Dominance, Pleiotropy and Metabolic Structure

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ABSTRACT

It is a common observation that most mutants have similar dominance relations for all the characters they are known to affect. As a model of pleiotropic effects we investigate a branched pathway where the two outputs represent two characters whose variation is affected by changes in any of the genetically specified enzymes in the system. We consider the effects on the phenotype (fluxes or intermediate metabolites) of substitutions at one locus represented by enzyme activities of the two homozygotes (mutant and wild type) and that of the heterozygote. Dominance indices for the characters pleiotropically connected by the metabolic system are calculated. We show that if enzymes behave 'linearly,' (first order), that is if saturation and feedback inhibition or other nonlinearities are absent, all fluxes and pools have identical dominance relations. The presence of such nonlinearity, however, leads to differences in dominance between different characters and we define the conditions where such differences can be important.

THE characters which geneticists measure must arise from the physiological and metabolic processes which occur within the organism. Genetic variation results in variation of the gene products of which many are catalytic proteins, *i.e.*, enzymes. Acting through the metabolism, enzymes 'control' the variables, *i.e.*, fluxes and metabolic pools, in a quantitative manner. Metabolism is often represented by the metabolic map. This map shows us how the enzymes are connected to one another by the metabolites they share. The substrate for one reaction is, in general, the product of another. Other interactions are from metabolites that act as effectors, positive or negative, for particular enzymes. The map does not, however, define the kinetic structure of the metabolic system, that is, give us any information on the rates at which substrates are converted from one to another. The measurement of these rates—the metabolic fluxes—and of the metabolite pool concentrations is the proper subject of 'quantitative metabolism.' The methodology of quantitative metabolism is to study the effects of varying the *parameters* of the system (*e.g.*, enzyme activities) on *variables* which may be measured (*i.e.*, fluxes and metabolite pool levels). Theoretical treatments of quantitative metabolism (KACSER and BURNS 1973; HEINRICH and RAPOPORT 1974; KACSER 1983; FELL and SAURO 1985; HOFMEYER, KACSER and VAN DER MERWE 1986) have provided expectations of the behavior of living systems (*e.g.*, FLINT, PORTEOUS and KACSER 1980; FLINT *et al.* 1981; GROEN *et al.*, 1982; MIDDLETON and KACSER 1983; STUART *et al.* 1986; WOODROW 1986; SALTER, KNOWLES and POGSON 1986; DYKHUIZEN, DEAN and HARTL 1987). Because, in principle, all enzymes affect all variables and since enzyme activities are under genetic control, fluxes and pool levels are quantitative characters or very closely related to them. In living systems the values of these characters are determined by the alleles which control enzyme activities and by the environment which controls the inputs and the external effectors to the metabolic system.

In a previous paper (KACSER and BURNS 1981), the methods of quantitative metabolism were applied to the question of the effects of finite changes in enzyme activity generated by allelic differences and led to a general analysis of dominance relationships. It was shown that there is a nonlinear relationship between flux or metabolite concentration and enzyme activity. The general expectation that "null" mutants at enzyme loci are 'recessive' is explainable in these terms without necessitating an evolutionary hypothesis of "modifiers" first proposed by FISHER (1928) (see *e.g.*, MIDDLETON and KACSER 1983; DEAN, DYKHUIZEN and HARTL 1986; CORNISH-BOWDEN 1987; KACSER 1987). On the other hand, small differences in the enzyme parameters of mutant and wild type were shown to result in an intermediate heterozygote phenotype. Here, we extend the KACSER and BURNS (1981) treatment to the problem of pleiotropic effects of enzyme variation.

Since the metabolic system is highly interactive, genetic variation at one locus will in principle affect all the characters. This, together with the interactive nature of development gives us a general expectation of pleiotropy. It does not, however, imply that any genetic variation affects all the characters in the same way. Intuitively, we would expect some characters to be 'close' to one another and others to be more

'distant' but the directions and extent to which different characters are affected by genetic variation and the types of response in such characters are not intuitively obvious. The purpose of the present study is to develop theoretical expectations for the effects of finite changes in genetically determined enzyme parameters on characters pleiotropically related in the metabolic system. In particular, expectations for the dominance relations will be derived. This will be related to the fact that, in a vast majority of cases, the dominance relations of a pair of alleles affecting one character are the same when the effects on a pleiotropically related character are considered.

THE MODEL

The dominance relationship of the three phenotypes in a diploid can be described by an index. The dominance index (D), first defined by WRIGHT (1934) and used by KACSER and BURNS (1981), gives a quantitative description of dominance. Using the symbols W, H and M for the values of the wild-type, heterozygote and mutant phenotypes, respectively, the index D is defined by:

$$D = \frac{W - H}{W - M}. \qquad (1)$$

Although D can take any value, the following useful conditions apply for limiting cases: (a) $W = H$, then $D = 0$. The mutation is fully 'recessive'; (b) $W - H = (W - M)/2$, then $D = 0.5$. The mutation gives an exact intermediate heterozygote phenotype; and (c) $H = M$, then $D = 1$. The mutation is fully 'dominant.'

For this study, where general properties are required, we use an abstraction of a living system displaying some important properties (Figure 1). This is a simple biochemical system showing pleiotropy, as two outputs will be affected by changes in any one enzyme. Although even an unbranched pathway will display pleiotropy, insofar as enzyme variation can affect the intermediate pools differentially, a branched system with two outputs is a more general case. The system will reach a steady state with input concentrations X_A and outputs X_B and X_C held at constant levels. The external concentrations of X_A, X_B and X_C are assumed to be controlled by the environment and are in no way affected by the activities of the metabolic system itself. If there is an electrochemical potential difference between X_A and X_B as well as X_C, there will be a net flow from the input to the outputs. The substrate S is utilised by enzymes in both of the branches and they therefore compete for S. At steady state the fluxes (J's) through each pathway are constrained by

$$J_A = J_B + J_C. \qquad (2)$$

The outputs J_B and J_C are 'correlated' characters as

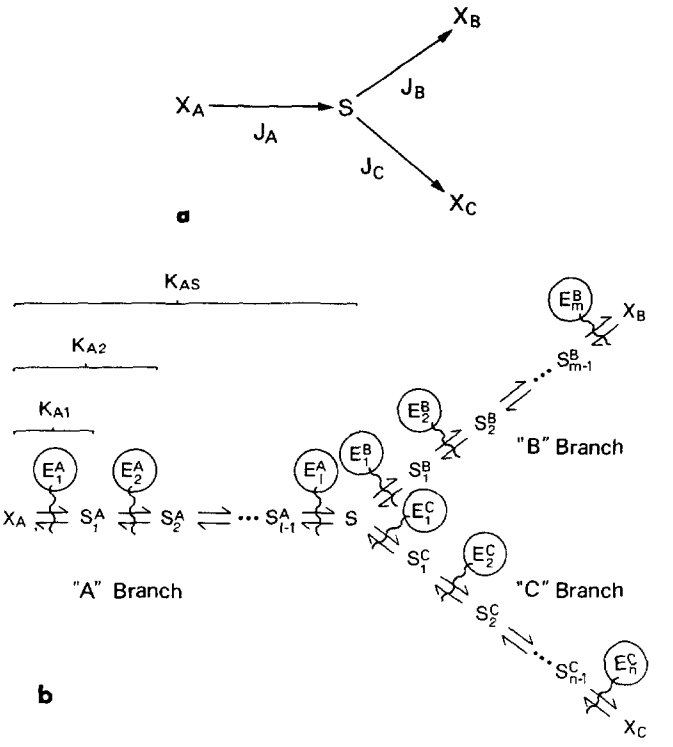

FIGURE 1 (a and b).—The branched system. The system is bounded by the external substances, X_A, X_B and X_C. The three symmetrical branches are labeled "A", "B" and "C". The point of divergence in the system is at substrate S which is catalysed by three enzymes E_1^A, E_1^B and E_1^C. The superscript on the enzyme and substrate symbols refer to the branch, and the subscript refers to the position in the branch. Equilibrium constants are similarly named. For example, $K_{A,1}$ refers to the equilibrium constant of the reaction $X_A \rightarrow S_1^A$, etc. For the unsaturated case, all the enzymes in each branch can be condensed to 'group enzyme activities,' A, B and C (see Appendix 1, Eq. 1.4).

are all the substrate pool levels including the concentration of S. Enzyme variation anywhere in the system will simultaneously affect all the characters though not necessarily to the same degree.

The unsaturated system

Figure 1b represents the general case of a branched pathway. By making the simplifying assumption that all the steps are monomolecular, and that saturation of the enzymes is absent, it is possible to derive a system of linear equations for the fluxes and pools (Appendix 1). As a further simplification the activities of the enzymes in each branch can be combined to give 'group enzyme activities' A, B and C (their exact definition is given in Appendix 1). For the unsaturated system defined in Figure 1b, there are therefore three linear rate equations for the three fluxes.

Since the system is symmetrical, the flows in any one branch can go on in either direction as long as the mass conservation constraint (2) is met. In the following sections, we define the following directions for positive fluxes: $X_A \rightarrow S$, $S \rightarrow X_B$, $S \rightarrow X_C$. We have three symmetrical branches—"A," "B" and "C" with

an arbitrary number of first order enzymes in each.

By solving the simultaneous equations, expressions for each of the fluxes J_A, J_B and J_C can be obtained in which the enzyme activities appear as parameters. Although a character, such as a flux or a pool, is a function of all the enzyme activities in the system, we are interested in the effect of variation in one enzyme only. This can be anywhere in the system. We consider the two-allele case: wild type, mutant and heterozygote for which three different values of one of the enzyme activities will apply. By inserting these three different enzyme values in each of the equations for J_A, J_B and J_C, the phenotypic values for the fluxes are obtained. Finally, insertion of these in to the expression for the dominance index (1) allows us to compare how changes at one locus affects the dominance relations for the pleiotropically related fluxes.

In Appendix 2 we give a general proof that for a system of unsaturated enzymes, of any complexity, the dominance index with respect to any one locus is exactly the same for all characters (fluxes or metabolite pools) affected by the allelic substitutions. Although the dominance indices are identical, the measured differences in the characters may be very different. If, *e.g.*, we find 'recessivity' in one character, say a flux, ($D \rightarrow 0$), then we shall find the same in another pleiotropically related character, say a pool. The underlying feature which generates these identities is that in a system with linear equations for each step, all the fluxes are linearly related to all the pools. This is illustrated in Figure 2 which is shown as a 'reflection diagram.' (See BURNS and KACSER 1977.) Such a diagram shows the effects of the independent variable (enzyme activity A in this case) on a dependent variable (metabolite S in this case) which, in turn, affects further dependent variables (the three fluxes). The decomposition into the functional components aids the understanding of the system.

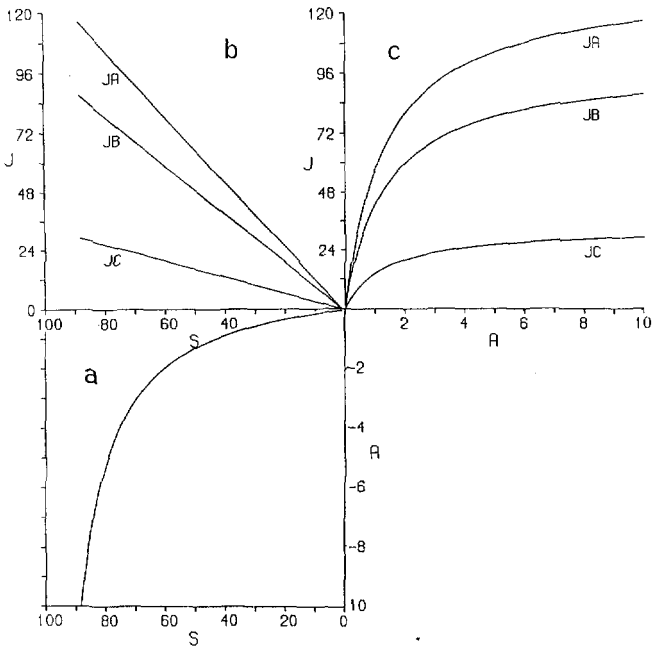

FIGURE 2.—Flux responses to changes in the enzyme activity of the common branch in a system with first order enzymes (all enzymes unsaturated). The effects are shown as a "reflection diagram" which 'decomposes' the effect into component functions of the final function. (*a*) How variation in the parameter A (group enzyme activity) changes the steady state value of the variable S (the branch point substrate). (see Figure 1). (*b*) Changes in S are now reflected in this panel which shows how changes in the variable affect the three fluxes dependent on it. This therefore shows how S is allocated from J_A to the two output fluxes J_B and J_C. (*c*) The flux changes are reflected back to A and show the resultant net effect of changes in A on the three fluxes. It will be noted that (*b*) shows linear relationships of S on J's. This implies that the Dominance Index (resulting from any three values of A) will be identical when measured in any of the three fluxes or in S. The relationships of the J's on A in (*c*) are simple hyperbolic functions. Any three values of A will give identical flux proportions in all three fluxes. It will also be noted that in (*b*) and (*c*) the fluxes are constrained by $J_A = J_B + J_C$.

The branched system with saturation

We now consider specifically the case of some degree of saturation in one of the branches. Let this be an enzyme E_j^B in the "B" branch. The system is represented in Figure 3.

The "A" and "C" branches are identical in structure to the nonsaturable system (Figure 1b). The saturable branch can be divided into three parts: (a) The "P" section of nonsaturable enzymes *proximal* to E_j^B. (b) The saturable enzyme E_j^B. E_j^B is saturable by either its product S_j or its substrate S_i or both, depending on the values of the Michaelis constants, M_j and M_i, respectively (see Eq. 1.1 in Appendix 1). (c) The "D" section of nonsaturable enzymes *distal* to E_j^B.

A quadratic expression for J_B is obtainable in terms of all the internal and external parameters (Appendix 3). The expression for J_c is also a quadratic and is also given in Appendix 3. These equations are difficult to manipulate algebraically, and, being non-linear, the matrix treatment does not apply. Their characteristics with respect to enzyme variation are better understood by evaluating specific parameter sets. (See *e.g.*, HOFMEYER 1986.) Branched pathways have been investigated previously [KACSER (1983) in terms of control analysis; LAPORTE, WALSH and KOSHLAND (1984) in terms of allocation of the fluxes; and SAURO, SMALL and FELL (1987) in terms of the matrix method giving branch distribution control coefficients].

Since we are interested in the possible effects of saturation on the dominance relations in J_B and J_C, we need to examine cases of 'high' and 'low' saturation. The degree of saturation may be described quantitatively by a simple saturation index, SAT:

$$\mathrm{SAT} = \frac{S_i/M_i + S_j/M_j}{1 + S_i/M_i + S_j/M_j}. \qquad (3)$$

SAT can take values as follows: (a) $S_i/M_i \ll 1$ and

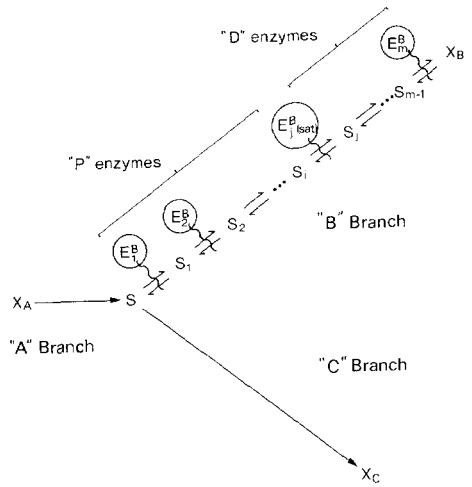

FIGURE 3.—The saturable branched system. As before there are three branches labeled "*A*", "*B*" and "*C*." The "*A*" and "*C*" branches are identical in structure to the branches as specified for the Figure 1b and are assumed to be unsaturated. The "*B*" branch consists of three parts. The linear "*P*" section is proximal to the saturable step $S_i \rightarrow S_j$ catalyzed by enzyme E_j. Distal to this step is the "*D*" portion of unsaturated enzymes. The nomenclature is the same as described in the legend to Figure 1.

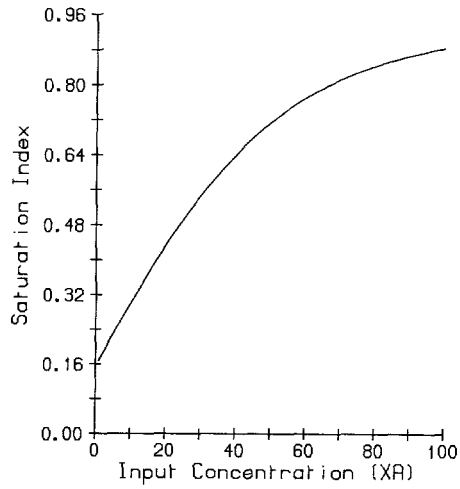

FIGURE 4.—Saturation response of E_j to X_A. The following parameters were used: X_A, varied from 1 to 100; $X_B = 1$; $X_C = 1$; $A = 2$; $P = 100$; $D = 100$; $C = 1$, $V_j = 100$; $M_i = 5$; $M_j = 1000$. All equilibrium constants were set to unity. These values were used to compute the fluxes and the pools, S_i and S_j, which are then inserted into Eq. 3.

$S_j/M_j \ll 1$ ('low' saturation, SAT $\rightarrow$ 0) and (b) $S_i/M_i \gg 1$ and/or $S_j/M_j \gg 1$ ('high' saturation, SAT $\rightarrow$ 1).

We investigate differences in saturation by modifying a parameter which will in turn affect the amount of saturation. To vary saturation we have chosen to simulate changes in X_A. As X_A increases, the saturation of E_j will increase (Figure 4). (An alternative method would have been to compare a series of enzymes with decreasing values of *M*.)

The dominance relations in such a system are investigated by evaluating the functions for J_B and J_C with different values of X_A. For any given value of X_A we now consider variation in one enzyme's activity

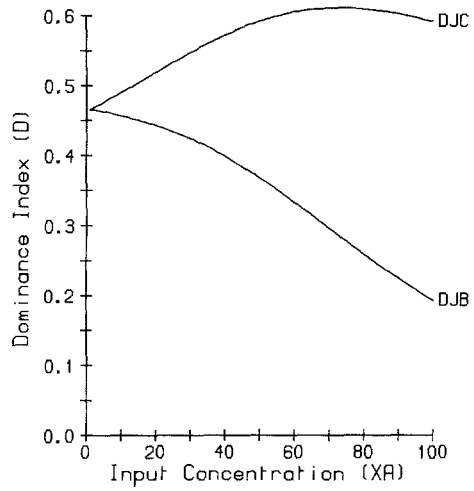

FIGURE 5.—Dominance indices as a function of saturation by varying X_A. The dominance indices of the two output fluxes with respect to enzyme variation occurring in the common ("*A*") branch. The parameters are identical to those used in generating Figure 4, except for *A*. The values for *A* were taken as: the mutant activity = 0, the heterozygote activity = 1 and the wild-type activity = 2.

and its effect on the fluxes. We obtain three J_B and J_C values, two homozygote values and the heterozygote. These are then used to evaluate the expressions for the dominance indices, D_{JB} and D_{JC} (Eq. 1), where the subscripts refer to the phenotype where the dominance index is measured. This procedure is repeated for different values of X_A (and hence different degrees of saturation of enzyme E_j).

Enzyme variation in the common branch: Figure 5 illustrates the dominance indices of J_B and J_C for enzyme variation in the "*A*" branch as X_A is modulated and hence as the degree of saturation changes. Clearly with high levels of saturation, the dominance indices can be quite different with, in this case, the saturable output, J_B more 'recessive' (smaller value of *D*). At low saturation, as the analysis of the unsaturated case predicts, the indices tend to equality. The differences in dominance which can occur are best explained in terms of the nonlinearity of the fluxes to substrate concentrations now present in the system. Since the "*C*" pathway is a chain of nonsaturable enzymes, J_C responds linearly to changes in the common substrate, *S*, but J_B responds nonlinearly due to the damping effect of the saturable enzyme. This is illustrated in Figure 6 which is shown as a "reflection diagram" for one value of X_A giving high saturation.

Figure 6a shows the effect of varying *A* enzyme on the common substrate *S*. Unlike the response in nonsaturated systems, where the relationship was hyperbolic (Figure 2), *S* shows an early 'accelerating' portion before approaching a plateau at high values of *A* (not shown). Figure 6b shows the effect of such changes in *S* on its differential allocation to the two output fluxes J_B and J_C. Clearly, J_C responds linearly to *S* (*cf.* section on nonsaturated systems), while J_B shows the effects

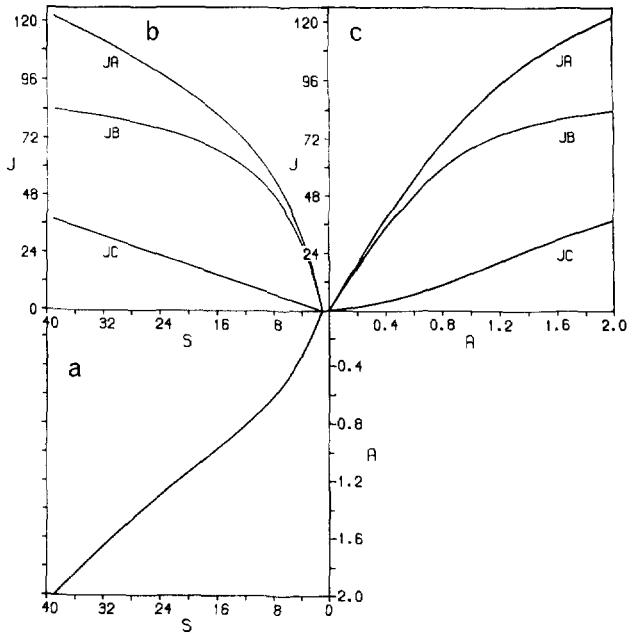

FIGURE 6.—Flux responses to changes in the enzyme activity of the common ("A") branch. Parameters as in Figure 4 with X_A at 100 (high saturation) and A varying from zero to 2. As explained in the legend to Figure 2, the effects are shown as a 'reflection diagram.' The "C" branch of first-order enzymes gives a linear flux response to S. The "B" branch, however, gives a damped response due to the presence of the saturable enzyme. This damping effect leads to the tendency for the "B" flux to give a 'more recessive' phenotype than the "C" flux (see Figure 5) and hence to different dominance indices.

of increasing saturation. Finally, Figure 6c shows the net effect of changes in A on the fluxes.

Thus, no matter which three values of S result from the three enzyme activities, the J_B phenotype will appear 'more recessive' than J_C. It is notable (Figures 5 and 6c) that in this case the flux in the nonsaturable branch, J_C, can give a Dominance Index greater than 0.5, *i.e.*, the mutant can therefore tend to be 'dominant' over the wild type (the heterozygote is nearer the mutant phenotype). This result, not previously observed, is due to the increasing slope of the J_C flux at low values of A (Figure 6c) in contrast to the monotonically declining change in J_B.

Enzyme variation in the nonsaturable branch: Figure 7 illustrates the dominance indices measured in the two fluxes for varying values of X_A where enzyme variation occurs in the nonsaturable branch. Here, we observe the opposite result from the effect of variation in the common branch. The flux measured through the nonsaturable pathway, J_C, is in this case a more recessive phenotype than that measured in the saturable branch. This result is also explainable in terms of the reaction of the fluxes to changes in the common substrate, S. This is illustrated in Figure 8. As in the case of variation in the common branch, J_B varies nonlinearly with S, higher values being damped due to the effect of saturation (Figure 6b). The flux in the

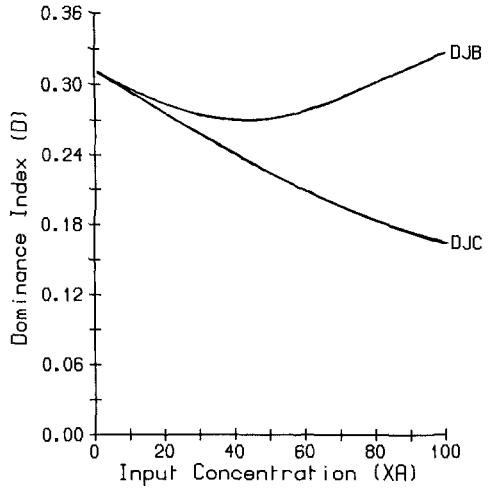

FIGURE 7.—The dominance indices of the two output fluxes with respect to enzyme variation occurring in the nonsaturable ("C") branch. Saturation is varied in the system by modulating the input X_A. The mutant enzyme activity is zero and the heterozygote activity is half wild type. The following parameters were used to generate the curves: $X_B = 1$; $X_C = 1$, $A = 1$; $P = 100$; $C = 10$ (wild type); $V_j = 50$; $M_i = 5$; $M_j = 1000$. All equilibrium constants were set to unity.

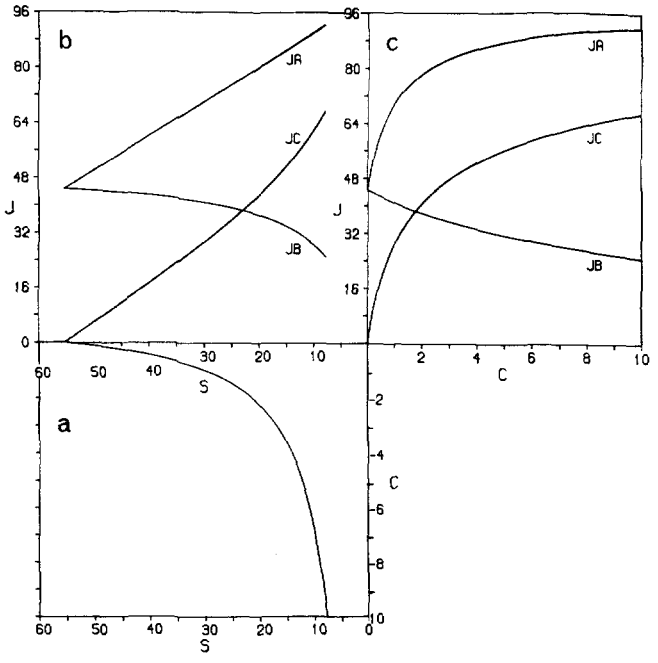

FIGURE 8.—Flux response to changes in enzyme activity in the competing "C" branch in the saturable system. This is a 'reflection diagram' (see Figure 2). Parameters as in Figure 7 with $X_A = 100$ and C varying from zero to 10. The "A" flux gives a linear response to changes in S, and "B" flux is damped due to the presence of saturation. The net result of these changes (c) is the tendency for J_C to give a more recessive phenotype than J_B.

common branch, J_A, is, however linear in S. Since $J_C = J_A - J_B$, J_C varies in this case nonlinearly with S, changes in which have been induced by enzyme variation in this pathway.

Enzyme variation in the saturable branch: In this case the numerical studies indicated, somewhat surprisingly, that the dominance indices measured in

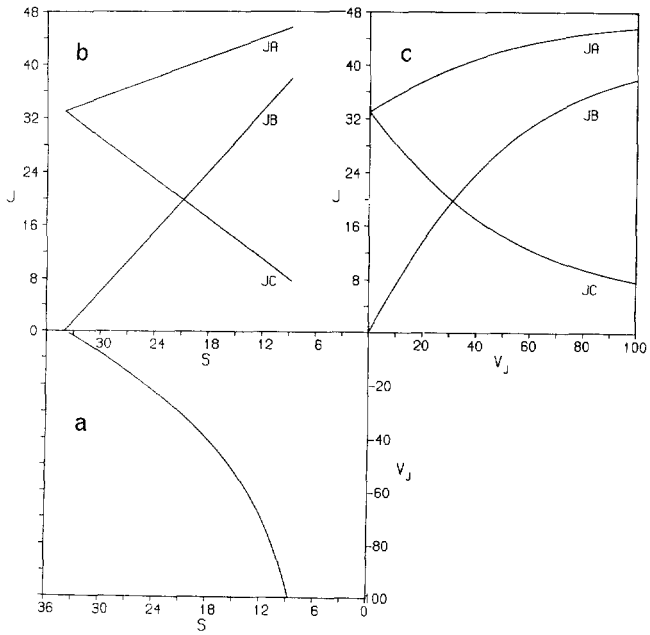

FIGURE 9.—Flux responses to change in enzyme activity in the saturable "B" branch. Parameters: $X_A = 100$, $X_B = 1$; $X_C = 1$; $A = 0.5$; $P = 100$; $D = 100$; $M_i = 10$; $M_j = 1000$, $C = 1$; and V_j varied from 0 to 100. All equilibrium constants were set to unity. Here the relationships of the fluxes to the branch point metabolite, S, are all linear, leading to identical dominance relations (compare Figure 2).

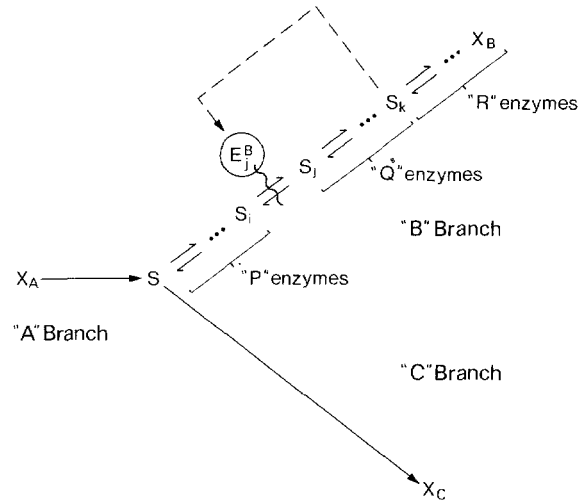

FIGURE 10.—The branched system with feedback inhibition. The structure is similar to the previously defined systems (Figures 1 and 3). The "A" and "C" branches are identical in structure to the linear system. The "B" branch consists of four parts. The "P" section is a chain of linear enzymes proximal to the step $S_i \rightarrow S_j$ catalyzed by enzyme E_j^B. This enzyme is inhibited by S_k, a substrate further up the chain. Proximal to S_k is the "Q" section of linear enzymes and distal is the "R" section. The equilibrium constants for the reactions $S \rightarrow S_i$, $S_j \rightarrow S_k$, and $S_k \rightarrow X_B$ are termed K_P, K_Q and K_R, respectively.

each character were identical although there was considerable saturation in the branch. This is so irrespective of whether variation occurs before, after, or at the saturable step. The explanation for this result lies in the linearity of the fluxes in the nonsaturable branches (J_A and J_C) with changes in S caused by enzyme variation in the saturable branch. Since the fluxes are constrained by $J_B = J_A - J_C$, J_B must also be linear with changes in S caused by enzyme variation in its own pathway (Figure 9). (Contrast Figures 6b and 8b.)

In our simulations we have assumed that a mutation of the saturable enzyme affects only the V_{max}, and not the K_m values. This is an unnecessary restriction as changes in any enzyme parameter will affect the phenotypes through changes in S. The behaviour of the indices will therefore be the same as discussed above.

Effect of feedback inhibition: Feedback inhibition is another mechanism which can cause nonlinear relationships between pools and fluxes. We have investigated the effect of a feedback inhibition loop (Figure 10) on the dominance relations of the output fluxes.

Again the common "A" branch and the "C" branch are identical in structure to the corresponding branches in the nonsaturable system (Figure 1b). The branch where feedback inhibition occurs is divided into three sections, P, Q and R with an arbitrary number of enzymes in each. The substrate S_k inhibits the enzyme E_j. S_k is distal to the product of E_j (S_j) in the chain.

Assuming steady state, quadratic equations are obtainable for fluxes through each branch (Appendix 4). The nonlinearity introduced by feedback inhibition leads to qualitatively similar behavior to the system with saturation in one branch (results not shown). As feedback increases (measurable by an index analogous to the saturation index), differences in dominance can occur, the directions of which are the same as the saturable case. The arguments to explain these phenomena are identical to those used to explain the effects of saturation.

DISCUSSION

As a limiting case, we have in the first place investigated dominance in systems of monomolecular transformations with no saturation or feedback inhibition. This is a reasonable approximation to 'real' systems when the metabolite concentrations are less than their respective Michaelis constants. Our conclusions apply to any arbitrary network of any structural complexity. An important result is that this type of system has identical Dominance Indices for all fluxes and pool levels with respect to variation of any of the enzymes. The reason for this behaviour is to be found in the fact that in such systems, the pools and fluxes are linearly related to one another. Real metabolic systems are not of course all monomolecular as many reactions involve splitting and combining substrates. For example, a bimolecular step in a divergent metabolic system could be the reaction $S_i \rightarrow S_j + S_k$, where S_j and S_k are the beginning of two further pathways.

This reaction is constrained by its stoichiometry and the rates of production of S_j and S_k must be identical. If two outputs arise from such stoichiometrically constrained fluxes, no question of competition for a shared metabolite arises. No difference in dominance relationship will therefore be observed, no matter what the saturation state or feedback conditions are.

For nonlinear systems we have had to use numerical simulations to assess the effects on independently variable fluxes. It has been shown that saturation at one enzyme can lead to differences in the dominance relations of two outputs. We have defined the directions of the differences in Dominance Indices for enzyme variation at different parts of the system. We have not explicitly considered systems with saturation in both the competing branches. The directions of any differences in dominance will depend on the relative saturation in each of the branches. The dominance relations in the presence of such nonlinear enzymes are dependent on their Michaelis constants as well as on the concentrations of external substances which affect the degree of saturation. Dominance and any possible differences are thus a function of the environment in which organisms are operating as well as of their genes.

The effect of saturation on the Dominance Indices is essentially through the resulting nonlinearity of some fluxes to substrate concentration. The presence of a feedback inhibition loop in the system can also lead to differences in the dominance between the outputs. The mechanism is essentially the same as for the case of saturation, with feedback inhibition introducing nonlinear relationships between fluxes and pools. This qualitative explanation of the behaviour of the saturable system and the system with feedback inhibition is robust to changes in parameters and consistent.

How do the above considerations relate to the dominance relations for pleiotropic effects of enzyme variation actually found *in vivo*? An enormous amount of work has gone into studies of human inborn errors of metabolism. These are frequently caused by near null-mutations at enzyme loci (HARRIS 1980). In most cases, heterozygotes are detectable if measurement of the enzyme activity is possible when it will show an activity which is usually the mean of wild type and mutant (STANBURY *et al.* 1983). In spite of this, the clinical phenotype of the heterozygote is 'recessive.' It has been shown that 'recessive' does not mean 'complete recessive' (*e.g.*, KNOX and MESSINGER 1958; BULFIELD and KACSER 1974; KACSER and BURNS 1981) as the heterozygote phenotype will invariably exhibit a small average difference from the wild type. Detection of heterozygotes will therefore depend on the ability to measure small differences and on the noise in the system. We have not, however, found any case in the literature where the heterozygote for a clinical phenotype is distinguishable from the wild type in the 'main effect' or any other manifestation of autosomal genes.

In Drosophila, a vast number of mutants are now known, many of which are null-mutants at enzyme loci. In these cases the situation appears to be the same as that for human inborn errors of metabolism—the mutations tend to be recessive for the 'main effect' and all the other pleiotropic effects (LINDSLEY and GRELL 1968).

There are, however, some cases in the literature where mutants show unequal dominance relations for different characters. In mice for example homozygotes for W^f alleles at the W locus are extensively depigmented, and there is no obvious pattern to the depigmentation (GUENET *et al.* 1979). Heterozygotes resemble the wild type except, however, for the presence of white spots on the forehead and belly. In these areas, the mutant allele is therefore 'dominant' for hair color, and in the rest of the animal, it is 'recessive.' The 'internal environment' of the gene in the different tissues must clearly be different. The two characters "pigment on dorsum" and "pigment on belly" can be compared to the example discussed in Figures 5 and 6 where the two branches would represent the melanin production in the two tissues. In the model X_A and X_B would be the same substance (melanin) in the two tissues. The enzymes would be the 'same' in the sense that they are specified by the same genes. The two tissues could however be different by, *e.g.*, sustaining different substrate concentrations and/or different activations or inductions of some of the enzymes. The effect of genetic substitution at one locus (W^f) could therefore have different consequences when the Dominance Index is measured in the two tissues.

A further example of differences in dominance relations occurs in the well known gene in pigs for halothane sensitivity (reviewed by WEBB 1981). Homozygotes for this allele are sensitive to the anaesthetic halothane, are stress susceptible, have improved meat colour and have improved performance for a number of economically important traits. Heterozygotes are not detectably different from the wild type for sensitivity to the anaesthetic, stress susceptibility, or meat colour, but are nearly intermediate for the other traits (*e.g.*, growth rate and carcass quality). The allele is therefore 'recessive' for some of the characters it affects, but is 'additive' for others. We have found one less clear cut example of this type of phenomenon in *Drosophila melanogaster*. The allele *scabrous-like* found in an abdominal bristle number selection line (HOLLINGDALE 1971) is a recessive semilethal, but has a substantial effect on bristles in the heterozygote. The biochemical bases of all these ef-

fects are of course obscure. On the whole, however, it would appear from the literature that most mutations, especially where the mutation is shown to be at an enzyme locus, have similar dominance relations for the characters they affect pleiotropically.

We must now enquire how relevant the conclusions of the very simple model are to the very much more complex 'real' metabolic system and to the 'characters' arising from its operation. The behaviour of our model has established four conditions which must be *simultaneously* satisfied if substantial differences in dominance of pleiotropically related characters are to be observed.

1. *Nonlinearity of metabolites to fluxes must occur.* The fact that, in principle, all enzymes are nonlinear converters and that feedbacks are frequent features of metabolism is not in itself a sufficient condition. Saturation must be high or the feedback function must be steep for significant deviation from linearity to occur. Evidence concerning *in vivo* saturation is very sparse. What there is (*e.g.*, FLINT, PORTEOUS and KACSER 1980; HESS 1973) suggests that most enzymes operate below or near their substrate Michaelis constants, although co-factors, such as NAD, appear to be present in saturating concentrations. 'Strong' nonlinearities may therefore be an exception rather than the rule.

2. *The allelic differences must not affect enzymes in the branch in which the nonlinearity occurs.* This condition eliminates a good fraction of loci for which dominance differences can be expected.

3. *The fluxes must not be stoichiometrically constrained.* Such fluxes cannot give rise to differential dominance indices with respect to any variation.

4. Finally, *the variation with respect to which the dominance indices are calculated, must affect a step which is reasonably sensitive to changes in enzyme activity.* This means that the heterozygote phenotype will have to show a clear difference from the wild type (*i.e.*, the mutant must not be effectively 'recessive'). If such complete recessivity of the flux through the enzyme obtains, it will necessarily imply that the pools (including the pool at the branch point) will show no variation in the heterozygote. Since any effect on other pleiotropic fluxes is only mediated via a change in the branch pool(s), such other fluxes will also show recessivity and no substantial difference in indices can arise. In terms of the concepts of control analysis (*e.g.*, KACSER and PORTEOUS 1987) this means that the affected step should have a reasonably high control coefficient (as indicated by the slope of the enzyme *vs.* flux relationship). It will be noted that the two cases (Figures 5, 6 and Figures 7, 8), where we demonstrated differences in Dominance Indices, had significant coefficients for the affected step. Thus the slope of J_A *vs.* A in Figure 6c, and the slope of J_C *vs.* C in Figure 8c, both have values 0.2 and higher.

In vivo such steps with high coefficients are relatively rare (KACSER and BURNS 1973, 1981). In our simulation, the enzymes in the branches were 'condensed' to a single step for which a high coefficient could easily be devised. In general, however, branches will have a number of enzyme steps and the magnitude of the overall coefficient will be divided among them all. Genetic variation affecting any one of these is therefore likely to act on a low coefficient step with consequently much smaller differences in the indices. Feed-back loops have, as one of their consequences, the lowering of all control coefficients inside the loop and proximal to it (KACSER and BURNS 1973; STUART *et al.* 1986). The stronger such feedback effects are, the more the system is buffered with respect to genetic and environmental changes.

It therefore appears, from our knowledge of the kinetic structure and from the experimental evidence, that the four necessary conditions for dominance differences are rather unlikely to be met and that the rarity of observed cases is consistent with our analysis.

There are also implications for the effects of enzyme activity variation on metric characters. Due to the nature of multienzyme systems, we expect that null or near-null mutants will be 'recessive' with respect to fluxes and pools. Small variations in activity on the other hand, will be 'additive' for these characters. Our results suggest that such small variations in enzyme activity will produce similar additivity for other, perhaps distantly pleiotropically related traits. We have not addressed the question of how variation occurring simultaneously at many enzyme activity loci affects the dominance variation present in populations. This question together with the problem of epistasis in multienzyme systems will be the subject of a future publication.

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LITERATURE CITED

BULFIELD, G. and H. KACSER, 1974 Histidinaemia in mouse and man. Arch. Dis. Child. **49:** 545–552.

BURNS, J. A. and H. KACSER, 1977 Allosteric Repression: an analysis. J. Theor. Biol. **68:** 199–213.

CORNISH-BOWDEN, A., 1987 Dominance is not inevitable. J. Theor. Biol. **125:** 333–337.

CLELAND, W. W., 1963 The kinetics of enzyme catalysed reactions with two or more substrates or products. I. Nomenclature and rate equations. Biochim. Biophys. Acta **67:** 104–137.

DEAN, A. M., D. E. DYKHUIZEN and D. L. HARTL, 1986 Fitness as a function of β-galactosidase activity in *Escherichia coli.* Genet. Res. **48:** 1–8.

DYKHUIZEN, D. E., A. M. DEAN and D. L. HARTL, 1987 Metabolic flux and fitness. Genetics **115:** 25–31.

FELL, A. and H. M. SAURO, 1985 Metabolic control and its anal-

ysis. Additional relationships between elasticities and control coefficients. Eur. J. Biochem. **148:** 555–561.

FISHER, R. A., 1928 The possible modification of the response of the wild type to recurrent mutations. Am. Nat. **62:** 115–126.

FLINT, H. J., D. J. PORTEOUS and H. KACSER, 1980 Control of the flux in the arginine pathway of *Neurospora crassa*. Biochem. J. **190:** 1–15.

FLINT, H. J., R. W. TATESON, I. B. BARTHELMESS, D. J. PORTEOUS, W. D. DONACHIE and H. KACSER, 1981 Control of the flux in the arginine pathway of *Neurospora crassa*. Biochem. J. **200:** 231–246.

GRAHAM, A., 1981 *Kronecker Products and Matrix Calculus with Applications*. Ellis Horwood, Chicester.

GROEN, A. K., R. VAN DER MERWE, H. V. WESTERHOFF, R. J. A. WANDERS, T. P. M. ACKERBROOM and J. M. TAGER, 1982 Control of metabolic fluxes. pp. 9–37. In: *Metabolic Compartmentation*, edited by H. FRIES. Academic Press, New York.

GUENET, J.-L., G. MARCHAL, G. MILON, P. TAMBOURIN and F. WENDLEY, 1979 Fertile dominant spotting in the house mouse. J. Hered. **70:** 9–12.

HARRIS, H., 1980 *The Principles of Human Biochemical Genetics*, Ed. 3. Elsevier, Amsterdam.

HESS, B., 1973 Organization of glycolysis: oscillatory and stationary control. Symp. Soc. Exp. Biol. **27:** 105–132.

HEINRICH, R. and T. A. RAPOPORT, 1974 A linear steady state treatment of enzyme chains. Eur. J. Biochem. **42:** 89–102.

HOFMEYER, J. H. S., 1986 Steady state modelling of metabolic pathways: A guide for the prospective simulator. Cabios Rev. **2:** 5–11.

HOFMEYER, J. H. S., H. KACSER and K. J. VAN DER MERWE, 1986 Metabolic control analysis of moiety-conserved cycles. Eur. J. Biochem. **155:** 631–641.

HOLLINGDALE, B., 1971 Analysis of some genes from abdominal bristle number selection lines in *Drosophila melanogaster*. Theor. Appl. Genet. **41:** 292–301.

KACSER, H., 1983 The control of enzyme systems in vivo: elasticity analysis of the steady state. Biochem. Soc. Trans. **11:** 35–40.

KACSER, H., 1987 Dominance not inevitable but very likely. J. Theor. Biol. **125:** 505–506.

KACSER, H. and J. W. PORTEOUS, 1987 Control of metabolism: what do we have to measure? Trends Biochem. Sci. **12:** 5–14.

KACSER, H. and J. A. BURNS, 1973 The control of flux. Symp. Soc. Exp. Biol. **27:** 65–104.

KACSER, H. and J. A. BURNS, 1981 The molecular basis of dominance. Genetics **97:** 639–666.

KNOX, W. E. and E. MESSINGER, 1958 The detection in the heterozygote of the metabolic effect of the recessive gene for phenylketonuria. Am. J. Hum. Genet. **10:** 53–60.

LAPORTE, D. C., K. WALSH and D. E. KOSHLAND, 1984 The branch point effect. J. Biol. Chem. **259:** 14068–14075.

LINDSLEY, D. L. and E. H. GRELL, 1968 Genetic variations of *Drosophila melanogaster*. Carnegie Inst. Wash. Publ. 627.

MIDDLETON, R. J. and H. KACSER, 1983 Enzyme variation, metabolic flux and fitness: alcohol dehydrogenase in *Drosophila melanogaster*. Genetics **105:** 633–650.

SALTER, M., R. G. KNOWLES and C. I. POGSON, 1986 Quantitation of the importance of individual steps in the control of aromatic amino acid metabolism. Biochem. J. **234:** 635–647.

SAURO, H. M., R. SMALL and D. FELL, 1987 Metabolic control and its analysis: extension to the theory and matrix method. Eur. J. Biochem. **165:** 215–221.

STANBURY, J. B., J. B. WYNGAARDEN, D. S. FREDRICKSON, J. L. GOLDSTEIN and M. S. BROWN, 1983 *The Metabolic Basis of Inherited Disease*. McGraw-Hill, New York.

STUART, F., D. J. PORTEOUS, H. J. FLINT and H. KACSER, 1986 Control of flux in the arginine pathway of *Neurospora crassa*: effects of co-ordinate changes of enzyme concentration. J. Gen. Microbiol. **132:** 1159–1166.

WEBB, A. J. 1981 The halothane sensitivity test. pp. 105–124. In *Proceedings of Symposium on Porcine Stress and Meat Quality*, Edited by T. FROYSTEIN, E. SLINDE and N. STANDAL. Norwegian Agricultural Food Research Society, As.

WOODROW, I. E., 1986 Control of the rate of photosynthetic carbon dioxide fixation. Biochim. Biophys. Acta. **851:** 181–192.

WRIGHT, S., 1934 Molecular and evolutionary theories of dominance. Am. Nat. **63:** 24–53.

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APPENDIX 1

Here we derive a set of equations for the fluxes in the nonsaturable branched system (Figure 1b). Consider the following monomolecular step within one branch, catalyzed by a Michaelis-Menten enzyme, at steady state.

$$\cdots \rightleftharpoons S_i \overset{E_j}{\rightleftharpoons} S_j \rightleftharpoons \cdots$$

The rate, v_j, of the reaction is given by

$$v_j = \frac{(V_j/M_i)(S_i - S_j/K_{i,j})}{1 + S_i/M_i + S_j/M_j}, \tag{1.1}$$

(CLELAND 1963), where V_j is the maximal velocity (V_{max}); M_i and M_j are the Michaelis constants (K_m) for the forward and backward reactions respectively; S_i and S_j are the concentrations of the substrate and product and $K_{i,j}$ is the equilibrium constant for the step which is, of course, independent of enzyme activity. When $S_i \ll M_i$ and $S_j \ll M_j$ (absence of saturation), Eq. 1.1 reduces to

$$v_j = e_j\,(S_i - S_j/K_{i,j}), \tag{1.2}$$

where $e_j = V_j/M_i$, the genetically determined enzyme activity.

Since the three branches of the system are symmetrical in structure, we can take the "A-pathway" (common branch) as an example. The steady state flux is obtained when all the individual rates in the branch are equal to one another and hence equal to the branch flux, *i.e.*, $v_1 = v_2 = v_3 = \cdots J_A$. All the intermediate pools will have time-invariant values. J_A is given by the solution of a set of linear simultaneous equations of the same form as Eq. 1.2:

$$J_A = e_1^A(X_A - S_1^A/K_{A,1}^A),$$
$$J_A = e_2^A\,(S_1^A - S_2^A/K_{1,2}^A),$$
$$\cdots$$
$$\cdots$$
$$J_A = e_l^A(S_{l-1}^A - S/K_{l-1,s}^A).$$

Solving these equations gives:

$$J_A = \frac{X_A - S/K_A}{1/e_1^A + K_{A,1}^A/e_2^A + \cdots K_{A,l-1}^A/e_l^A}, \tag{1.3}$$

where $K_A = K_{A,1}^A \cdot K_{1,2}^A \cdot K_{2,3}^A \cdot \cdots K_{l-1,S}^A$, *i.e.*, the equilibrium constant, $K_{A,S}$ between X_A and S. Similarly the equilibrium constant between X_A and S_{l-1} is $K_{A,l-1}$, etc. (Figure 1b).

Expression (1.3) can be reexpressed by grouping the sum of the reciprocals of enzyme activities and the equilibrium constants in Eq. 1.3 into a 'group enzyme activity,' A:

$$J_A = A(X_A - S/K_A), \tag{1.4}$$

where

$$^1/A = \sum_{i=1}^{l} K_{A,i-1}/e_i^A$$

and the first term contains $K_{A,0} = 1$.

Since each branch on the system has the same structure, we can write down similar expressions for the fluxes in the two competing branches:

$$J_B = B(S - X_B/K_B), \tag{1.5}$$

$$J_C = C(S - X_C/K_C), \tag{1.6}$$

where B and C represent the group enzyme activities of the two branches and K_B and K_C are the equilibrium constants between S and X_B and X_C, respectively. The three linear equations can be solved by eliminating S. We obtain expressions for each of the fluxes in terms of external parameters (X_A, X_B and X_C) and of the internal (enzyme) parameters A, B and C. Each expression will be a *different* function of all the parameters.

APPENDIX 2

Proof of the identity of the dominance indices of all the variables with respect to variation at one enzyme in an unsaturated system.

The steady-state system is fully described by the three linear rate equations (Appendix 1) (1.4), (1.5) and (1.6) and by definition (2). There are four consistent equations in four unknown variables J_A, J_B, J_C and S. Explicit solutions for the variables can be obtained from the matrix:

$$\begin{bmatrix} K_A/A & 0 & 0 & 1 \\ 0 & -1/B & 0 & 1 \\ 0 & 0 & -1/C & 1 \\ 1 & -1 & -1 & 0 \end{bmatrix} \begin{bmatrix} J_A \\ J_B \\ J_C \\ S \end{bmatrix} = \begin{bmatrix} X_A K_A \\ X_B/K_B \\ X_C/K_C \\ 0 \end{bmatrix}$$

or $\quad M \qquad z \qquad = \qquad y.$

The solution to the column vector z is obtained from

$$z = M^{-1}y. \tag{2.1}$$

Any system of monomolecular nonsaturable enzymes can be expressed in these terms. Importantly (see Appendix 1), each enzyme activity term is first order and only occurs once in the matrix M. Defining the nonsaturable system in these terms simplifies the algebraic treatment of the simultaneous effect of changing one enzyme activity on all the fluxes and pools which are pleiotropically related to each other.

We consider the two-allele case, 'wild type,' mutant and heterozygote. If e is the wild type enzyme activity (in any units), we can write the wild-type phenotype as $f(e)$, where $f(e)$ is the measured value of one character. We can also express the homozygote mutant enzyme activity as $(e + m)$ and the phenotype as $f(e + m)$. (For example, in the case of a 'null' mutant, *i.e.*, the mutant allele is a 'loss-of-function' mutation, the value of m would be $-e$, hence $e + m = 0$. The phenotypic value, $f(e + m)$ in such a case would not necessarily be equal to zero since not all characters are equally dependent on a single enzyme activity.) The heterozygote enzyme activity can be expressed as $(e + \lambda m)$ and the corresponding phenotype as $f(e + \lambda m)$. The value of λ would, in most cases be 0.5 (*heterozygote enzyme activity* is intermediate between wild type and mutant (see *e.g.*, KACSER and BURNS 1981; MIDDLETON and KACSER 1983), though of course, not the heterozygote phenotype. If we specify the three enzyme activities, the three phenotypic functions, $f(e)$, $f(e + m)$ and $f(e + \lambda m)$, can be calculated from the kinetic equations or the matrix (2.1). The f function could, for example, be one of the branch fluxes, say, J_B. Similarly, taking another function of the same three enzyme activities (the character, say, flux J_C) we can obtain $g(e)$, $g(e + m)$ and $g(e + \lambda m)$. These functions are now inserted into the Dominance Index definition giving:

$$D_f = \frac{f(e) - f(e + \lambda m)}{f(e) - f(e + m)}, \tag{2.2}$$

$$D_g = \frac{g(e) - g(e + \lambda m)}{g(e) - g(e + m)}. \tag{2.3}$$

Equations 2.2 and 2.3 can be expanded as a Taylor's series:

$$D_f = \frac{f(e) - [f(e) + \lambda m f'(e) + \lambda^2 m^2 f''(e)/2! + \cdots]}{f(e) - [f(e) + m f'(e) + m^2 f''(e)/2! + \cdots]}, \tag{2.4}$$

$$D_g = \frac{g(e) - [g(e) + \lambda m g'(e) + \lambda^2 m^2 g''(e)/2! + \cdots]}{g(e) - [g(e) + m g'(e) + m^2 g''(e)/2! + \cdots]}. \tag{2.5}$$

From (2.1), $z = M^{-1}y$, it follows that

$$\frac{\partial z}{\partial e} = \frac{\partial M^{-1}}{\partial e} y + M^{-1} \frac{\partial y}{\partial e}$$

(see *e.g.*, GRAHAM 1981) where e is an enzyme activity occurring in matrix M. Noting that the vector y contains no elements with enzyme activity parameters (and therefore its derivative is zero) we can reexpress the derivative of the inverse matrix, and obtain

$$\frac{\partial z}{\partial e} = - M^{-1} \frac{\partial M}{\partial e} M^{-1} y. \tag{2.6}$$

Similarly the second derivative is given by

$$\frac{\partial^2 z}{\partial e^2} = 2M^{-1} \frac{\partial M}{\partial e} M^{-1} \frac{\partial M}{\partial e} M^{-1} y. \tag{2.7}$$

Since e occurs only once and linearly in M, $\partial M/\partial e$ is an elementary matrix with nonzero element at the position of enzyme activity e multiplied by a scalar. Turning now to Eq. 2.7, the term

$$\frac{\partial M}{\partial e} M^{-1} \frac{\partial M}{\partial e}$$

is the same elementary matrix multiplied by a different scalar. Clearly therefore, expressions (2.6) and (2.7) are proportional to one another as are higher order derivatives. Thus for any complexity of a linear system,

$$\frac{f'(e)}{g'(e)} = \frac{f''(e)}{g''(e)} = \frac{f'''(e)}{g'''(e)} = \cdots = \text{constant}. \tag{2.8}$$

Relation (2.8) together with Eqs. 2.4 and 2.5 imply that:

$$D_f = D_g.$$

Therefore, for a system of any structural complexity having unsaturated enzymes, the dominance index for any character will be identical to that for any other character.

APPENDIX 3

Here, we derive functions for the fluxes of the branched pathway with saturation in the "B" branch (see Figure 3). At steady state, relations (1.4) and (1.6) give the fluxes in the linear "A" and "C" pathways. For J_B there are three equations:

$$J_B = P(S - S_i/K_P), \tag{3.1}$$

$$J_B = D(S_j - X_B/K_D), \tag{3.2}$$

where P and D represent the (linear) 'group enzyme activities' in the "P" and "D" sections, and K_P and K_D are the equilibrium constants for the reactions $S \rightarrow S_i$ and $S_j \rightarrow X_B$, respectively. From Eq. 1.1 we can also write down the flux for the step with

saturation containing the relevant Michaelis constants.

$$J_B = \frac{V_j/M_i(S_i - S_j/K_{i,j})}{1 + S_i/M_i + S_j/M_j}. \tag{3.3}$$

The variable S terms can be eliminated from the above three equations and from (1.4) and (1.6) to yield a quadratic in J_B.

$$\begin{aligned} J_B^2[M_i/(DM_j) - K_P\,(1/P + 1/(AK_A + C))] \\ + J_B[V_j/(DK_{i,j}) + X_BM_i/(M_jK_D) \\ + V_jK_P(1/P + 1/(A/K_A + C)) + M_i \\ + K_P(AX_A + CX_C/K_C)/(A/K_A + C)] \\ + X_BV_j/K_{i,j}K_D - V_jK_P(AX_A + CX_C/K_C)/(AK_A + C) = 0. \end{aligned}$$

For an asymptotically stable steady state this has only one positive real root.

The expression for J_C is obtained by eliminating S from Eqs. 2, 1.4 and 1.6. We obtain

$$J_C = \frac{X_A - X_C/K_{AC} - J_B/A}{1/A + 1/CK_A}, \tag{3.5}$$

where K_{AC} is the equilibrium constant for the reaction $X_A \rightarrow X_C$. The root of Eq. 3.4 (if known) can then replace J_B in (3.5) giving an expression for J_C in terms of parameters only.

APPENDIX 4

We derive equations for the fluxes in the branched system with feedback inhibition. At steady state, the set of simultaneous equations defining the fluxes in the system, defined in Figure 10 is given by (2), (1.4) and (1.6) together with four equations for J_B:

$$J_B = P(S - S_i/K_P), \tag{4.1}$$

$$J_B = Q(S_j - S_k/K_Q), \tag{4.2}$$

$$J_B = R(S_k - X_B/K_R), \tag{4.3}$$

where the equilibrium constant (K) terms are as defined in the legend to Figure 10 and P, Q and R represent the 'group enzyme activities' of the "P," "Q" and "R" sections respectively. A simple expression for the rate of the reaction $S_i \rightarrow S_j$ catalyzed by E_j can be

$$J_B = \frac{(V_j/M_i)(S_i - S_j/K_{i,j})}{1 + S_k/K_I} \tag{4.4}$$

(CLELAND 1963), where S_k is the concentration of the metabolite which acts as an allosteric inhibitor, and K_I is the inhibition constant. The solution to the set is a quadratic as follows:

$$\begin{aligned} J_B^2/(R/K_I) + J_B[1 + X_B/(K_RK_I) + V_jK_P/(PM_i) \\ + V_j/(QM_iK_{i,j}) + V_j/(RM_iK_{i,j}K_Q) \\ + V_jK_P/(M_i(A/K_A + C))] + V_jX_B/(M_iK_{i,j}K_QK_R) \\ - V_jK_P(AX_A + CX_C/K_C)/(M_i(A/K_A + C)) = 0. \end{aligned}$$

The flux through the "C" pathway is given, as before, by Eq. 3.5.