Do Deleterious Mutations Act Synergistically? Metabolic Control Theory Provides a Partial Answer

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ABSTRACT

Metabolic control theory is used to derive conditions under which two deleterious mutations affecting the dynamics of a metabolic pathway act synergistically. It is found that two mutations tend to act mostly synergistically when they reduce the activity of the same enzyme. If the two mutations affect different enzymes, the conclusion depends on the way that fitness is determined by aspects of the pathway. The cases analyzed are: selection for (1) maximal flux, (2) maximal equilibrium concentration (pool size) of an intermediate, (3) optimal flux, (4) optimal pool size. The respective types of epistasis found are: (1) antagonistic, (2) partly synergistic, (3–4) synergism is likely to predominate over antagonism. This results in somewhat different predictions concerning the effect of metabolic mutations on fitness in prokaryotes and eukaryotes. The fact that bacteria are largely clonal but have often a mosaic gene structure is consistent with expectations from the model.

EPISTASIS between deleterious mutations can be very important, since it affects the mutational load. For example, if deleterious recessive mutations show synergistic epistasis (the reduction in fitness caused by two mutations acting together is larger than the sum of the two reductions when they act alone), the mutational load of sexual populations is reduced relative to that of asexual ones (KIMURA and MARUYAMA 1966), and this effect can be as strong as to compensate for the twofold cost of sex (KONDRASHOV 1988). Furthermore, if epistasis results in approximately truncation selection, diploidy is favored over haploidy (KONDRASHOV and CROW 1991). Data on this topic are distressingly scarce, including those on human populations (reviewed by KONDRASHOV 1988).

Metabolic control theory (KACSER and BURNS 1973, 1979) has been successful in describing the ways that metabolic performance (such as flux) depends on enzyme activity. Experiments on bacterial metabolism, for example, are in good accord with the theory (DEAN, DYKHUIZEN and HARTL 1986; DYKHUIZEN, DEAN and HARTL 1987). The theory has been found useful in deriving conditions for dominance (KACSER and BURNS 1981) and pleiotropy (KEIGHTLY and KACSER 1987) in metabolism. I show that metabolic control theory is applicable to problems of epistasis as well. The simple formulae for flux and pool are largely taken from CLARK (1991), who established a connection between metabolic control theory and quantitative genetics. Epistasis can be defined on an additive and a multiplicative basis. On the additive basis, it is the convexity or concavity of the fitness–number of mutations plot that defines antagonistic ("diminishing return") and synergistic epistasis (Figure 1), whereas on the multiplicative basis, it is the convexity or concavity of the *logarithmic* fitness that is decisive. For synergism, the multiplicative definition covers a larger number of cases than the additive definition, simply because a linear plot (no epistasis) in the latter qualifies as synergistic in the former. For the theories of sex, using difference equations, it is the multiplicative basis which is relevant [see Charlesworth (1990) for review]

METHOD

Mutations affecting the same enzyme

It is interesting to consider first the effects on fitness of two mutations of the same enzyme. In principle, these effects can act synergistically or antagonistically. In order to avoid terminological confusion, I will use the word "interaction" rather than "epistasis" in this case.

Fitness directly proportional to flux: Imagine an unsaturated, linear metabolic pathway. The flux (J) through the system depends on the activity of a single enzyme (E) as: $J = aE/(1 + bE)$, i.e. enzyme activity saturates the pathway (a and b are constants). If it is assumed that mutations act additively on the activity scale (GILLESPIE 1978), they will necessarily act synergistically on flux. If flux is directly proportional to fitness, then synergistic interaction is guaranteed. This conclusion is modified when mutations do not affect

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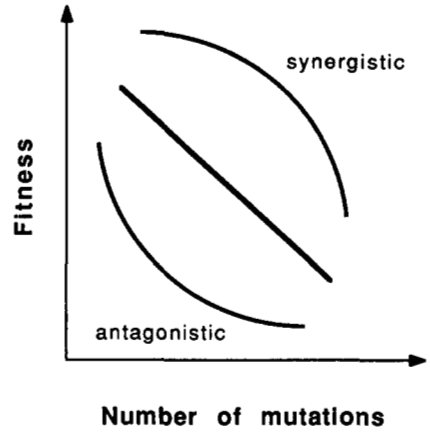

FIGURE 1.—The definition of epistasis on additive basis.

activity additively. A simple model for this is as follows. Take $E = Ce^{G/(kT)}$, where G is the (positive) binding energy between the enzyme's active site and the nonreacting part of the substrate, k is Boltzmann's constant and T is the thermodynamic temperature (FERSHT 1977). Substituting this into the formula for flux, the $J - G$ plot becomes a sigmoid curve whose point of inflection is found from $\partial^2 J/\partial G^2 = 0$, yielding $G^* = kT \ln(1/(bC))$. If this is negative, we see only the saturation part of the sigmoid curve when $G > 0$, which must be so. Suppose that mutations act approximately additively on the G scale. This is reasonable if we assume that they are eliminating hydrogen-bonding sites one after the other, for example. Then, synergism is always guaranteed in the region $G > G^*$, i.e. in the saturation region where $\partial^2 J/\partial G^2 < 0$. If fitness is directly proportional to flux, and the wild-type enzyme acts close to its highest possible performance in the pathway, synergistic interaction among mutations affecting the same enzyme must be found for mildly deleterious mutations. The same is true for the multiplicative basis.

Fitness directly proportional to metabolic pool size: Here we want to maximize the concentration S of an intermediate in the pathway. S increases from b/d to a/c with increasing activity of an upstream enzyme as: $S = (b + aE)/(d + cE)$. Enzyme activity depends on G as before. The results are very similar to the previous case: The $S - G$ curve has an inflection point $G^* = kT \ln(d/(cC))$. The condition $G > G^*$ prevails again for synergism. Thus, synergism is again guaranteed for mildly deleterious mutations, which applies to the multiplicative basis as well.

Selection for optimal flux: Here fitness is assumed to depend on J as follows: $w = \exp[-(J - J_{opt})^2/(2\sigma^2)]$, where J_{opt} is the optimal flux, and the strength of selection increases as σ^2 decreases. Setting $w = 1$ and using the formula for E one can always find G_{opt}. The $w - G$ plot is either skewed, or close to a saturation curve, depending on whether J_{opt} is small or large, respectively. In the latter case, selection on enzyme activity is effectively directional (CLARK 1991). Analysis of the curves reveals that there is synergistic epistasis whenever G is between G_{opt} and the two inflection points. There is no simple analytic expression for the point of inflection, however. For the multiplicative basis, the inflection points of the $\ln(w) - G$ plot are: $kT \ln[a/(bC(a - bJ_{opt})) + [a^2 - abJ_{opt} + b^2 J_{opt}^2]^{1/2}/(bC(-a + bJ_{opt})]$ and $kT \ln[a/(bC(a - bJ_{opt})) - [a^2 - abJ_{opt} + b^2 J_{opt}^2]^{1/2}/(bC(-a + bJ_{opt})]$, between which synergism is ensured. (One may wonder whether the formula applied for stabilizing selection begs the question. Two points are relevant. First, the applied formula is just convenient to use, but any other alternative must have a maximum and inflection points. Second, selection acts directly on flux, rather than on enzyme activity. It is a straightforward consequence of metabolic control theory that, nevertheless, interaction between mutations is found. (The same applies to all examples of stabilizing selection in this paper). Of course, synergism holds only if both mutations happen to be located on the *same* side of the curve, away from the maximum. With no basis in the distribution of mutations, this would happen in only half of the cases by chance alone. However, it is reasonable to assume that J_{opt} implies high enough E so that most mutations will cause a reduced G, and consequently reduced E.

Selection for optimal pool size: Here $w = \exp[-(S - S_{opt})^2/(2\sigma^2)]$, where S_{opt} is the optimal pool concentration. S depends on E, and in turn on G as before. The analysis and the results are analogous to the preceding case. Setting $w = 1$ and using the formula for E one can always find G_{opt}. The $w - G$ plots are again either skewed or close to saturation. The conclusion is that synergism is ensured in the neighborhood of G_{opt}, bounded by the inflection points. For the multiplicative basis, i.e. for $\ln(w)$, these are at $G = kT \ln[-(bc - ad)/(cC(a - cS_{opt})) + [b^2c^2 - abcd + a^2d^2 - bc^2dS_{opt} - acd^2S_{opt} + c^2d^2S_{opt}^2]^{1/2}/(cC(-a + cS_{opt}))]$ and $kT \ln[-(bc - ad)/(cC(a - cS_{opt})) - [b^2c^2 - abcd + a^2d^2 - bc^2dS_{opt} - acd^2S_{opt} + c^2d^2S_{opt}^2]^{1/2}/(cC(-a + cS_{opt}))]$.

Mutations affecting two enzymes

Fitness directly proportional to flux: The formula for flux in this case is $J = a/(1/E_1 + 1/E_2 + b)$, where the subscripts refer to enzyme 1 and 2. Of course it is true that each of the enzymes saturates the flux when varied alone. For all cases with two enzymes the sign of the mixed partial derivative $K = \partial^2/(\partial E_1 \partial E_2)$ will be decisive. In the present case, the criterion for synergistic epistasis on the multiplicative basis is $K[\ln(w)] < 0$ (*cf.* GILLESPIE 1978). Assuming $w = J$, we can check for the criterion. It turns out that epistasis is always antagonistic, however one defines it.

Fitness directly proportional to pool size: It makes a difference whether the substrate is after, between,

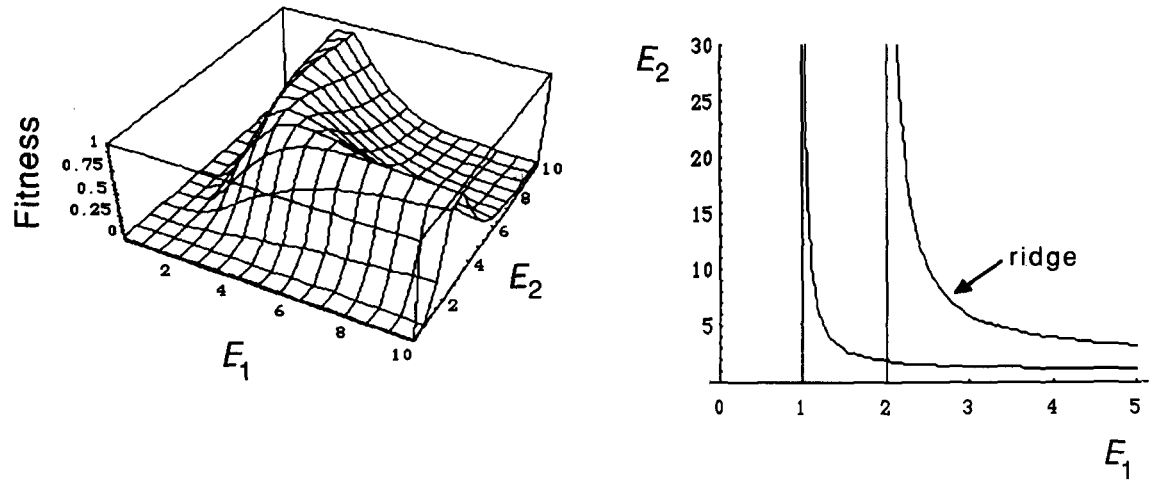

FIGURE 2.—Selection for optimal flux. (Left) Fitness surface. $w = \exp[-(J - J_{opt})^2/(2\sigma^2)]$, $J = a/(1/E_1 + 1/E_2 + b)$. $J_{opt} = 3$, $a = 3$, $b = 0.5$, $\sigma = 0.447$. (Right) The first curve from the left shows the boundary for synergism on a multiplicative basis: $E_2 = 2E_1J_{opt}/(3aE_1 - 2J_{opt} - 2bE_1J_{opt})$, the second curve is that of the ridge: $E_{2,opt} = E_1J_{opt}/(aE_1 - J_{opt} - bE_1J_{opt})$.

or after the two enzymes of which the activities are varied. I consider these cases in turn.

Both enzymes upstream of the substrate: As shown in APPENDIX 1, the relevant formula is $S = a/(1/E_1 + 1/E_2 + b)$, which is isomorphic to that for flux. This is hardly surprising: the two enzymes increase the flux through the pathway, as a result of which S accumulates. Thus, analogously, epistasis is always antagonistic.

Substrate between the two enzymes: The relevant formula is $S = (aE_1 + bE_2)/(cE_1 + E_2)$. Here enzyme 1 is upstream, enzyme 2 is downstream from the substrate, $b < 1$ and $a > bc$. S increases with E_1 and decreases with E_2 (see CLARK 1991). Because of this, synergism is now defined as $K(w) > 0$ or $K[\ln(w)] > 0$. Assuming $w = S$, these two criteria are satisfied if $cE_1 > E_2$ and $acE_1^2 > bE_2^2$, respectively. It is apparent that the latter relation is less stringent, as it should be. Considering that it is likely that $E_1 \gg E_2$ for the wild-type, we have synergistic epistasis for mildly deleterious mutations.

Both enzymes downstream of the substrate: As shown in APPENDIX 2, the relevant formula is $S = (a/E_3 + b/E_2)/(c/E_3 + d/E_2 + e)$ where with $a > c$, $b > d$ and $bc = ad$. Both enzymes decrease the steady-state concentration of the substrate. The mixed partial derivative is always negative, hence synergistic epistasis is ensured. Note that this is a somewhat peculiar case: if fitness is in fact directly proportional to S, the optimal downstream enzyme activities must be as low as possible. Then, it is the *increase* of activity which is deleterious: synergism applies to this case.

Selection for optimal flux: Fitness depends on flux as in the one-enzyme case. The fitness surface has a curved ridge, which has a hyperbolic projection in the $E_1 - E_2$ plane [Figure 2, left, and *cf.* CLARK (1991)]. Because of this, when $w = 1$, $E_1 - E_2$ cannot vary independently (Figure 2, right). In the multiplicative case, synergism is ensured by $K[\ln(w)] < 0$, which is valid in a large region of the $E_1 - E_2$ plane (Figure 2, right). Thus, if the wild-type is a point on the ridge, synergism will be encountered if both activities either decrease or increase. If one increases and the other increases, the double mutant will be closer to the ridge and thus have a higher fitness than any one of the single mutants, so the considerations do not apply. There are three possibilities: (i) if J_{opt} is sufficiently low, mutations will tend to increase the low wild-type enzyme activities. (ii) If J_{opt} is high, mutations will tend to reduce the high wild-type enzyme activities. In both cases synergism will prevail. (iii) For intermediate values of J_{opt}, about half of the double mutants will be antagonistic and the other half will be synergistic. It is reasonable to assume, however, that as regards enzyme activities, J_{opt} is always high enough that case (ii) is realized and synergism is prevalent. For the additive basis, the region of synergism is restricted to the area along the ridge, bounded by the inflection lines.

Selection for optimal pool size: Fitness depends on pool size as in the corresponding one-enzyme case.

Both enzymes upstream of the substrate: The fitness surface has a similar shape to that shown in Figure 2, left, which is hardly surprising given the mathematical isomorphism to the corresponding flux case. The conclusion is similar as well: synergism is ensured in the neighborhood of the ridge of the fitness surface.

Substrate between the two enzymes: The fitness surface has a ridge which projects to the $E_1 - E_2$ plane as a straight line [Figure 3, left, and *cf.* CLARK (1991)]. Because $w = 1$ when $S = S_{opt}$, there is again a tight relationship between enzyme activities along the ridge (Figure 3, right). On the multiplicative basis, the region of synergism, $K[\ln(w)] > 0$, is bounded by two straight lines on either side of the ridge (Figure 3,

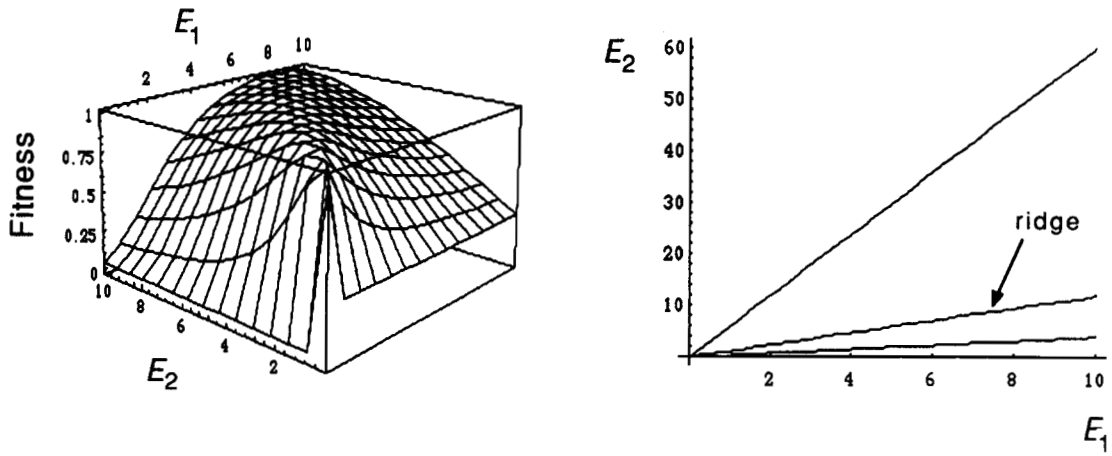

FIGURE 3.—Selection for optimal pool size. (Left) Fitness surface. $w = \exp[-(S - S_{opt})^2/(2\sigma^2)]$, $S = (aE_1 + bE_2)/(cE_1 + E_2)$. $a = 2$, $b = 0.2$, $c = 2$, $S_{opt} = 0.7$, $\sigma = 0.2$. (Right) The middle line is that of the ridge: $E_2 = (cE_1S_{opt} - aE_1)/(b - S_{opt})$, the two others demark the synergistic region on the multiplicative basis, with equations: $E_2 = -(E_1(-c + (a - cS_{opt})/(b - S_{opt}))) + (E_1[a^2 - abc + b^2c^2 - acS_{opt} - bc^2S_{opt} + c^2S_{opt}^2]^{1/4})/(-b + s_{opt})$ and $E_2 = -(E_1(-c + (a - cS_{opt})/(b - S_{opt}))) - (E_1[a^2 - abc + b^2c^2 - acS_{opt} - bc^2S_{opt} + c^2S_{opt}^2]^{1/4})/(-b + S_{opt})$.

right). There are again three cases: (i) when S_{opt} is sufficiently low E_1 is low an E_2 is high for the wild-type, and mutations will tend to increase E_1 and reduce E_2. (ii) when S_{opt} is high enough, E_1 is high and E_2 is low for the wild-type, and mutations will tend to decrease E_1 and increase E_2. Both cases allow for synergism if mutations are mildly deleterious. (iii) For intermediate values of S_{opt}, it is expected that roughly half of the double mutants will show antagonistic and half of them will show synergistic epistasis (*cf.* Figure 3, right). On the additive basis, synergism is again possible, but the permissible region is smaller along the ridge.

Both enzymes downstream of the substrate: Despite the differences in the location of the substrate, analysis using (2.1) and (2.3) for the steady-state substrate concentration reveals that the fitness surface is similar to that shown in Figure 2, left. Consequently, synergism is guaranteed on the inside of the outer hyperbola in Figure 2, right.

DISCUSSION

It is unfortunate that data seem to be insufficient to test these predictions thoroughly. Although there are experiments that validate metabolic control theory for the one enzyme–selection for maximal flux case (*e.g.* DEAN, DYKHUIZEN and HARTL 1986), I am unaware of studies dealing with single and double mutants in analogous situations. Experiments using site-directed mutagenesis would be welcome. As to the two-enzyme cases, DYKHUIZEN, DEAN and HARTL (1987) provided the only data, selecting for maximal flux in *Escherichia coli* chemostat cultures. They had various activity mutants of the β-galactoside permease and the β-galactosidase enzymes. Their fitness estimates are consistent with my result that antagonistic epistasis is bound to appear. In general, CLARK (1991) calls attention to the fact that in the case of human metabolism, for example, selection is unlikely to focus on flux: human inborn metabolic defects are mostly due to the excessive accumulation of intermediates [$S > S_{opt}$; *cf.* BROCK and MAYO (1978)], predominantly because of the loss of activity in a certain enzyme, although cases when $S < S_{opt}$ are also known (*e.g.*, albinism and G6PD deficiency). All these mutations result in a breakdown of metabolic homeostasis, indicating that selection is for optimal pool sizes (CLARK 1991). Systematic analyses of single and double mutants would again be welcome.

The above models have several limitations. One is the lack of generalization to many mutations, possibly using the methods of quantitative genetics (*cf.* CLARK 1991). Another is the fact that I used the classical formulae from metabolic control theory, assuming linear pathways, nonsaturable enzymes, and the lack of other nonlinearities (KACSER and BURNS 1973, 1979). Nevertheless, if the fitness-flux, fitness-pool, flux-activity, and pool-activity functions retain the same *shape* as in the cases treated here, the conclusions will remain the same. Assuming that this is so, some interesting suggestions can be made.

For prokaryotes, starvation is a rather common state, which implies that fitness will be determined by the *flux* of a nonsaturated pathway metabolizing the limiting nutrients, similar to the situation in chemostat cultures (*cf.* DEAN, DYKHUIZEN and HARTL 1986; DYKHUIZEN, DEAN and HARTL 1987). It seems that the growth rate of *E. coli* is controlled by metabolism, because the macromolecular biosynthetic apparatus is subsaturated with substrates and catalytic components (JENSEN and PEDERSEN 1990; MARR 1991). Consequently, mutations affecting different enzymes are likely to show antagonistic epistasis, which by itself would render sexual recombination disadvantageous

(KIMURA and MARUYAMA 1966). This would imply, in terms of the mutation theory of sex (KONDRASHOV 1988), that bacterial sex is infrequent not only because the genome size of bacteria is small, but also because the nature of epistasis between genes is unfavorable. On the other hand, within-gene interaction is likely to be synergistic, thus recombination *within* genes occurring *once in a while* should be favorable; this is the pattern of recombinants in bacteria: sex is "localized", resulting in a mosaic structure of genes (MAYNARD SMITH 1990; MAYNARD SMITH, DOWSON and SPRATT 1991). In fact REDFIELD (1988) has shown that transformation can reduce the mutational load when mutations interact synergistically, despite the fact that it is non-reciprocal, and often the donors are dead cells. Note also that the amount of DNA taken up does not exceed a maximum of 5–10% of the genome (SMITH, DANNER and DEICH 1981).

On the other hand, eukaryotes are more likely to have synergistic epistasis between genes, since selection for *optimal flux* or (even more) *optimal pool size* rather than maximal flux is probably much more prevalent. Consistent with this is the observation that eukaryotes are *K*-selected on the whole relative to prokaryotes (CARLILE 1982). This may help to explain why, besides their larger genome size, eukaryotes have ritualized their sex life in the form of meiosis and syngamy, with nonlocalized allelic exchange. It must not be forgotten, however, that advanced eukaryotes have many important fitness components not directly related to metabolism. Also, the buffering mechanisms of organismal physiology may modify the patterns of realized epistasis.

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APPENDIX 1

Here I derive the dependence of the steady-state concentration of a substrate on the activities of two upstream enzymes. Consider, following KACSER and BURNS (1973, 1979), an enzymatic pathway of the form:

$$X \overset{E_1}{\rightleftharpoons} S_1 \overset{E_2}{\rightleftharpoons} S_2 \overset{E_3}{\rightleftharpoons} Y.$$

It can be shown that the number of steps in the pathway does not modify the results qualitatively. The flux flows from left to right. The enzymes operate below saturation; the reaction velocities are as follows:

$$v_1 = E_1(X - S_1/K_1) \tag{1.1}$$

$$b = E_2(S_1 - S_2/(K_1K_2)) \tag{1.2}$$

$$v_3 = E_3(S_2 - Y/(K_1K_2K_3)) \tag{1.3}$$

where K_i is the equilibrium constant of the ith reaction. In steady-state, all the velocities must be equal. It is also assumed that X and Y are kept constant. From this it follows that the steady-state concentration of the second intermediate is:

$$S_2 = \frac{K_1^2K_2K_3XE_1E_2 + E_3Y(E_1 + E_2K_1)}{K_3E_1E_2 + K_1K_2K_3E_3E_1 + K_1^2K_2K_3E_3E_2}. \tag{1.4}$$

The mixed partial derivative $\partial^2S_2/(\partial E_1\partial E_2)$ is positive provided

$$K_1^3K_2^2K_3X - Y > 0, \tag{1.5}$$

which is consistent with the assumption that the flux

flows from left to right. The numerator of (1.4) is approximated well by its first term if

$$K_1^3K_2^2K_3X \gg K_1K_2E_3Y(1/E_2 + K_1/E_1) \quad (1.6)$$

which is consistent with (1.5) and the usual assumption that $K_1^3K_2^2K_3$ is large enough. Keeping only the first term in (1.4) and dividing both the numerator and the denominator by E_1E_2 we obtain a formula of the form:

$$S_2 = a/(1/E_1 + 1/E_2 + b). \quad (1.7)$$

Note, however, that all the qualitative results remain valid using the more accurate formula (1.4) instead of (1.7).

APPENDIX 2

Here follows the derivation of the steady-state concentration of S_1, depending on the activities of two downstream enzymes. Reaction velocities (1.1–1.3) remain the same and equal to each other. Thus we obtain:

$$S_1 = \frac{K_1K_2K_3XE_1(E_2 + E_3K_1K_2) + E_2E_3Y}{K_3K_2(E_1(E_2 + K_1K_2K_3) + K_1^2K_2E_3E_2)}. \quad (2.1)$$

The last term of the numerator can be omitted provided

$$K_1K_2K_3XE_1(E_2 + E_3K_1K_2) \gg E_2E_3Y \quad (2.2)$$

which is consistent with condition (1.5). The same condition guarantees that the mixed partial derivative of the expression is negative. Using this approximation we can arrive at a formula of the following form:

$$S_1 = \frac{a/E_3 + b/E_2}{c/E_3 + d/E_2 + e} \quad (2.3)$$

with $a > c$, $b > d$ and $bc = ad$.