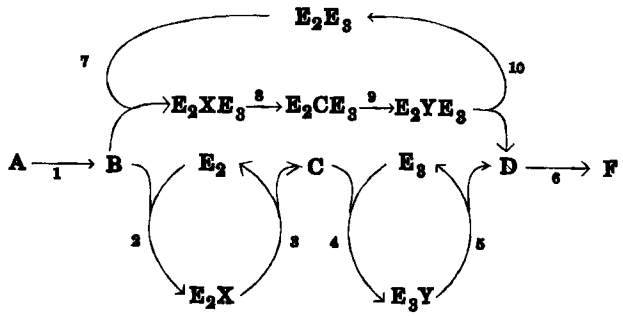

Fig. 10. Pathway model containing a static channel. Reactions are represented by arrows that indicate the forward direction of flow. All steps are considered to be reversible. The reactions of both enzymes 2 and 3 were broken down into elementary reactions: steps 2 and 3 for enzyme 2, steps 4 and 5 for enzyme 3. E_2 and E_3 are free forms of enzyme. E_2X, E_3Y are 'normal' enzyme complexes and E_2E_3 is the binary enzyme complex without bound intermediate. Steps 1 and 6 react according to reversible Michaelis-Menten kinetics, and steps 2, 3, 4, 5, 7, 8, 9 and 10 are chemical reaction steps (with two associated kinetic constants each). In all simulations shown [A] = 10 and [F] = 0.1

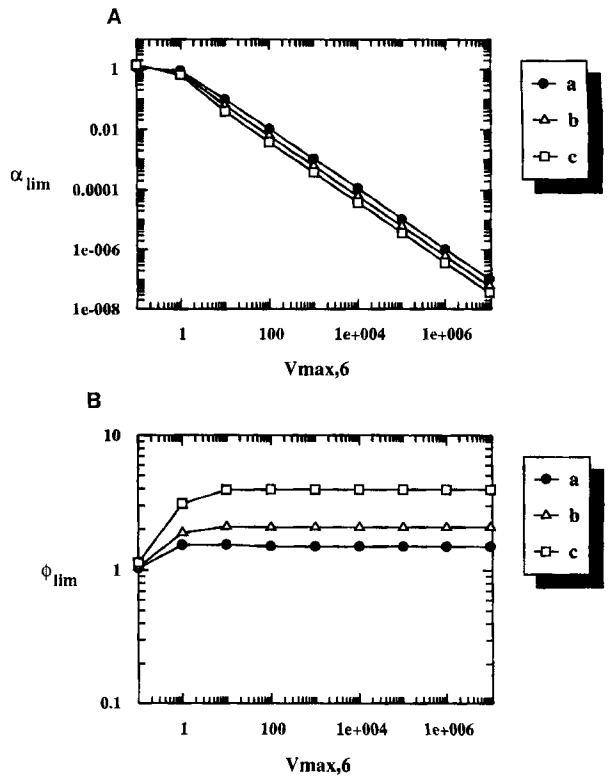

Fig. 11. Effect on α_{lim} and ϕ_{lim} of V^f_{max} of step 6 when the channel is a static channel, at different normalised concentrations of E_2E_3. Parameters for steps 1–6 as in Fig. 4, except that V_{max} of step 6 was varied as indicated. For steps 7, 8, 9 and 10 the rate constants (p) are zero or 10^6. $[E_2E_3]_{total}$/[all E_2 moieties] was varied as follows: (a) 25%, (b) 50%, (c) 75%.

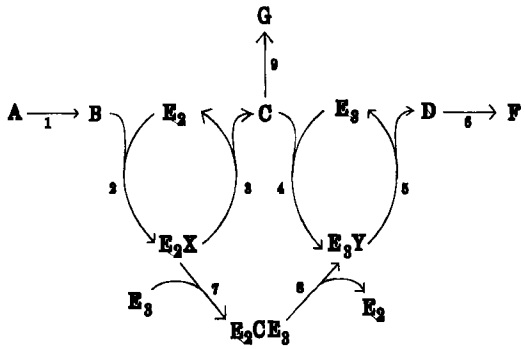

Fig. 12. Model of a dynamic channel with a branch from the bulk form of metabolite C. Reactions are represented by arrows that indicate the forward direction of flow. All steps are considered to be reversible, except step 6 which is irreversible. The reactions of both enzymes 2 and 3 were broken down 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, steps 6 and 9 according to irreversible Michaelis-Menten kinetics 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 [F] = 0

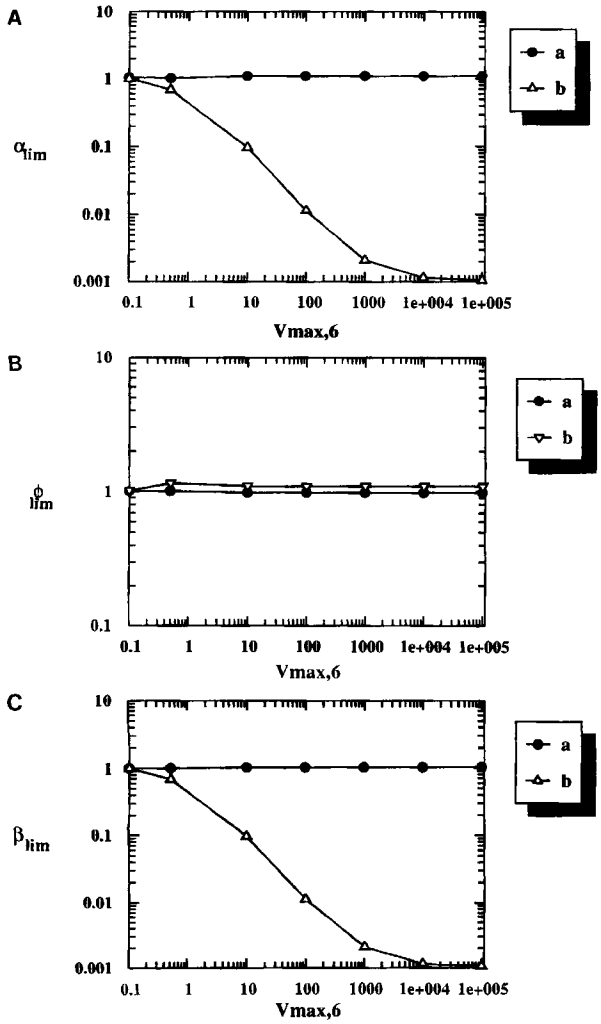

Fig. 13. Dependence of α_{lim} (A), ϕ_{lim} (B) and β_{lim} (C) on the V_{max} of step 6 for a dynamic channel with a branchpoint. Parameters: step 1 as in Fig. 2; step 6 K_m = 0.07818, V_{max} varied as indicated; steps 2, 3, 4, 5, 7 and 8 as in Fig. 2 (for a) or Fig. 4 (for b). Step 9: K_m = 0.5, V_{max} = 0.5 for (a), K_m = 1, V_{max} = 100 for (b).

to the data in Fig. 5 conditions a and c) on the ability or otherwise of the channel to decrease the pool concentration (Fig. 13A) at constant flux (Fig. 13B), and that the decrease in the bulk concentration of C is accompanied by a proportionate decrease in the flux through the branch (Fig. 13C). It may be mentioned that the magnitudes of the leak pathway at low values of $V_{max,6}$ were such that more than 80% of the total flux went via the leak pathway.

DISCUSSION

Much of metabolism *in vivo* may be organised such that intermediary metabolites are passed from enzyme to enzyme without becoming solvated by the bulk of the aqueous cytoplasm, and do not exhibit 'pool' behaviour. The evidence for this phenomenon (variously referred to as 'micro-compartmentation', 'channelling' or 'localised coupling') is both structural and functional, and has recently been reviewed *in extenso* (e.g. Srivastava and Bernhard, 1986; Welch and Clegg, 1986; Srere, 1987; Jones, 1988; Keleti et al., 1989). If this type of behaviour does indeed occur, the question arises as to what possible advantages it might have for the cell.

One widely held view is that it is attractive for a cell to keep the free concentrations of some metabolites low. The advantage of this if the metabolites are labile is obvious; in addition, the presence of metabolic pathways potentially competing for free metabolites may constitute a reason for keeping the concentration of the free metabolites low. Moreover, as pointed out by Atkinson (1969, 1977), the cell may have a limited solvent capacity, and certainly this solvent capacity is also constrained by the requirements of osmotic balance (Savageau, 1976; Heinrich et al., 1987; Schuster and Heinrich, 1987, 1991; Schuster et al., 1991). At all events, the idea that the channelling of an intermediate metabolite in a metabolic pathway can reduce its pool size is widely held. However, only recently have attempts been made to substantiate this idea by calculating, for well-defined model systems, whether more channelling is indeed accompanied by a lowered concentration of free metabolite. Conflicting results have been obtained, in that Westerhoff and colleagues (1984) found that channelling could decrease pool sizes significantly, whilst more recently Cornish-Bowden (1991) argued from his own simulations that "channelling has no effect on the free concentration of a channelled intermediate in a pathway".

Since both of these previous studies used rather limited sets of parameter values, we assumed that the apparent conflict between them might reflect the fact that it is the exact conditions under which channelling is increased that determines whether the free metabolite concentration does or does not decrease. In the present work, we therefore set out, more systematically than had previously been done, to study the relationship between the extent of channelling and the concentration of the free intermediate.

To facilitate comparison with the results obtained by Cornish-Bowden (1991), we focussed on the model (our Fig. 1) that he studied. Comparison of the steady-state properties of this pathway under conditions in which little or no flux went through the channel with conditions in which most flux went through the channel was carried out with the aid of the simulation program GEPASI (Letellier et al., 1990; Mendes, 1991).

On increasing the fraction of flux through the channel in Fig. 1, Cornish-Bowden (1991) found hardly any decrease in the free concentration of intermediate. He then generalized this finding to the effect that "it is unlikely that varying [other]

rate constants could bring about very large changes in the intermediate concentration".

However, Cornish-Bowden (1991) studied the effects of only two types of ways of varying the extent of channelling in the model of Fig. 1. In the first, he increased the channelling flux by increasing the forward and reverse rate constants of steps 7 and 8 by the same factor (p). In his second way of varying the extent of channelling, Cornish-Bowden (1991) again increased k_{+7}, k_{-7}, k_{+8} and k_{-8} by the same factor, but then decreased each of the rate constants of steps 2, 3, 4 and 5 by the same factor (q). However, there are numerous other parameters which may be changed and which may affect the ability of the channel to decrease the pool.

Notwithstanding, one cannot change rate constants in kinetic schemes at will. First, any change must be consistent with the thermodynamic requirement of microscopic reversibility. This imposes a strict constraint on the rate constants of steps 3, 4, 7, and 8 of Fig. 1. Second, for a given metabolic pathway, the identity of the metabolites A, B, C, D, and F should remain fixed as one changes the extent of channelling (since the latter supposedly only occurs as a result of changes in the enzymes). Consequently, for a given metabolic pathway, the equilibrium constants for conversion(s) of these metabolites should not be affected by the parameter changes.

Nonetheless, for a given pathway structure, the standard free energies of the metabolites themselves may affect the extent to which channelling can decrease the pool. To this end, 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, by changing the standard free energy differences between B and C and between C and D, decrease the pool size to less than one half of that observed in the absence of the channel (Fig. 3). Similarly, by changing the equilibrium constant for the reaction $E_2X \rightarrow E_3Y$, we also found that channelling could decrease the pool size significantly, under conditions of a constant total flux (Fig. 4).

The model studied by Cornish-Bowden possessed two important constraints: not only, by using symmetrical rate constants, did it maintain the overall equilibrium constant for the channel reaction at a value of 1 (see above), but, in addition, 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 (thus allowing any E_3Y formed via the channel to leak back and fill up the pool from the product side). In contrast, for instance, to the constraint of microscopic reversibility, neither of these constraints is necessary. We have already seen (Fig. 4) that relaxing the first of these constraints can cause channelling to decrease the pool.

By relaxing both of these constraints (Fig. 5), we found that incorporating a channel into a pathway of identical stucture to that described could in fact decrease the concentration of the pool intermediate to one thousandth of that observed in the absence of the channel, under conditions in which the net flux through the pathway was barely changed. Similar behaviour was obtained when step 6 was made reversible (Fig. 6) or was replaced by a first-order reaction which could not be saturated (Fig. 7). It is therefore evident that channelling can indeed have a substantial effect on the free concentration of a channelled intermediate in a pathway (Westerhoff et al., 1984), and that the effectiveness of the channel is governed by the kinetic properties of the entire system.

Under our conditions, the effectiveness of the channel in decreasing the concentration of the pool intermediate, and the role of the V_{max} of step 6 in controlling this, did not depend upon the concentration of the starting substrate.

Having seen that channelling may or may not be accompanied by a decrease in the concentration of the free metabolite C, one may wish qualitatively to understand when and why [C] does in fact decrease. First, we note that $[E_2]_{total}$ and $[E_3]_{total}$ must be constant. In the simulations of Fig. 2, the case focussed on by Cornish-Bowden (1991), channelling did not significantly change the concentrations of either E_2X, E_2, E_3Y or E_3, and hence those of E_2CE_3 and C were also essentially constant (Fig. 9A). By contrast, as the rates of steps 7 and 8 are increased to enhance channelling in our simulation in Fig. 5, conditions c, $[E_2X]$ drops substantially (Fig. 9B) and hence the rate of step 3 (filling up the pool) decreases. Under these conditions, $[E_2]$ is essentially constant, and thus $[E_2CE_3]$ is raised to fulfil the conservation of enzyme moieties. However, when the channel flux is modulated indirectly (at constant total flux), by changing the rate constants for steps 3 and 4 (Tables 1 and 2), it is step 3 that we should decrease in order to effect a decrease in the pool concentration from the relevant reference value.

In the above arguments no back flux from E_3Y to C was considered. For Fig. 2, k_{-4} exceeds k_5, but for the simulations in which we found [C] to decrease with channelling k_5 exceeded k_{-4}. In these terms we can also understand why, at the lower values for the rate constants of step 6, [C] is little decreased by the addition of the channel: because of the back reaction from D to E_3Y the back flux to C becomes significant. In contrast, lowering [D] by stimulating its removal towards F stops this, and thus permits channelling to decrease the pool.

The model system studied thus far was a dynamic channel, i.e. one in which a necessary condition for the enzymes to form a complex is that one of them has already bound the common intermediate. It was of interest, therefore, to study a 'static' channel, in which this condition is absent, and in which (in our terminology) an E_2E_3 complex can form in the absence of the common intermediate. We found (Fig. 13) that the 'static' channel (Fig. 12) could be even more effective than the dynamic one in decreasing the concentration of a pool intermediate, ultimately (at high values of $V_{max,6}$) to what appear to be arbitrarily low levels.

Although it may be argued that channels might be more useful in unbranched pathways (Srere, 1987), Cornish-Bowden (1991) developed the idea that a channel might be beneficial to the cells if C (or a metabolite thereof) was toxic at 'high' concentrations. To this end, he extended his study to a case in which (bulk) C could be consumed by side-reactions (our Fig. 12). We found (Fig. 13) that the addition of this extra 'leak' pathway had little effect on the ability or otherwise of the channel to decrease the pool.

In conclusion, we have seen that, as expected by the metabolic control analysis (Kacser and Burns, 1973; Heinrich and Rapoport, 1974; Kell and Westerhoff, 1986; Westerhoff and van Dam, 1987; Kell et al., 1989; Cornish-Bowden and Cárdenas, 1990), which indicates that metabolic control is a systemic property of all enzymatic (and other) parameters in the system of interest, it is not possible to make conclusions about the effectiveness of channelling by studying only some of the relevant parameters. When these are studied systematically, it is possible to show not only that channelling can in fact decrease the pool size in a simple, model channel, but also why it does so. In particular, the equilibrium constant of the channelled reaction and the rate at which its product is removed are especially critical in determining the effectiveness of a metabolite channel in decreasing pool concentrations.

Overall, our analysis suggests that one should indeed study this type of system using metabolic control analysis (Kell and Westerhoff, 1990; Welch and Keleti, 1990); such a study, including a study of the dynamic control of channelled pathways, will form the subject of a subsequent communication.

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