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Dominance is not Inevitable

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In a diploid organism, a mutant gene that results in elimination of an enzyme activity in the homozygote is almost universally found to be recessive, so that the heterozygote phenotype is virtually indistinguishable from the wild type. It has been argued (H. Kacser & J. A. Burns, *Genetics* **97**, 639-666 (1981)) that there is no need to look to evolution for an explanation of this phenomenon, as it is an inevitable consequence of the low control coefficients for metabolic flux possessed by nearly all enzymes. However, it is possible to envisage pathways in which every enzyme is more than half-saturated, so that moderate changes in the concentration of any enzyme result in substantial changes in metabolic flux. Such behaviour can occur, for example, if the limiting rates of the enzymes decrease as one proceeds along the pathway and the precursor concentration is large compared with the Michaelis constants of all the enzymes. Consequently one does require an explanation in terms of natural selection of why such pathways are apparently not observed in nature.

1. Introduction

In his classic study of inheritance in the garden pea, Mendel (1866) discovered the phenomenon of dominance, whereby in diploid organisms the phenotype for a heterozygote for a particular gene is commonly indistinguishable from that of one or other homozygote, even though the phenotypes of the homozygotes may differ greatly. For genes that are lethal in homozygotes, the phenotype of the heterozygote is usually normal, so that wild-type genes are dominant with respect to lethal mutants.

Fisher (1928, 1930) attempted to explain the general occurrence of dominance in terms of the existence of "modifiers" that would cause the heterozygote phenotype to resemble that of the wild type even though in the absence of the modifiers it would be intermediate between the two homozygote phenotypes.

Much more recently, Kacser & Burns (1981) have pointed out that explanations of this kind are in general unnecessary, because the control of the flux through a metabolic pathway is shared between all of the enzymes in the organism, especially those within the pathway concerned. The amount of control attributable to any one enzyme is normally very small, therefore, and the system can tolerate substantial changes in the activity of an enzyme with virtually no change in flux. Thus even if the occurrence of a mutant gene results in a 50% decrease in the activity of the gene product in the heterozygote the metabolic capability will be virtually unaltered. However, in a homozygote mutant reduction of an enzyme activity to zero will

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certainly result in a change in phenotype unless alternative catalysts exist, as provided, for example, by isoenzymes.

Although the argument of Kacser & Burns (1981) appears in general to be correct, it is stated in a rather extreme form, and it does not, I believe, entirely eliminate the need for discussion in tems of natural selection. In particular, Kacser & Burns (1981) make the following claim:

> The notion of "excess" enzyme... has no meaning in the context of a systemic property such as flux. No system of catalyzed reactions can be conceived where *every* enzyme has "just enough" activity. All enzymes are "in excess" or have "safety factors" by the test that quite substantial reduction in any one activity hardly affects the output.

This claim is incorrect, as such systems can not only be conceived but can be defined precisely, as I shall show in this paper. That they are systems that any biochemist would criticize as unrealistic does not affect the argument, because the reason why they are unrealistic is that nature appears not to have chosen to implement them; this is a consequence of natural selection, not mathematics.

2. Theory

The modern theory of the steady state in metabolic pathways derives from the work of Kacser & Burns (1973) and Heinrich & Rapoport (1974). Both groups, together with others, have recently agreed to use a common set of terms and symbols (Burns *et al*, 1985), which differ from those used in the earlier papers, including the one in which Kacser & Burns (1981) discuss the biochemical basis of dominance. In the agreed system the effect of any enzyme concentration e on the metabolic flux J is expressed by its *flux control coefficient*, C_e^J, defined as follows

$$C_e^J = \frac{\partial \ln J}{\partial \ln e}.$$

This coefficient thus expressed the fractional change in J that results from an infinitesimal fractional change in e, other enzyme concentrations and other parameters of the system being held constant. In earlier work, Kacser & Burns (1973, 1981) used the term *sensitivity* for flux control coefficient and gave it the symbol Z; Heinrich & Rapoport (1974) expressed the same concept in the term *control strength*, with the symbol C.

The crucial property of flux control coefficients is embodied in the summation theorem (Kacser & Burns, 1973; Heinrich & Rapoport, 1974), whereby the sum of flux control coefficients (for a single flux) for all the enzymes in the system is unity. As the number of enzymes in any real system is normally very large, this means that the average flux control coefficient is close to zero. Although negative flux control coefficients are not impossible, negative flux control coefficients of appreciable magnitude are rare and do not invalidate the argument.

It follows from the summation theorem that small changes in the concentrations of most enzymes will have no detectable effect in a metabolic system. In discussing dominance, however, we are not concerned with small changes in enzyme activity but with changes of the order of 50%. The conclusion can only be extended to

changes of this magnitude by assuming that the system is "well behaved", i.e. that large changes in flux control coefficient do not occur in response to moderate changes in enzyme concentration. For linear pathways in which all enzymes are far below saturation this is certainly true, and Kacser & Burns (1981) show, for example, that in such a pathway of nine enzymes reduction of an enzyme concentration by 50% has negligible effect (Fig. 4 of their paper). However, if a pathway operates in conditions where all of its enzymes are more than half-saturated, it is quite possible for an individual flux control coefficient to change from less than 0·2 to more than 0·8 when the enzyme concentration decreases by about 20%. Consequently, it is by no means inevitable that the genes for the enzymes catalysing such a pathway will in general be dominant with respect to mutations that cause complete loss of enzyme activity in homozygotes. On the contrary, it is quite possible for *all* of the genes to show intermediate behaviour.

3. Computation

The simulations described in the next section were carried out on a Casio FX-700P programmable calculator, using a short program in Basic. This method is much slower and more inflexible than use of a main-frame computer, but permits work to be carried on in places where main-frame computers are not readily available, for example in buses. A much more flexible and user-friendly program in Fortran for simulating metabolic pathways is described and reproduced in full elsewhere (Cornish-Bowden, 1987).

4. Example

Consider a set of ten enzymes $E_1, E_2, \ldots, E_{10}$, catalysing a linear pathway of reactions that convert a precursor A into an end product Z via nine intermediates $M_1, M_2, \ldots, M_9$, each step having an equilibrium constant of 5 in favour of its product, so the pathway as a whole has an equilibrium constant of 5^{10}, or about 10^7. Suppose, moreover, that each enzyme individually obeys reversible Michaelis-Menten kinetics, i.e. that its rate v_j is given by an equation of the following form

$$v_j = V_j(m_{j-1} - m_j/K_j)/(K^{\mathrm{f}}_{\mathrm{m}} + m_{j-1} + m_j K^{\mathrm{f}}_{\mathrm{m}}/K^{\mathrm{r}}_{\mathrm{m}})$$

in which m_j is the concentration of M_j (or of A or Z for $j = 0$ or 10 respectively), V_j is the limiting rate of the forward reaction, K_j (= 5 in every case, as indicated above) is the equilibrium constant, $K^{\mathrm{f}}_{\mathrm{m}}$ and $K^{\mathrm{r}}_{\mathrm{m}}$ are the Michaelis constants for the forward and reverse reactions respectively, which are set to 1 and 5 respectively for every reaction. If $V_j = 5$ for the first reaction, decreasing in arithmetic progression to 3·65 for the tenth (i.e. $V_j = 5{\cdot}15 - 0{\cdot}15j$, in general), then the steady-state flux for the pathway may be calculated to be 2·82 for concentrations $a = 10$ and $z = 0$ for the precursor and end product respectively.

As all of the enzymes in this example are operating at more than half saturation (with v_j/V_j ranging from 0·56 for $j = 1$ to 0·77 for $j = 10$) it establishes immediately that it is possible to conceive of a pathway in which no enzyme is present in excess.

More important, however, is the change in flux that results from decreasing any enzyme concentration. This is shown in Fig. 1, where it may be seen that although in the wild type every enzyme lies on a plateau, so that small changes in enzyme concentration are virtually without effect, the plateau is much narrower than implied by Kacser & Burns (1981). The concentration of no enzyme can be decreased by more than 25% without producing a substantial decrease in flux, and at 50%, the value most relevant to discussion of dominance in diploid organisms, the decreases in flux range from 22% for E_3 to 35% for E_{10}, with a median of 25%. The change from plateau to narrow gorge occurs most abruptly for E_{10}: this enzyme has the smallest flux control coefficient, 0·06, in the wild type, but it increases steeply from 0·19 when its concentration is 15% below wild type to 0·82 at a concentration 30% below wild type, as plotted also in Fig. 1.

The changes in flux shown in Fig. 1 are smooth and undramatic, but they are accompanied by very large changes in the metabolite concentrations. For example, m_9 increases more than ten-fold (from 8·29 to 84·2) when the concentration of E_{10} changes from 85% to 70% of its wild-type value. At 50% activity it is 5680, about 1700-fold greater than the value of 3·40 in the wild type. These effects are smaller than those that can occur in the presence of an uncompetitive inhibitor (Cornish-Bowden, 1986), but they are nonetheless very large and might well be intolerable in a living organism. Consequently the phenotypic effects of the change in flux may well be less important than those of the changes in metabolite concentration. Accordingly, if such a pathway existed in a real organism mutations might easily

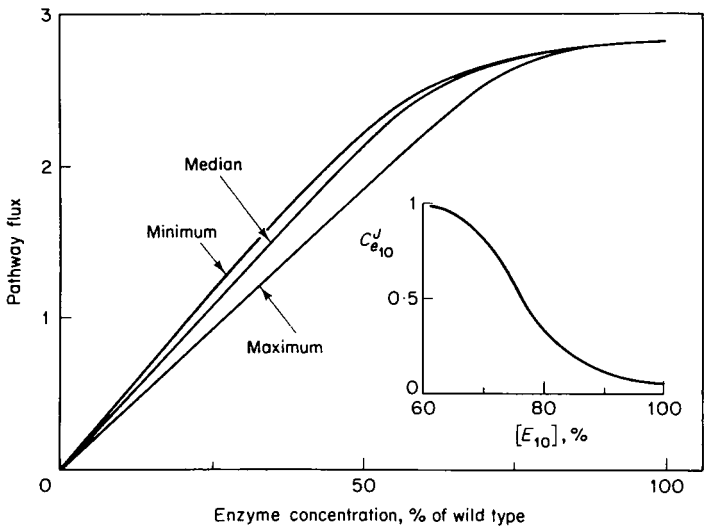

FIG. 1. Pathway in which every enzyme is close to saturation. The variation in flux brought about by decreasing the concentration of any *one* enzyme below the wild-type value is shown for the pathway of ten reactions defined in the text. The three curves represent the minimum effect (top curve), median effect (middle) and maximum effect (bottom). No curve consistently refers to the same enzyme: variation of the tenth enzyme concentration, for example, produces the maximum effect between 0 and 78%, but the minimum effect between 82% and 100%. The *inset* shows the change in the flux control coefficient for E_{10} as its concentration is decreased to 60% of the wild-type value.

prove lethal in heterozygotes as well as homozygotes, even though consideration of flux alone suggests a heterozygote phenotype closer to normal than to that of the mutant homozygote.

The example illustrated in Fig. 1 shows by no means the most extreme kind of behaviour that is possible. One can devise pathways of any length in which every enzyme is as close to saturation as one wishes. In Fig. 1, for example, each step had an equilibrium constant of only 5 in favour of product, and the ratio of Michaelis constants for forward and reverse reactions was 0·2. If these values are made 10 and 0·01, respectively, and if all of the limiting rates are equal but the forward Michaelis constants decrease in geometric progression from 0·149 for the first enzyme to 0·0188 for the tenth, then concentrations of $a = 10$ for the precursor and $z = 1$ for the end product generate a state in which every enzyme is working at 90% of its limiting rate. Moreover, for any enzyme, the flux is essentially proportional to the enzyme concentration in the range 0 to 87% of the wild-type value.

5. Discussion

Kacser & Burns (1981) cite a large body of experimental data in support of their contention that real organisms behave as they suggest: most real enzymes operate well below saturation, and harmful mutations are normally recessive; quantitative traits, however, i.e. ones for which the differences between enzyme activities are slight, do not show dominance, but show a heterozygote phenotype intermediate between the two homozygote phenotypes. Perhaps the most striking evidence that their general thesis is correct is to be found in organisms such as fungi that reproduce almost exclusively as haploid organisms but can be induced to behave as polyploids in the laboratory. That these organisms also show almost universal recessivity of mutants, even though one can hardly believe that selection is the explanation for properties that would virtually never be needed in the wild state, is strong support for the idea that the explanation of recessivity and dominance must be sought in biochemical rather than evolutionary considerations.

One cannot, however, dismiss natural selection entirely, because the results in Fig. 1 show that pathways in which all of the enzymes exist in amounts that are barely sufficient are not impossible. The fact that they do not, apparently, occur in nature is therefore a consequence of selection, not mathematics. One must therefore enquire why pathways of the kind considered here would be expected in general to be harmful. The most obvious problem that they present is that they lead to highly unstable intermediate concentrations, so that small variations in the activity of any enzyme can produce very large fluctuations in metabolite concentration. Moreover, such fluctuations can be avoided very easily by evolving pathways in which the limiting rates V_j do not decrease smoothly or remain constant as one proceeds along a pathway, but instead are appreciably higher than the required flux. No evolution of new enzymes is required, and there is no obvious cost in the form of less effective control.

In general it appears undesirable for enzymes to operate near saturation in the living organism, but two exceptions may be mentioned. For an enzyme at the true

initial step of metabolism, i.e. one such as pepsin in the stomach that is concerned with digestion of food, saturation provides a means of maintaining an almost constant flux of digestion products without also requiring continuous feeding (Cornish-Bowden, 1976). Second, saturation of an enzyme at a branch point can ensure that slight changes in the concentration of its substrate are reflected in very large changes in the flux through the other branch: this is the "branch point effect" suggested by LaPorte *et al.* (1984) to be responsible for regulating flux through the glyoxalate pathway in *E. coli*. In both of these examples, the requirement is for the first step in a sequence to operate at saturation; it is not obvious that there are any advantages in pathways such as the one illustrated in Fig. 1 that operate with all enzymes close to saturation.

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REFERENCES

BURNS, J. A., CORNISH-BOWDEN, A., GROEN, A. K., HEINRICH, R., KACSER, H., PORTEOUS, J. W., RAPOPORT, S. M., RAPOPORT, T. A., STUCKI, J. W., TAGER, J. M., WANDERS, R. J. A. & WESTERHOFF, H. V. (1985). *Trends Biochem. Sci.* **10**, 16.

CORNISH-BOWDEN, A. (1976). *J. mol. Biol.* **101**, 1.

CORNISH-BOWDEN, A. (1986). *FEBS Lett.* **203**, 3.

CORNISH-BOWDEN, A. (1987). In: *Constraint and Control in Metabolism.* (Porteous, J. W., ed.). Cambridge: Cambridge University Press.

FISHER, R. A. (1928). *Am. Nat.* **62**, 115.

FISHER, R. A. (1930). *Biol. Rev.* **6**, 345.

HEINRICH, R. & RAPOPORT, T. A. (1974). *Eur. J. Biochem.* **42**, 89.

KACSER, H. & BURNS, J. A. (1973). In: *Rate Control of Biological Processes.* (Davies, D. D., ed.). pp. 65–104. Cambridge: Cambridge University Press.

KACSER, H. & BURNS, J. A. (1981). *Genetics* **97**, 639.

LAPORTE, D. C., WALSH, K. & KOSHLAND, D. E., JR. (1984). *J. biol. Chem.* **259**, 14068.

MENDEL, G. (1866). *Verhandlung des naturforschenden Vereines in Brünn* **4**, 3.