THE MOLECULAR BASIS OF DOMINANCE

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ABSTRACT

The best known genes of microbes, mice and men are those that specify enzymes. Wild type, mutant and heterozygote for variants of such genes differ in the catalytic activity at the step in the enzyme network specified by the gene in question. The effect on the respective phenotypes of such changes in catalytic activity, however, is not defined by the enzyme change as estimated by *in vitro* determination of the activities obtained from the extracts of the three types. *In vivo* enzymes do not act in isolation, but are kinetically linked to other enzymes *via* their substrates and products. These interactions modify the effect of enzyme variation on the phenotype, depending on the nature and quantity of the other enzymes present. An output of such a system, say a flux, is therefore a systemic property, and its response to variation at one locus must be measured in the whole system. This response is best described by the sensitivity coefficient, **Z**, which is defined by the fractional change in flux over the fractional change in enzyme activity.

$$\mathbf{Z} = \frac{dF}{F} \Big/ \frac{dE}{E} .$$

Its magnitude determines the extent to which a particular enzyme "controls" a particular flux or phenotype and, implicitly, determines the values that the three phenotypes will have. There are as many sensitivity coefficients for a given flux as there are enzymes in the system. It can be shown that the sum of all such coefficients equals unity.

$$\sum_{i=1}^{n} \mathbf{Z}_i = 1 .$$

Since n, the number of enzymes, is large, this summation property results in the individual coefficients being small. The effect of making a large change in enzyme activity therefore usually results in only a negligible change in flux. A reduction to 50% activity in the heterozygote, a common feature for many mutants, is therefore not expected to be detectable in the phenotype. The mutant would therefore be described as "recessive". The widespread occurrence of recessive mutants is thus seen to be the inevitable consequence of the kinetic structure of enzyme networks. The *ad hoc* hypothesis of "modifiers" selected to maximize the fitness of the heterozygote, as proposed by FISHER, is therefore unnecessary. It is based on the false general expectation of an intermediate phenotype in the heterozygote. WRIGHT's analysis, substantially sound in its approach, proposed selection of a "safety factor" in enzyme activity. The derivation of the summation property explains why such safety factors are automatically present in almost all enzymes without selection.

FOLLOWING the rediscovery of MENDEL's 1866 paper, dominance was taken as a fact of life until FISHER (1928, 1930) posed and attempted to answer the question of its possible evolution. Because the vast majority of mutants are recessive, he postulated selection of "modifiers" at other loci acting on the expression of the heterozygote. This would make its phenotype, which was assumed to be intermediate when it first arose, approach that of the wild type, which, by definition, is the fittest phenotype. His view has generated even to this day (*e.g.*, CHARLESWORTH and CHARLESWORTH 1979; CALIGARI and MATHER 1980) a large body of literature. Only a few of these papers are concerned with the physiological or biochemical mechanism of the phenomenon; outstanding among these is the seminal paper by WRIGHT (1934). Putting forward a physiological explanation of dominance, WRIGHT criticized FISHER's view with respect to the assumptions made and the magnitude of the selection coefficients required (see also HALDANE 1930; PLUNKETT 1933). It is not the purpose of this paper to review the arguments in this controversy (see CROSBY 1963; SHEPPARD and FORD 1966; SVED and MAYO 1970; CHARLESWORTH 1979), but to put forward an analysis of the problem in terms of more recent evidence on the control of gene expression and the behavior of multi-enzyme systems. We shall show that the recessivity of mutants is an inevitable consequence of the kinetic properties of enzyme-catalyzed pathways and that no other explanation is required.

The most investigated genes are those that control either the nature or quantity of catalytic proteins: enzymes. These may comprise the largest class of genes, many of which are known only by some morphological or other functional change in the phenotype. While there has been rapid progress in the identification of specific enzymes controlled by such genes, there are undoubtedly some genes whose products are not enzymes (*e.g.*, histones, immunoglobulins, crystallins), although in many cases their role is quasicatalytic insofar as they affect the rate of a process (*e.g.*, hemoglobin, rRNA, repressors). Our treatment is based on the gene-enzyme relationship and extends to all catalytic gene products. How far it may be applicable to noncatalytic products will be discussed later.

The mechanism of gene expression has been the subject of considerable investigation. The general conclusions, insofar as enzymes are concerned, are quite clear. The genetic specification, encoded in the DNA sequence, is transcribed and translated into amino acid sequences, whose folded tertiary or quaternary structure determine the catalytic efficiency (turnover number), substrate specificity and substrate binding (Michaelis constants). Further genetic determinants exist, well documented in prokaryotes, whose peptide products (repressors) interact at specific DNA sites and affect the rate of transcription and, hence, the quantity of particular enzymes synthesized. Small ligand molecules (co-repressors), often products of metabolism, can interact with such repressor molecules to alter their binding to DNA (repression or induction). For eukaryotic organisms, the mechanisms of control of enzyme quantity are less clear, but the phenomenon is well documented. In addition, some small metabolites can act directly on the catalytic properties of the enzymes themselves (inhibition or activation).

The result of mutational events anywhere in the genome can alter the kinetic parameters of catalysis *via* the processes sketched out above. The variation in catalytic activity can be brought about in several ways: (1) by a change in the turnover number or the Michaelis constant, or both, as would result from a mutational change in the structural gene, (2) by a change in enzyme concentration resulting from changes of gene dose or as a consequence of changes in repression or induction due to alterations in the structural gene or elsewhere in the genome, or (3) by changes in inhibition or activation caused by alteration of enzyme structure or by changes in the concentration of the ligands.

Since all these net catalytic variations are representable as an equivalent change in enzyme concentration, we can conveniently describe the mutational consequences in these terms. The recent discovery of introns, sequences of DNA within genes that are excised prior to translation, does not affect the conclusion that the net effect of mutation on the phenotype is mediated *via* an alteration in the rate of one metabolic transformation. When, as geneticists, we consider substitutions of alleles at a locus, as biochemists, we consider alterations in catalytic parameters at one enzyme step.

Multi-enzyme systems and fluxes

The organism, viewed as an enzyme system, consists of a large array of specific and saturable catalysts organized into diverging and converging pathways, cycles and spirals all transforming molecular species and resulting in a flow of metabolites. Enzymes in such a system do not act in isolation. The substrate of one enzymic step is produced by the activity of another (except for "first" enzymes), and the product of this enzymic step is the substrate for at least one step (except for "end products"). This kinetic linking results in the net flux across any enzyme step being affected by the activities of its neighboring enzymes and hence, in principle, by those of all others. The flux through each part of the metabolic map is therefore seen to be dependent on the kinetic parameters of all the enzymes and, hence, on the genetic specification of the whole genome. Fluxes are therefore systemic properties. Some of these fluxes will be "outputs" (*e.g.*, waste products, milk solids, etc.); others will have "internal" products, used in growth and maintenance, such as muscle proteins, fats or pigments. The fluxes are thus seen to be closely related to the characters or phenotype insofar as we measure or observe the outcome of the activities of the biochemical network. Instead of actual fluxes (*i.e.*, mol/time), the integral of such a flux over a given time could be chosen (*e.g.*, laying down of insoluble pigment). The effect on the phenotype of altering the genetic specification of a single enzyme, with which the problem of dominance is concerned, is, as we have seen, unpredictable from a knowledge of events at that step alone and must involve the response of the system to alterations of single enzymes when *they are embedded in the matrix of all other enzymes.* Experimentally, this involves not a detailed study of a single enzyme (which is the subject matter of enzymology), but experiments *in vivo* where we can estimate the effect of known changes in one enzyme activity on a flux that is in some way dependent on this enzyme.

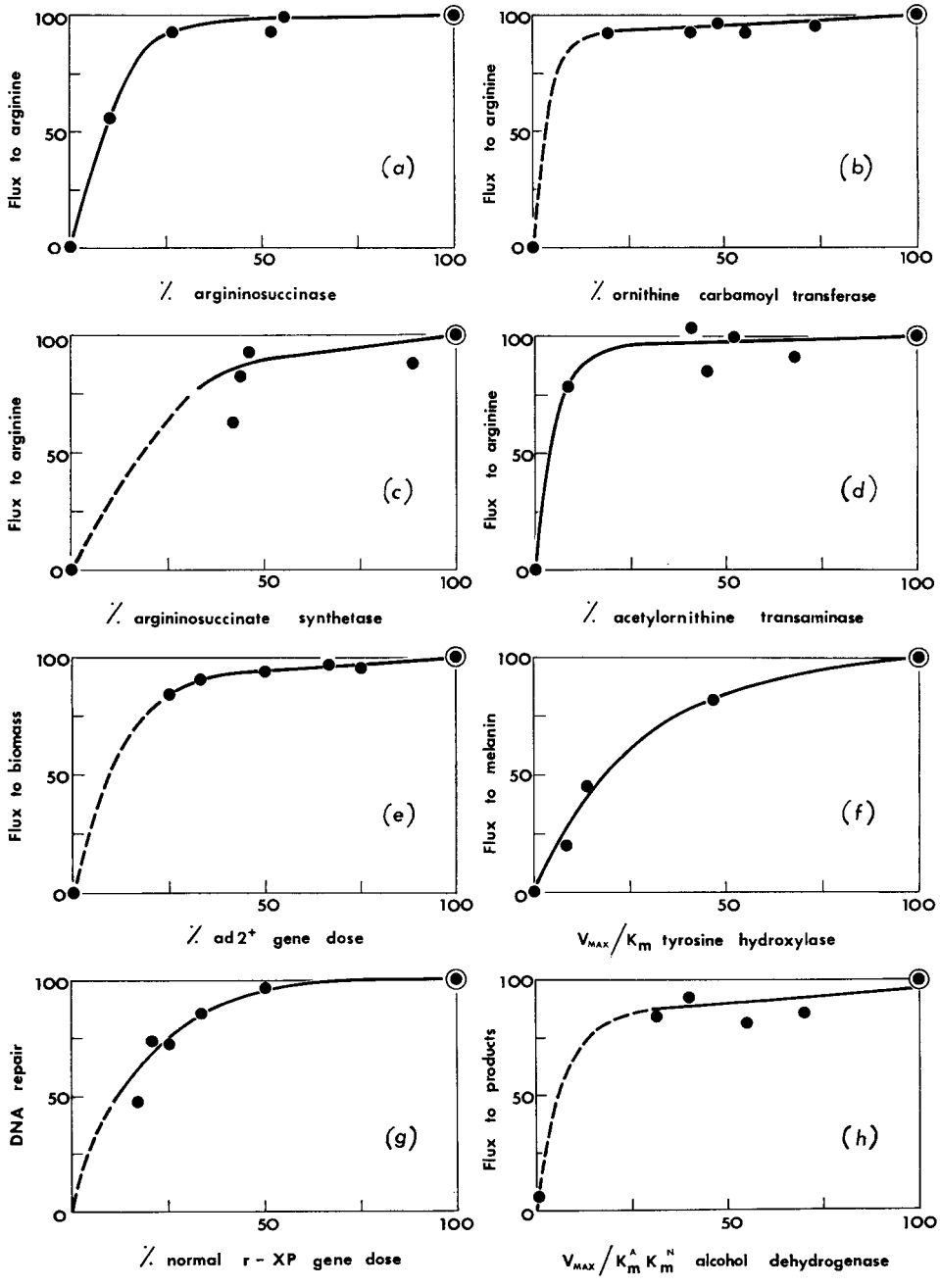

FIGURE 1.—In all figures, the circled point represents the wild-type activity, which is scaled to 100. The curves are fitted by eye. (a) to (d), Neurospora heterokaryons: Each figure represents a series of heterokaryons with different nuclear ratios of null to wild-type nuclei. The two extreme points are the mutant and wild-type homokaryon, respectively. Heterokaryons are constructed by varying the input ratio of mutant to wild-type conidia. Enzyme activity was measured by extracting mycelium during exponential growth. The flux to arginine was measured by estimating

A few examples from experimental data, ranging from microorganisms to man, are shown in Figure 1. Two important properties are common to all of the systems illustrated. First, the relationship of enzyme activity to flux is nonlinear: the lower the enzyme activity, the greater the effect on the flux of a given change in activity. Second, the "wild-type" activity lies on or near the plateau of the relationship. It will be noted that, in Figure 1 (a), (b), (c) and (d), we show four successive enzyme steps in the arginine pathway, suggesting that every enzyme in the pathway shares the above-mentioned aspects. The wide range of organisms and types of enzymes indicate that this is a universal phenomenon. The subsequent analysis will show that this is indeed the expected behavior.

If the flux represents a phenotype, it is easy to see how dominance results from the shapes of the curves. If the enzyme activity of a heterozygote is midway between those of the two homozygotes, then the phenotype will be nearer that of the wild-type homozygote. This, however, does not explain dominance. Two questions have to be answered: why do the curves have this shape, and why is the wild type on the plateau of the curve? To answer these questions, we have to introduce two important properties of enzyme systems, namely the sensitivity coefficient and the summation property. These will now be briefly explained.

Sensitivity coefficients

The problem of explaining the behavior of *in vivo* systems in terms of the biochemical properties of its parts meets with a number of difficulties. Enzymological investigations enable us to obtain algebraic formulations for individual enzyme steps. It is therefore, in principle, possible to write down the equations for every step in the system (formidable as this may be) and obtain a set of simultaneous equations. Since each of the steps is represented by a nonlinear differential equation, there is no analytical solution to the set that would represent the whole system.

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the sum of the pool arginine, the protein arginine and the urea production. In (a), the mutant is at the *arg-10* locus having null activity in argininosuccinase. (b) *arg-12* locus, ornithine carbamoyl transferase. (c) *arg-1* locus, argininosuccinate synthetase. (d) *arg-5* locus, acetylornithine transaminase (TATESON 1972). (*e*): Saccharomyces mutant at the *ad-2* locus: Triploid and tetraploid strains were constructed having various doses of null and wild-type alleles at the *ad-2* locus, while the rest of the genome was isogenic. The locus specifies phophoribosyl aminoimidazole carboxylase. The flux to biomass is the exponential growth constant (REICHERT 1967; 1968). (*f*): Mouse mutants at the albino locus: The extreme points represent the *cc* (albino) and *CC* (wild) homozygotes, respectively. Intermediate points are $c^{ch}c^{ch}$ and albino heterozygotes (cc^{ch}, cC). Enzyme activities and melanin production were estimated in skin extracts of 5–6 day old mice. K_m = Michaelis constant for tyrosine of the different active enzymes (ANDREWS 1974). (*g*): Tissue culture heterokaryons between normal nuclei and nuclei from a *Xeroderma pigmentosum* patient: DNA repair was estimated by thymidine incorporation (GIANELLI and PAWSEY 1976). (*h*): Drosophila mutants at the *Adh* locus: The extreme points represent the Adh^{null} (*n-2*) allele and the Adh^{Fd} allele, respectively. Intermediate points are various "fast" and "slow" alleles and their heterozygotes with "null." The flux was measured as counts of CO_2 and fat production by exposing adult flies to radioactive ethanol vapour. K_m^A = Michaelis constant for ethanol, K_m^N = Michaelis constant for NAD of the different active enzymes (MIDDLETON 1980).

It is, however, possible to obtain some general answers by applying systemic concepts and methodologies (KACSER and BURNS 1973, 1979). In brief, these consist of applying a small change, ΔE_i, to, say, the concentration of any one of the enzymes, E_i, and measuring the net effect, ΔF_j, on any flux, F_j, anywhere in the system. Figure 2 illustrates the pathway to be considered.

It is convenient to consider the respective fractional changes $\Delta F_j/F_j$ and $\Delta E_i/E_i$. A comparison of the enzyme change and its effect, *i.e.*, the ratio $\Delta F_j/F_j/\Delta E_i/E_i$, represents the effectiveness of E_i in "controlling" the flux F_j. Since the dependence of F on E is, in general, nonlinear, the ratio will depend on the magnitude of the imposed ΔE_i. If we reduce the value of $\Delta E_i \rightarrow 0$, we obtain, in the limit, a differential that is independent of step size and represents the true local "sensitivity" of that flux to changes of this particular enzyme. This sensitivity can be formally expressed as follows:

$$\frac{\Delta F_j}{F_j} \Big/ \frac{\Delta E_i}{E_i} \longrightarrow \frac{dF_j}{F_j} \Big/ \frac{dE_i}{E_i} = \frac{d\ln F_j}{d\ln E_i} = \mathbf{Z}_i^j \ .$$

This ratio of the fractional response in a flux over the fractional change in an enzyme causing the response is a constant for a particular system and will, in general, depend on all the parameters of this system. Its magnitude takes account of all the adjustments and changes in the rest of the system that may have occured as a result of the change. The ratio is called the sensitivity coefficient and given the symbol Z (KACSER and BURNS 1973). The sensitivity coefficient will be recognized as the tangent of the F *vs.* E relationship at a particular point (multiplied by the scaling factor E/F).

$$\frac{dF}{F} \Big/ \frac{dE}{E} = \frac{dF}{dE} \times \frac{E}{F} = \mathbf{Z}$$

It will be noted that the magnitude of the coefficient varies with the enzyme value. As will be shown, it also depends on the values of all the other enzymes.

The magnitude of the sensitivity coefficient represents the "importance" of the enzyme in its potential to influence the flux. If, for example the **Z** value is approaching 1, *i.e.*, if a small fractional change results in an almost identical change in the dependent flux, the enzyme may be said to be "fully controlling." **Z** values approaching zero, on the other hand, could be described as "noncontrolling." The sensitivity coefficients can of course take any value between these extremes. It is therefore not useful to introduce such a classificatory terminology, and for the same reason such terms as "rate limiting," "pacemaker" and "key

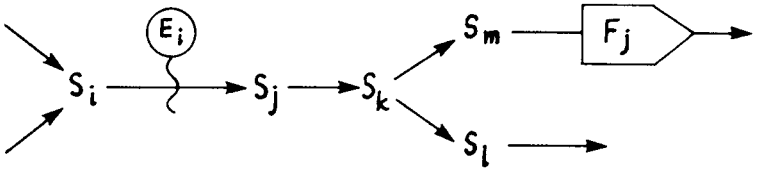

FIGURE 2

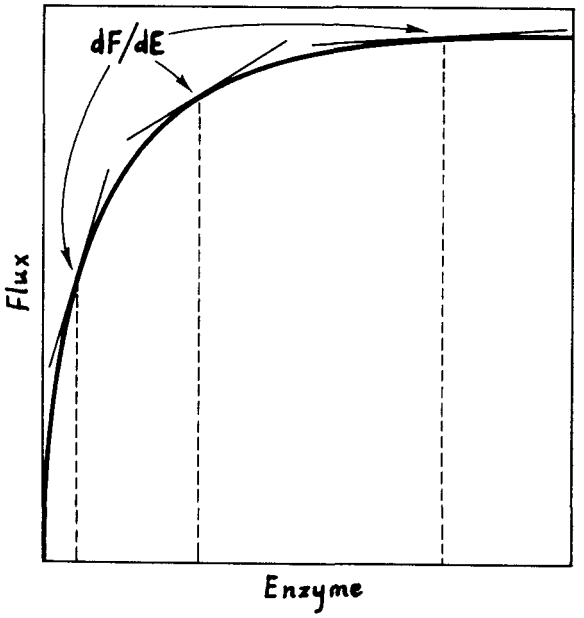

FIGURE 3.—Change of sensitivity coefficient with change in enzyme activity. The curve has been derived from equation (1) (see also Figure 4).

enzymes" are likely to be misleading. The magnitude of the sensitivity coefficient gives a precise measure of the particular enzyme's role when acting within the whole system. It is a measure of the likely effect of a given gene-dependent change in the catalytic properties of an enzyme on the magnitude of a given enzyme-dependent character.

The nonlinearity of the flux-enzyme relationship arises from the necessary interactions between successive steps of a "chain" of catalyzed reactions. Because of the kinetic linking *via* the shared substrates/products of adjacent enzymes, the effects of changing catalytic activity at one step tends to be buffered by the response to this of the other enzyme steps. This is illustrated in Scheme 1, which represents an easily calculated example of a simple pathway of unsaturated enzymes. It consists of n enzyme-catalyzed steps with a "source," X_1, and a "sink," X_n, both being held constant.

Scheme 1

$$\overbrace{\phantom{X_1 \rightleftharpoons S_2 \rightleftharpoons S_3 \rightleftharpoons S_4 \rightleftharpoons \cdots \rightleftharpoons S_{n-1} \rightleftharpoons X_n}}^{K_{1n}}$$

$$\overbrace{}^{K_{14}}$$

$$\overbrace{}^{K_{13}}$$

$$\overbrace{}^{K_{12}}$$

$$X_1 \underset{E_1}{\rightleftharpoons} S_2 \underset{E_2}{\rightleftharpoons} S_3 \underset{E_3}{\rightleftharpoons} S_4 \rightleftharpoons \cdots \rightleftharpoons S_{n-1} \underset{E_n}{\rightleftharpoons} X_n \,.$$

Such an open system will come to a steady state when the flux, *i.e.*, the rate of production of X_n, is constant ($\frac{dX_n}{dt} = F$). At the same time, the intermediate pools, S, will reach time-invariant steady state values ($\frac{dS_i}{dt} = 0$).

At steady state, each step will be represented by an expression of the form

$$v_i = \frac{V_i/M_i(S_i - S_j/K_{ij})}{1 + S_i/M_i + S_j/M_j} ,$$

where v = the rate at which S_i is transformed to S_j, V = maximal velocity (V_{max}) M = Michaelis constant (K_m)[1], K = equilibrium constant (see, *e.g.*, CLELAND 1963). Making the assumption of "no saturation," *i.e.*, $S_i \ll M_i$ and $S_j \ll M_j$, we obtain

$$v_i = V_i/M_i(S_i - S_j/K_{ij}) .$$

These now give a set of linear differential equations in S that are soluble (KACSER and BURNS, 1973). At steady state, the flux through such a pathway of "unsaturated" enzymes is given by

$$F = \frac{(X_1 - X_n)K_{1n}}{\frac{M_1}{V_1} + \frac{M_2K_{12}}{V_2} + \frac{M_3K_{13}}{V_3} + \ldots\ldots \frac{M_nK_{1n}}{V_n}} , \qquad (1)$$

where the subscripts of M and V denote the enzyme number and the K terms are the equilibrium constants whose subscripts denote the substrates. Each additional enzyme adds one more term to the denominator. Equation (1) is of the form:

$$F = \frac{C_x}{1/E_1 + 1/E_2 + \ldots 1/E_i \ldots + 1/E_n} , \qquad (1a)$$

where C_x represents the environmental parameters, which are constant. The E terms are composite, but each represents the genetically determined parameters of one enzyme. They are proportional to enzyme concentration and are modifiable by mutation. If any one enzyme activity is reduced to zero, the flux will fall to zero (metabolic block). It is seen that the flux depends on the values of all enzyme terms. The relationship of the flux to changes in any one enzyme is hyperbolic and depends on the number of enzymes, n. The flux through the pathway consisting of a single enzyme ($n = 1$) is, of course, linear with enzyme concentration, the well-known property of the Briggs-Haldane formulation. As the number of enzymes, n, between the first substrate and final product is increased, the effect of variation in any one of them becomes more nonlinear, as shown in Figure 4.

We obtain the partial differential of the flux with respect to any one enzyme to yield the sensitivity coefficient as follows.

[1] The symbols V and M are used instead of the two traditional ones, given in brackets, to aid the clarity of the formulation.

FIG 4

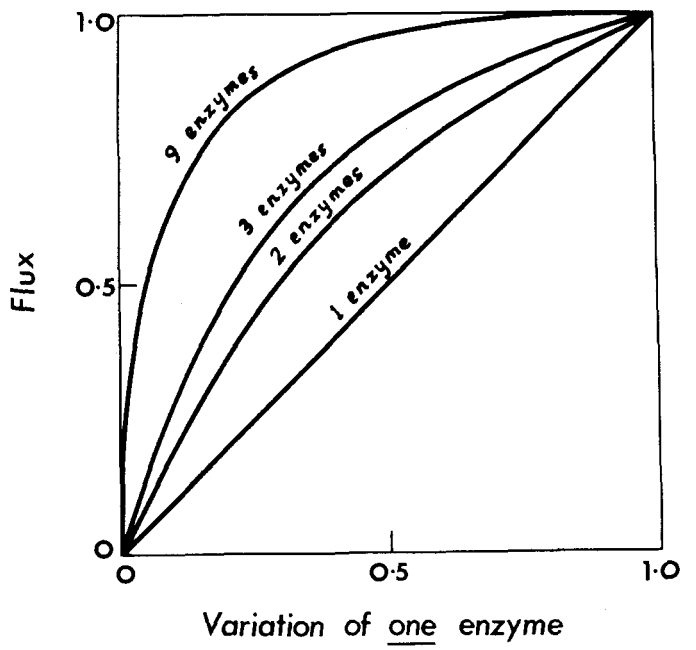

FIGURE 4.—Effect of number of enzymes on the flux response. The fluxes were calculated from equation (1). For convenience, all enzyme parameters were set to unity and the flux for each value of n, the number of enzymes scaled to unity. One of the V values was then modulated from 1 to 0 and the corresponding fluxes calculated.

$$\mathbf{Z}_i = \frac{\partial F}{\partial V_i} \times \frac{V_i}{F} = \frac{\dfrac{M_i K_{1i}}{V_i}}{\dfrac{M_1}{V_1} + \dfrac{M_2 K_{12}}{V_2} + \ldots\ldots \dfrac{M_i K_{1i}}{V_i} + \ldots\ldots \dfrac{M_n K_{1n}}{V_n}}, \qquad (2)$$

which is of the form:

$$\mathbf{Z}_i = \frac{1/E_i}{1/E_1 + 1/E_2 + \ldots 1/E_i + \ldots 1/E_n}. \qquad (2a)$$

This formulation shows that the sensitivity depends on both the enzymic values of the modulated enzyme (M_i/V_i) and those of all the others whose effects appear in the denominator. It is therefore a systemic property. This simple, algebraically soluble case demonstrates this basic property. It should be emphasized that, for algebraic convenience, we have used (and shall use in what follows) a simple straight chain of enzymes. The conclusions apply equally to systems of interlocking pathways and cycles, except that the formulations become more tedious to handle. The general case of complex pathways with saturable catalysts and other nonlinear interactions, such as feedbacks, has been investigated by simulation

and shows the same general property (KACSER and BURNS 1973). Finally, experimental manipulation of *in vivo* systems gives estimates of the magnitude of particular coefficients (as illustrated in Figures 1 and 3) that can be obtained by interpolation.

It is clear that there exists a sensitivity coefficient for a given flux with respect to every enzyme in the system. For the flux F_j, these are:

$$\mathbf{Z}^j_1,\ \mathbf{Z}^j_2,\ \mathbf{Z}^j_3\ .\ .\ .\ .\ .\ .\ .\ .\ .\ .\ \mathbf{Z}^j_n$$

Furthermore, every other flux (F_k, F_l, etc., for more complex systems having branches) has another set of sensitivities, equal in number, but different in magnitude.

$$\mathbf{Z}^k_1,\ \mathbf{Z}^k_2,\ \mathbf{Z}^k_3\ .\ .\ .\ .\ .\ .\ .\ .\ .\ .\ \mathbf{Z}^k_n$$

$$\mathbf{Z}^l_1,\ \mathbf{Z}^l_2,\ \mathbf{Z}^l_3\ .\ .\ .\ .\ .\ .\ .\ .\ .\ .\ \mathbf{Z}^l_n\ ,$$

etc.

In principle, each flux is affected by all the enzymes and all fluxes by any one enzyme. We can therefore view the system in terms of a matrix of sensitivities representing the response characteristics of the phenotype to possible changes in the genetic specification of each of the enzymes.

The summation property

Inspection of equation (2a) shows that each coefficient is likely to be small since the numerator contains only one term out of the n terms of the denominator. Furthermore, since each coefficient is one of the terms over their sum, it follows that, if all the coefficients of a flux are summed over all enzymes, the value will be unity:

$$\sum_{i=1}^{n} \mathbf{Z}^j_i = 1\ .$$

For any given flux, the sum of all the coefficients with respect to every enzyme in the system = 1.

This property, shown here for the case of a set of soluble equations, is equally true for a set of nonlinear (saturable) enzymes in a system of any structural complexity. The proof was given in a previous publication (KACSER and BURNS 1979)[2], and need not be repeated here. The property has important consequences for our understanding of the response of a flux to changes in one of the enzymes.

It follows that the larger the number of enzymes in the system, the smaller each coefficient will be. The average value will in fact be $1/n$, where n = the number of enzymes. Taking this number at between 3000 and 5000, we expect the average coefficient to be between 10^{-3} and 10^{-4}. There is, however, likely to be a distribution of coefficient values over all the enzymes. For any given flux, most enzymes will be very many steps removed from it. Their coefficients, individually,

[2] In this publication, the summation formulation was misprinted, suggesting summation over all fluxes. The present formulation is correct.

are therefore likely to be smaller than average, *i.e.*, negligibly small. The sum of such a large number of very small coefficients may, however, not be negligible.

On the other hand, there will be a group of enzymes, those more directly involved in the pathway, whose coefficients will be larger than average. Which enzyme steps are so involved is ill defined. Apart from those obviously concerned with the flux of metabolites, there are strong interactions with, *e.g.*, steps involved in the synthesis of co-factors or bimolecular condensations involving other pathways or inhibitions/activations from areas of metabolism not normally associated with the pathway. The number of such enzymes could be between 20 and 100, giving an expectation for their average coefficient of the order of 10^{-2} with, again, a distribution among these. This is still a small number for each of the coefficients and implies, as we have seen experimentally, that substantial reductions in any one enzyme activity will have only marginal effects on the flux. At the same time, equation (2a) shows that the coefficient increases with reduction in one enzyme activity in a hyperbolic manner. The finding of the "wild type" near the plateau ($Z \ll 1$) and the non linearity, exemplified in Figure 1, are therefore seen to arise directly from the above analysis. The notion of "excess" enzyme, a not infrequent explanation, has therefore 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. In the limit, it is possible for one coefficient to approach unity when, by the summation property, all others approach zero.

We have already remarked that very low coefficients will often be attached to enzymes kinetically "distant" from the particular flux considered. This is the reason for the well known observation that mutations in a different part of the map may have no detectable effect on one character even when another character is seriously affected. This apparent independence of most characters makes simple Mendelian genetics possible, but conceals the fact that there is universal pleiotropy. All characters should be viewed as "quantitative" since, in principle, variation anywhere in the genome affects every character.

In structurally complex systems, there will be many points of divergence when a common substrate is the source of two pathways. Alternations in enzymes of one of them will inversely affect the flux in the other, *i.e.*, they will have negative coefficients. Since the summation property applies to the whole system, such negative coefficients imply that some positive coefficients can be greater than unity. However, the contribution of such branch points in generating negative coefficients is much less pronounced than would appear at first, since most divided pathways, including catabolic ones, eventually re-enter the synthetic system *via* some common pool. This means that the coefficient, apparently negative for a branch, will in fact be positive. Only pathways that exit the system will have negative coefficients with respect to some metabolically linked fluxes.

An important further consequence of the coefficient as a systemic property is that epistasis is a necessary consequence. Again, inspection of equation (2) shows that the magnitude of the coefficient is altered both by changes in its "own" en-

zyme as well as by changes in any of the other terms in the denominator, *i.e.*, by changes in any other enzyme. A large change in one of these latter will alter the coefficient so that a subsequent change in its "own" enzyme will have a different effect on the flux. For the new constellation of enzymes, however, the summation property still holds, since the only changes have been in the distribution of the magnitudes of the coefficients and, of course, the magnitudes of certain fluxes. Substitution at one locus therefore alters the effects of substitutions at another.

Nature and detection of mutants

We now come to the relationship of the systemic properties of enzyme networks to the character differences between wild type and mutant. We shall consider what mutationally caused changes in enzyme activity are likely to be detected as segregating alleles. At one extreme is the class of "null" mutants where by virtue of the complete absence of enzyme activity in mutant homozygotes the flux is zero. Many auxotrophs in micro-organisms, pigmentless mutants in plants or animals and a number of inborn errors in man and other mammals fall into this class. These mutants shade into the class where we find a small finite activity (*e.g.*, "extreme dilution" in the mouse, "null Adh" in Drosophila), usually less than 3% of normal, and where the effect on the flux and its further consequences are sufficiently obvious by inspection of the affected character.

There is no general rule linking the magnitude of changes in flux with detectability as a mutant. Identification of a mutant will vary from character to character, from observer to observer and depend on the method used to measure the phenotype. Environmental noise will play a part. Coefficients of variation attributable to the environment vary widely, for example, from about 3% for linear measures in Drosophila to about 20% for milk production in cattle. In general, however, the well-known mutants are those whose measured phenotype is unambiguously different from the wild type.

Figure 5 gives an impression of where these "detectable" mutants lie in relation to the flux-enzyme curve. There is, of course, a grey area, where in some instances mutants will belong to the "detectable" class, in others, to the "undetectable." It will be noted, however, that there is an asymmetry, in that possible mutants with higher activity than that of the wild type (normal) will almost always evade detection because their phenotypic effect is very small. Mutants in the undetectable class, however, if they exist, will appear as quantitative genes whose variation in a population will constitute the genetic component of the variance.

The asymmetry referred to above is reinforced by the nature of protein molecules as catalysts. Independent of the effect on the system, any changes in the genetic specification of the protein is much more likely to reduce the catalytic activity than to enhance it. Although, in the course of evolution, such enhancement must undoubtedly have taken place, it is mutation from an existing, highly special configuration of the present form (or forms) that is at issue. Random changes in the genes will in the vast majority of events, lead to reduction in catalytic function. Quite independent of any question of detectability, therefore, most

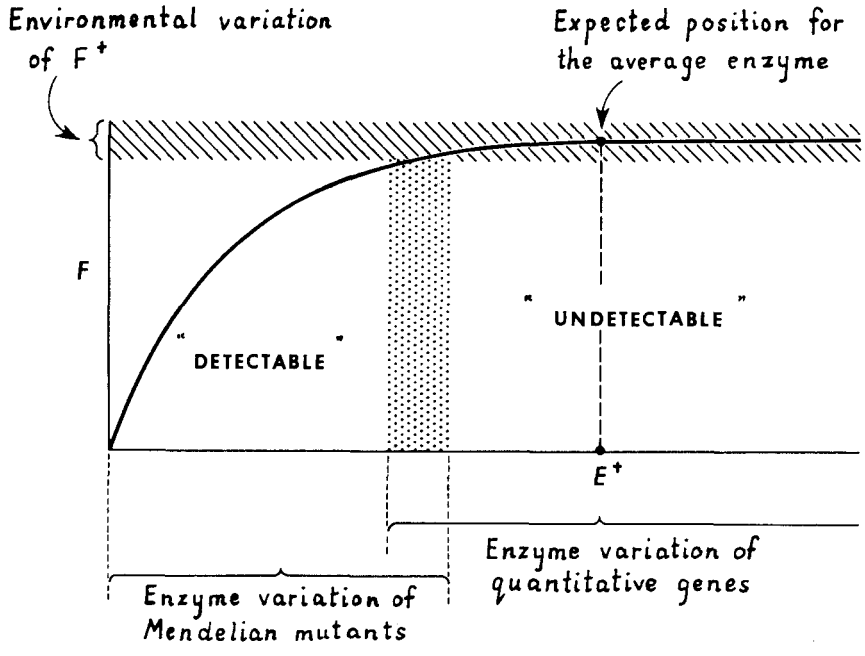

FIGURE 5

mutations will tend to fall in the direction of the detectable range. Thus, even if a particular coefficient at the wild-type level is reasonably large (and therefore not typical of the average coefficient), mutations to increased fluxes that can be detected are unlikely to occur.

It should, at this juncture, be pointed out that there are additional constraints on the catalytic effect of mutation. The kinetic parameters of enzymes are subject to a thermodynamic constraint made explicit in the Haldane relation (CLELAND 1963). For a monomolecular transformation, this takes the form of:

$$\frac{\overrightarrow{V_{max}}\ \overleftarrow{K_m}}{\overrightarrow{K_m}\ \overleftarrow{V_{max}}} = K_E \ ,$$

where the arrows symbolize the parameters for the forward or backward reaction, respectively. The equilibrium constant, K_E, for substrate and product is, of course, independent of the nature of any catalyst, being determined solely by the nature of the substrate and product. The V_{max} and K_m values, on the other hand, are interaction constants between molecule and catalyst and hence are affected by the nature of the enzyme. They are kinetic equivalents of the genetic parameters. The Haldane relation shows that mutational alterations will in principle affect all four phenomenological constants of the enzyme such that the above product remains constant. Changes in the constants are therefore not independent. In particular, it is the ratio V_{max}/K_m, relevant to our flux formulation, that may show divergent or compensatory changes in its two components. V_{max} change deter-

minations, so frequently used as a predictor of selective advantages, are therefore likely to be misleading, unless accompanying changes in the Michaelis constants are also estimated. (see Figure 1 f and h.)

In summary, then, we can state that the class of detectable mutations, *i.e.*, those whose phenotype and segregation are unambiguous, will be found to have a very substantial reduction in effective enzyme activity. There are other types of mutation that will escape our detection as phenotypically distinguished variants. These are, for example, the quantitative genes already referred to and the large number of electrophoretic mutants, which are found by an *in vitro* test and in almost all cases have, individually, no detectable functional consequences on the phenotype.

The quantiative description of dominance

Each character has predictable changes in its measured value, provided the response curve with respect to a particular enzyme change is known (Figure 6). This will also determine the value of the heterozygote in relation to those of wild type and mutant, provided the enzyme activity of the heterozygote is known. It is an observational fact that intermediacy of enzyme activity is almost universally found (see, *e.g.*, HARRIS 1975). This point will be discussed in detail later.

Depending on the form of the response curve, a given fractional change may have different consequences. More importantly, the value of **Z** at the wild type, indicated by the slope, will determine the phenotypic value of the heterozygote. In both cases, we compare equal changes in enzyme activity with the phenotypic changes represented by F. In Figure 6(a), the heterozygote W/M carries a flux only slightly smaller than W/W. Unless discrimination is very fine, these two phenotypes would be indistinguishable and M would be described as "recessive."

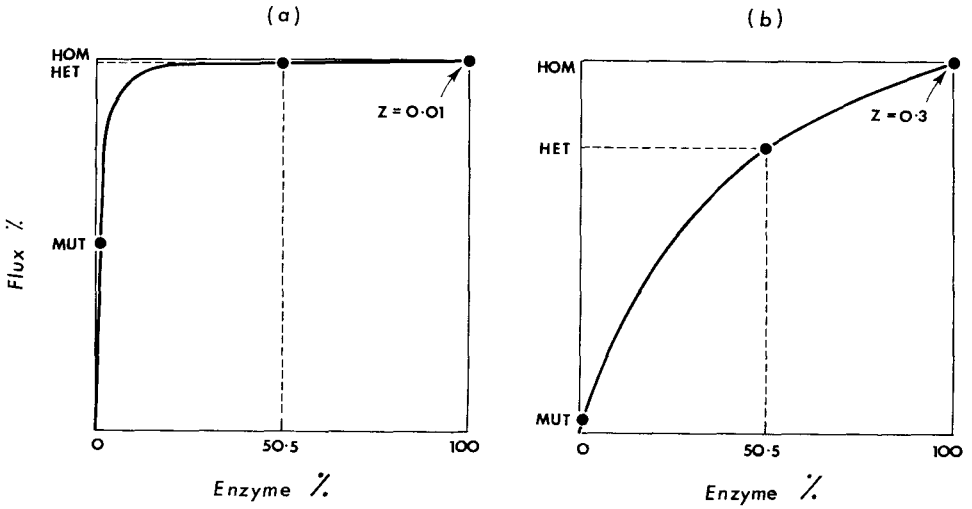

FIGURE 6.—Flux response to changes in enzyme activity. Two cases were calculated by equations (1) and (6). (a) $Z = 0.01$; (b) $Z = 0.3$. The three points shown correspond, in both cases, to the enzyme activities: 100% (wild type), 1% (mutant), 50.5% (heterozygote).

In the (b) case, the heterozygote is clearly different and somewhere intermediate between the two homozygotes and is therefore described as "partial dominance" or, by a curious convention, as "dominant" in the classificatory schemes for Man and Drosophila.

It is obvious that there is no qualitative difference between the two cases and that we should find some quantitative expression which would differentiate between them. Dominance is neither a "property" of genes nor a "property" of characters as SHEPPARD and FORD (1966) described it. Dominance is a shorthand term to describe the *relationship* of the phenotypes of three genotypes. These phenotypic measures can be used to define the Dominance Index, **D**.

Using the symbols WIL and MUT for the phenotypic values of the two homozygotes W/W and M/M, respectively, and HET for that of the heterozygote, W/M, then:

$$\mathbf{D} = \frac{\text{WIL} - \text{HET}}{\text{WIL} - \text{MUT}} \quad . \tag{3}$$

The scale of **D** runs continuously from 0, when HET = WIL (M is "recessive") through $\mathbf{D} = 0.5$ when WIL − HET = ½ (WIL − MUT) ("intermediacy"), to $\mathbf{D} = 1$ when HET = MUT (W is "recessive").

This index, first used by WRIGHT (1934) is preferred, for reasons that will appear later, to the equally valid "degree of dominance" used by FALCONER (1960). In practice, zero or near zero values for **D** are frequently found, which justifies the somewhat imprecise use of the term "recessive allele," particularly in the teaching of elementary genetics. For the purpose of our further analysis, however, we must relate the quantitative changes in enzyme activity to the quantitative consequences on the phenotype. The dominance index gives this quantitative measure of the phenotypic relationship.

Taking the flux as a measure of the phenotype, we can write the dominance index as the relationship of the fluxes:

$$\mathbf{D} = \frac{F_{WW} - F_{WM}}{F_{WW} - F_{MM}} \quad . \tag{3a}$$

It is intuitively obvious that there is a relationship between the value of the sensitivity coefficient of the wild type, whose magnitude describes the differential response at one point, and the dominance index, whose magnitude reflects the often large changes in enzyme and flux.

This relationship is easily established for the case of the linear equations discussed before. Equation (1a) can be further simplified to

$$F = \frac{C_1 E_i}{C_2 + E_i} \tag{1b}$$

when changes in only one enzyme are considered. In these terms, the sensitivity coefficient (equation 2a) becomes

$$\mathbf{Z}_i = \frac{C_2}{C_2 + E_i} \quad . \tag{2b}$$

If we now define the ratio of enzyme activities, e, of mutant and wild-type homozygotes as

$$e = \frac{E_{MM}}{E_{WW}} , \tag{4}$$

the enzyme activity of the heterozygote, as the mean of the two activities, will be

$$E_{WM} = E_{WW}\left(\frac{1+e}{2}\right) . \tag{5}$$

Substitutions of (4) and (5) into (1b) gives the respective fluxes for the three genotypes, and using (2b) and definition (3a) we obtain

$$\mathbf{D} = \frac{\mathbf{Z}(1-e)+e}{\mathbf{Z}(1-e)+e+1} . \tag{6}$$

The index is therefore seen to be dependent on the infinitesimal change represented by the sensitivity coefficient at the wild-type level, as well as on the finite change in enzyme activity represented by the ratio e.

The magnitude of the index: dominance expectations

Three extreme cases can be considered. (i) If there is a complete or almost complete elimination of enzyme activity in the mutant, $e \ll 1$, then as $e \to 0$,

$$\mathbf{D} \to \frac{\mathbf{Z}}{\mathbf{Z}+1} ,$$

and with values of $Z \ll 1$ (expected average)

$$\mathbf{D} \approx \mathbf{Z} \approx 0 .$$

This means that, for a large class of mutations, the mutant phenotype will show almost complete recessivity (*cf.* Figure 6a) in agreement with observation. No dominance modification is involved. The near identity of heterozygote and wild type is the automatic outcome of the pathway kinetics when dealing with most detectable mutations. The molecular basis of this recessivity is therefore seen to reside in the interactive nature of enzyme systems of which the low value of most sensitivity coefficients is a reflection. Recessivity is thus the normal expectation and does not require *ad hoc* hypotheses to explain its universal occurrence.

(ii) For small differences in enzyme activity between alleles, *i.e.*,

$$e \approx 1 ,$$

the mutant phenotype will not, in general, be seen to segregate. Such differences will nevertheless constitute a source of variability in populations if a number of such loci are involved. For this condition, equation (6) gives

$$\mathbf{D} \approx 0.5 ,$$

i.e., intermediacy of heterozygote phenotypes irrespective of the magnitude of **Z.** This is in agreement with the general observation of lack of dominance in many quantitative traits (ROBERTSON 1967) and especially when individual loci are investigated (MUKAI *et al.* 1972; CHARLESWORTH 1979). While such loci will be principally involved in any response to selection, being mainly additive with a

small dominance component, they do not account for inbreeding depression. In wild populations, we would expect alleles spanning the whole range of enzyme variation to be present. Those alleles that, when homozygous, seriously affect fitness or any of its components (see later) are likely to be very low on the enzyme scale and hence on the phenotypic scale as well (see Figure 5). Because of this large effect on fitness, their gene frequency will be kept very low by natural selection. Thus, they will be present almost exclusively as heterozygotes and, as we have seen in case (i), will be almost completely recessive. The combination of low frequency and recessivity will make their effects appear as "quantitative genes."

On inbreeding, on the other hand, the random fixation of alleles in individual lines will rapidly increase the frequency of these homozygotes and, since the effect is directional, will result in lowering of the phenotypic value. This is the familiar argument that deleterious recessive genes are responsible for inbreeding depression.

(iii) Finally, we must consider those rarer cases where the coefficient is very much higher than its expected average, *i.e.*, **Z**, say, higher than 0.3. For this, equation (6) gives

$$\mathbf{D} \rightarrow 0.5 \ ,$$

i.e., intermediacy, for all values of *e*. (*cf.* Figure 6b). In this class fall the well-known cases of heterozygotes between sets of "lower" alleles in a series. Thus, in the guinea pig (WRIGHT 1960) and the mouse (GRÜNEBERG 1952; see also Figure 1d), color mutants all show recessivity with respect to the highest (wild type) pigmented type, while among the lighter-shaded alleles (which have lower enzyme activities) the heterozygotes have intermediate values. The reduction in enzyme activity results in movement down the response curve, giving rise to increased values of **Z** and hence to the consequences on the dominance index. FISHER's explanation, namely that rarity of such heterozygotes has given little opportunity for modifier selection, is thus seen to be an unnecessary explanation.

Intermediate dominance will also be expected at those enzyme loci whose wild-type sensitivity coefficients are untypically high (*cf.* Figure 6b). From our expectation of a distribution of values, we should find an occasional coefficient in the high range. This would occur most probably in short pathways and is quite consistent with our general analysis. The occurrence of intermediate dominance has, on the other hand, been an embarrassment to FISHER's scheme, and sometimes elaborate hypotheses have been produced to account for its existence.

Instead of expressing the index in terms of enzyme activity movements represented by *e*, it is possible to formulate it in terms of the phenotypic ratio, *p*, of mutant to wild type, $p = \frac{\text{MUT}}{\text{WIL}}$.

Since $$e = \frac{p\mathbf{Z}}{p(\mathbf{Z}-1)+1} \ ,$$

it follows that $$\mathbf{D} = \frac{\mathbf{Z}}{\mathbf{Z}(1+p)-p+1} \ . \qquad (7)$$

This formulation is useful for cases where the underlying enzymatic basis of phenotypic differences is suspected, but not demonstrated, as will be discussed later in connection with fitness. Equation (7) behaves in the same way as equation (6) when e is replaced by p in the arguments.

Pools as characters

The analysis has so far dealt with the flux-enzyme relationship, which was equated with the corresponding character-gene relationship. It is, however, possible to regard the substrates, *i.e.*, the steady-state intermediate pools, as measurable characters. Thus, for example, fat can be regarded as an end product of a long and complex pathway beginning at glucose:

$$\text{glucose} \longrightarrow \text{fat} \ ,$$

or, since there are degradative steps from fat leading to other products, fat can be viewed as an intermediate:

$$\text{glucose} \longrightarrow \quad \text{fat} \longrightarrow \ .$$

Similarly, many inborn errors of metabolism in man are more likely to produce their clinical manifestation by the accumulations of intermediates rather than by the reduction in flux, which may be only slight (HARRIS 1975; BULFIELD, WHITEHOUSE and KACSER, submitted for publication).

The summation property, explained above as

$$\sum_{i=1}^{n} \mathbf{Z}_i^j = 1 \ ,$$

applies to the *flux* sensitivities with respect to alterations in the enzymes. There is, however, a parallel matrix of substrate sensitivities, **S**, relating the concentration changes of any intermediate pool to the fractional changes in any enzyme.

$$\mathbf{S}_i^j = \frac{dS_j}{dE_i} \times \frac{E_i}{S_j} \ .$$

This means that the steady state concentration of any one pool will be affected by changes in every enzyme in the system. For these substrate sensitivities, a second summation property applies (KACSER and BURNS 1979[3]; HEINRICH and RAPOPORT 1975), which states that the sum of these sensitivities equals zero:

$$\sum_{i=1}^{n} \mathbf{S}_i^j = 0 \ .$$

Enzyme changes distal to the pool have negative coefficients (decrease in enzyme increases the pool), while enzymes proximal to the pool have positive coefficients (decrease in enzyme decreases the pool). The two sets of distal and proximal coefficients balance out, and their sum equals zero. The question of the dominance relations for such pool-dependent characters therefore requires special consideration.

[3] See footnote 2.

Let us consider an intermediate pool, S_i, being affected by changes in one enzyme activity anywhere in the system. The pool concentrations for S_i corresponding to the three genotypes will be designated S_{WW}, S_{MM} and S_{WM}, respectively.

Let us divide the pathway into two moieties, one proximal, the other distal to the the pool, S_i, as shown in Figure 7. Irrespective of the number of enzymes, their nature and quantities, we would have only two equations representing the system:

$$v_p = f(E_p)\ (X_1 - S_i/K_{1i})$$

$$v_d = f(E_d)\ (S_i - X_n/K_{in})\ ,$$

where v_p and v_d are the rates for the two moieties and $f(E_p)$ and $f(E_d)$ are complex functions of the proximal and distal enzymes, respectively. At steady state, the flux through both will, of course, be equal and equal to the pathway flux, F:

$$v_p = v_d = F.$$

Let us consider a locus whose enzyme acts in the distal moiety. A change in the enzyme will alter the function $f(E_d)$, but not $f(E_p)$. Since X_1 is constant, changes in flux can be occasioned only by changes in S_i (and possible changes in other pools in the rest of the system). Considering the formulation for the flux through the proximal part, the fluxes corresponding to the three genotypes will be:

$$F_{WW} = f(E_p)\,(X_1 - S_{WW}/K_{1i}) \tag{8}$$

$$F_{WM} = f(E_p)\,(X_1 - S_{WM}/K_{1i}) \tag{9}$$

$$F_{MM} = f(E_p)\,(X_1 - S_{MM}/K_{1i})\ . \tag{10}$$

From definition (3a),

$$\mathbf{D} = \frac{F_{WW} - F_{WM}}{F_{WW} - F_{MM}}\ , \tag{3a}$$

and using 8, 9 and 10 we find:

$$\mathbf{D} = \frac{S_{WW} - S_{WM}}{S_{WW} - S_{MM}}\ . \tag{11}$$

The Dominance Index for the pool is therefore seen to be the same as for the flux with recessivity expectation for major mutants and intermediacy for minor ones. The same conclusion is reached if substitutions proximal to S_i are considered. For the linear case, then, it is equally admissable to use fluxes or pools as "characters", or any functions proportional to these systemic variables.

While flux measurements are difficult to obtain, particularly in higher organisms, pool values are much more accessible. Figure 8 shows the pool values

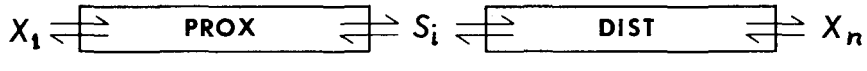

FIGURE 7

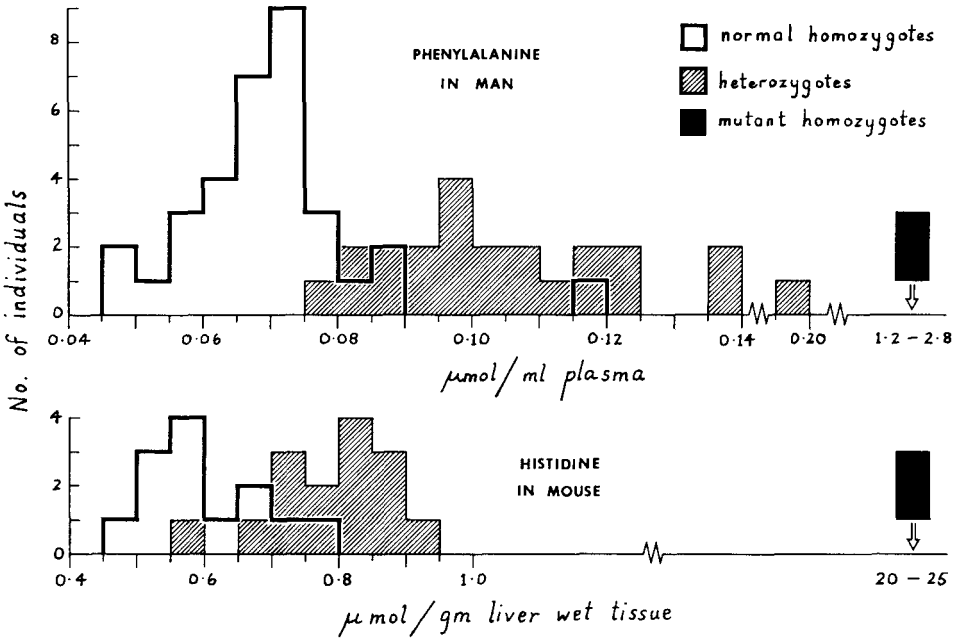

FIGURE 8.—Pool concentrations of two inborn errors of metabolism. (a) Phenylketonuria (phenylalanine hydroxylase) in man: phenylalanine concentrations in plasma (KNOX 1958). (b) Histidinemia (histidase) in the mouse: histidine concentrations in the liver (BULFIELD and KACSER (1974). In both cases the accumulations in the mutants is given as a range, while the normal and heterozygotes values are shown as histograms.

in two inborn errors in man and mouse, respectively. The mutant homozygote value is at least an order of magnitude larger than the wild-type and heterozygote values, which overlap. At the same time, it is evident that the dominance index is not exactly zero since there is a small but significant difference between the means of wild-type and heterozygote values. Whether this persists in the more complex clinical manifestations is difficult to assess in view of the problems of exact measurements at that level. The recessivity of almost all inborn errors whose enzymatic basis is established in man and mouse at the pool, flux and clinical level suggests that this is a very general conclusion. Unlike Fisher's hypothesis, which postulates modification of the deleterious heterozygote phenotype by gene action anywhere in the physiology, the present analysis predicts that the effects will be buffered at the point of the mutant's action.

The relation between enzyme concentration and pool phenotype can be further explored by making use of natural variation in enzyme activity. In murine histininemia, males and females differ in hepatic enzyme levels in both *WW* and *WM* animals (KASCER, BULFIELD and WALLACE 1973). Furthermore, histidase activity is virtually zero at birth and begins to increase after 2 to 3 days and reaches its maximum at about 14 days. Thus, by using enzyme differences due to genotype, sex and age and measuring the simultaneous histidine concentrations, the relation between the enzyme and the substrate in the pathway can be

established (Figure 9). The near identity of the *WW* and *WM* histidine values can therefore be seen to be due to the very low slope between enzyme values of 0.3 and 0.1. At lower values, the slope increases considerably, giving the high levels seen in the mutant. The "recessivity" of the mutant allele is therefore due to the strong nonlinearity that becomes important only at enzyme values between *WM* and *MM*.

It should be noted that, just as in the case of flux variations (Figure 1), pool variations show a monotonic relationship with enzyme concentration. There are no thresholds. Unlike the flux coefficients (**Z**), however, the pool coefficients (**S**), derivable from Figure 9, are negative, since we are dealing with the response

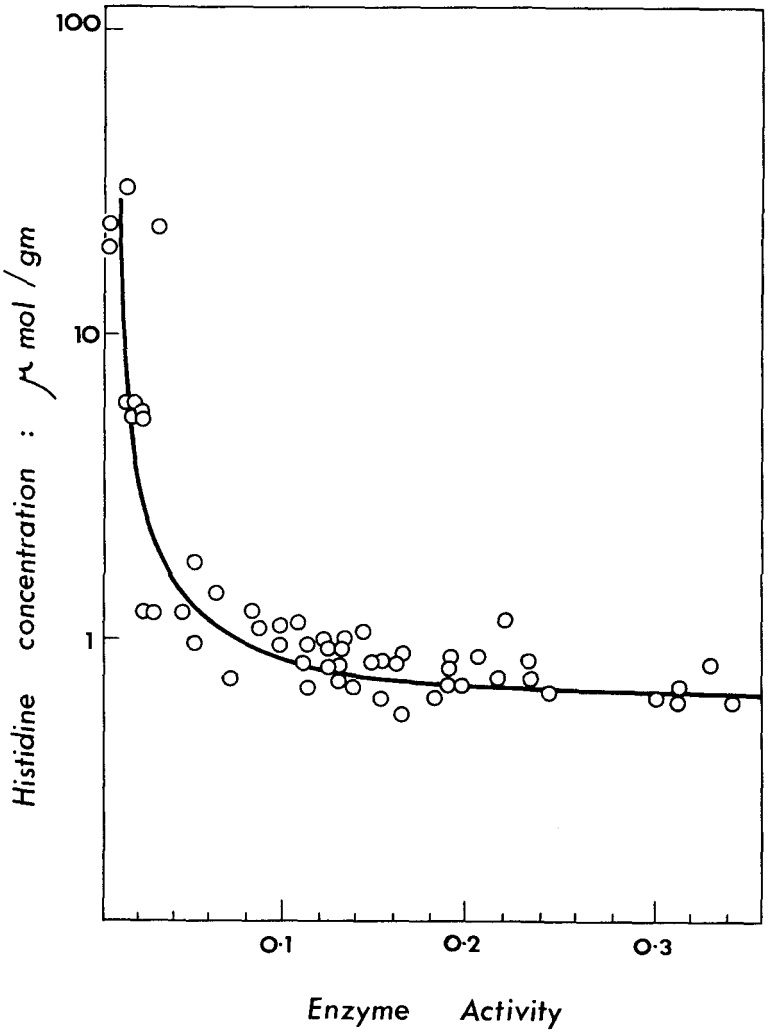

FIGURE 9.—Murine hepatic histidase and and histidine. Livers of homozygous wild-type or mutant (*his/his*) mice and heterozygotes were extracted at various ages and in both sexes. Histidase was assayed and histidine concentrations determined (KACSER, BULFIELD and WRIGHT 1979).

of the substrate proximal to the modulated enzyme. The dominance index, on the other hand, is positive and very small (*cf.* Figure 8).

Fitness

There is no inevitable identity of the effect of two alleles on the dominance index for a particular character and that for fitness (as Fisher assumed), just as two pleiotropically related characters may have different dominance indices if they involve different pathways. We have shown that the mechanism resulting in recessivity operates to buffer the metabolic consequences of enzyme variation. Consequently, the heterozygote usually differs very little from the wild type when pool sizes and fluxes are measured. Other and further effects of these minor differences, on viability for example, would, therefore generally, be expected to correlate with the metabolic differences. The approach of the fitness of the heterozygote to that of the wild type is thus seen to be a consequence, rather than a cause, of a character's recessivity.

Measuremens of viabilities in Drosophila (MUKAI *et al.* 1972; SIMMONS and CROW 1977) show that lethal alleles are almost completely recessive, while mildly deleterious alleles have intermediate heterozygote viabilities. This is contrary to the implications of Fisher's model, as was shown by CHARLESWORTH (1979). The agreement of this result with our analysis, on the other hand, gives support to the proposition that variation in even such a complex character as fitness has its origin in metabolic differences, even though in most cases the molecular basis is unknown.

We have argued that, on thermodynamic grounds, mutations to increased enzyme activity are unlikely, but that, if they occurred, the effect on the phenotype would be very small. There is, however, one class of events, more frequent than such mutations, that would result in doubling of enzyme activity. This is the well-documented class of duplications of small chromosomal regions, tandem or transposed. The massive study of LINDSLEY *et al.* (1972) in *D. melanogaster* is pertinent to this question. Using *Y*-autosome translocations, these authors were able to produce a large number of small duplications and deficiencies spanning the whole of chromosomes *2* and *3* and to study their effects in heterozygous condition. They were thus able to compare one, two and three doses of segments of the chromosome that, on average, contain tens of bands (and hence loci). Their general conclusions are well in line with our expectations. Of the 86% of the autosomal complement examined, only 4.6% failed to produce viable offspring as monosomics (deletions), while only a single duplication ($< 0.5\%$) had this effect. This is well in line with our expectations of low dose sensitivity. The finding that the effects of hyperploidy are much less pronounced than those of the corresponding hypoploidy is, of course, another prediction of our analysis (see Figure 5). Furthermore they conclude that their data imply that very few loci of *D. melanogaster* can produce dominant phenotypes by change in dosage.

It should be stressed that their experimental method results in the simultaneous change of dose of many loci (and enzymes?) so that the results constitute a more severe test than the single-locus studies discussed above. Although there

were small effects on viability of both deletions and duplications, these were attributed to the additive effect of many loci in such segments and not to the action of individual aneuploid loci. The dose insensitivity of the major portion of the whole genome is consistent with the expectations from the summation property.

Modifiers

We have seen that the notion that dominance requires an evolutionary explanation is not supported by the evidence on the behavior of enzyme systems. At the same time, it was shown that all systemic variables (fluxes, pools and coefficients) are subject to general epistatic effects. In particular, the sensitivity coefficient for one enzyme (and hence locus) is subject to alteration by changes in any of the other enzymes (loci). Such changes in the coefficient would be reflected in changes of the dominance index. It is thus seen that every enzyme is, in principle, a "modifier" of the dominance relationships of every other enzyme. The possibility of selection for dominance modification is therefore inherent in the kinetic organization of enzyme systems. Evidence that such selection can be achieved in the laboratory (FORD 1940; OHH and SHELDON 1970) is therefore fully in agreement with expectations. It is thus perfectly possible that a *particular* dominance relationship has been the subject of evolutionary modification. This, however, is a historical question and hence not subject to experimental verification by reference to present observations. Populations that have diverged from each other by isolation, followed by selection or drift, may have different sets of alleles at many loci. Crosses between such populations may show changes in dominance relations simply because they are a re-assortment of different sets of enzyme activities quite unconnected with any selection for dominance in the past. While we cannot make unambiguous assertions about the dominance at any single locus, the evidence does not support the idea that an evolutionary mechanism is responsible for the vast majority of known cases. Since recessivity of mutants is the expected outcome, natural selection has no work to do, even if it could be shown that the "wild type" is the best of all possible phenotypes.

The finding of almost universal recessivity of mutants in fungi and prokaryotes, usually determined in heterokaryons, is further evidence for our proposition. These organisms, overwhelmingly haploids, spend most of their time reproducing vegetatively and have only short and infrequent diploid sexual phases, if any at all. The difficulties in assigning realistic selection coefficients for modification in well-established diploids are compounded if such arguments are applied to haploid species.

The nature of our modifiers is also different from those postulated by Fisher. Far from being a special class of genes, acting only on the phenotype of the heterozygote, our modifiers are the ordinary "working enzymes" with metabolic functions. Wright's criticism of Fisher's view on these grounds is therefore fully justified. Inspection of equations (1) and (2) shows that changes in the value of the coefficient (and hence the index) by changes in other enzymes will be accompanied by changes in the wild-type flux. There is therefore no "pure"

dominance modification unaccompanied by other modifications of the phenotype, even if they may be minor ones. Furthermore, the phenotype of the mutant homozygote must also be affected in the same direction as that of the heterozygote with the exception of absolute null alleles, where alterations in other enzymes have, of course, no effect on the complete block of the pathway. Modification is thus seen to take its place as an expected phenomenon, inherent in the nature of enzyme networks.

Enzyme activities in heterozygotes

In the preceding treatment it was assumed that the enzyme activity of the heterozygote was the mean of the activities of the two homozygotes. While this is most frequently found experimentally, it is not a universal result. The principal points requiring special consideration are: changes in Michaelis constants, negative feedbacks and complementation.

The simplest case is the heterozygote with one of the chromosomes carrying a null allele. Unless modifying factors operate (see below), the activity will be exactly 50% of wild type. Enzyme activity will be proportional to the dose of the active allele.

If the turnover number (and hence V) of the mutant is not zero, the heterozygote will have an enzyme activity equal to the mean of the two homozygotes, provided that there are no significant differences in the Michaelis constants of the two enzymes. Even when there are differences, it will be the mean as long as both enzymes are substantially unsaturated, *i.e.*, the mean of $(V/M)_{WW}$ and $(V/M)_{MM}$. The character "enzyme activity" will show co-dominance.

If one or both enzymes have significant saturation, the general case is complex, and the effective activity could lie nearer to one or the other homozygotes. It can, however, not fall outside the range of both, *i.e.*, there is no overdominance.

More important is the effect of negative feedback. It is not uncommon that a late product of a pathway acts as a signal for repression or inhibition of earlier enzymes. If as a result of a change in flux in the heterozygote this signal concentration also changes, the effect would be to partially compensate for the altered activity. The buffering inherent in the pathway is therefore enhanced by this additional mechanism, thus re-inforcing the tendency to recessivity (see KACSER and BURNS 1973).

Intragenic complementation, most thoroughly documented in haploid fungi (*e.g.*, FINCHAM 1966), is still imperfectly understood. It can occur with enzymes composed of several identical peptide subunits (oligomeric enzymes). If two different peptide types aggregate, as would be the case in a heterozygote, the resulting enzyme activity can be less, equal to or more than the mean of that of the homomeric "parents". Since we have, as yet, no translation rules to predict this structure-function relationship, such cases must fall outside our kinetic treatment. If the heterozygote activity is higher than that of both homozygotes, we would describe this as overdominance *at the enzyme level.* For the case where both alleles are null mutants, such overdominance would, of course, result in growth—the well-known phenomenon of intragenic complementation in fungi.

On the other hand, if both alleles have substantial activities, as for "electrophoretic alleles", it should be noted that, whatever the overdominance of the heterozygote activity may be, it will lie farther towards the plateau on the response curve. In view of the low average sensitivity coefficient, such overdominance at the enzyme level is unlikely to be reflected in substantial changes in phenotype. The suggestion that complementation could be the mechanism for heterosis (*e.g.*, FINCHAM 1972) is therefore unlikely to be the general explanation.

Saturation

In this analysis we have used the device of describing in some detail the properties of a chain of monomolecular reactions catalyzed by unsaturated enzymes. A simple pathway was chosen because it makes the algebra less tedious, and monomolecularity and unsaturation lead to a set of linear differential equations with analytical solutions. This restriction is, however, not essential to the argument. We have shown (KACSER and BURNS 1973, 1979; HEINRICH and RAPPOPORT 1975) that any system containing, say, bimolecular steps, saturation of enzymes, feedbacks, divided pathways and cycles gives the same summation properties of fluxes and pools. The expectation of a low average sensitivity coefficient is, therefore, quite general.

The precise relationship to the dominance index [equations (6) and (7)], however, will be less sure because of the absence of an explicit expression for the flux in these nonlinear cases. Computer simulation has shown that the neglect of these factors only marginally affects the arguments described. Saturation can, however, affect the relative values of the sensitivity coefficients in a pathway, and external nutrient changes can reverse such values dramatically (KACSER and BURNS (1973). How commonly and under what conditions enzymes are highly saturated *in vivo* is difficult to assess. The paucity of data perhaps reflects the experimental difficulties of determining this. Simple calculations of cell volumes in conjunction with Michaelis constants, determined *in vitro*, are not an adequate basis for arriving at the true saturation values. These will depend on the *in vivo milieu*, which includes the effective kinetic space available to the enzyme in the heterogeneous compartments of the cell.

It seems, however, more likely that most enzymes operate under conditions of low saturation (CORNISH-BOWDEN 1976; FERSHT 1977; FLINT, PORTEOUS and KACSER 1980) for which the algebraic treatment presented here is a good approximation.

General conclusions

It was stated at the outset that our analysis would deal with enzyme systems, *sensu strictu*, but would also encompass quasicatalytic gene products. Our ability to draw quantitative conclusions about systemic variables (the characters) is based on the assumption that the function of the *individual* gene product can be described by an algebraic function of the type:

$$\text{Rate} \propto \text{gene product concentration } (e.g., V \propto [E]) \ .$$

It is a condition for the summation property. This will apply also to such products as hemoglobin where we deal with rates of oxygen adsorption or other similar quasicatalytic components.

The class of noncatalytic gene products raises the question of how their function should be described. In the absence of any clear evidence that they enter into some functional relationship with other processes (such as parts of membranes that act quasicatalytically), we would expect them to act co-dominantly. Until more investigations reveal the precise mechanism of their involvement, they must remain outside our framework.

It is possible that the so-called "pattern genes" fall into this class. It is likely that these control the timing of certain rate processes, as well as the tissues in which these occur (*e.g.*, ABRAHAM and DOANE 1978). Since this area is equally devoid of precise biochemical information, we can contribute little to the discussion of their dominance relationships. It is, however, evident that, in many instances, such alleles show the familiar recessivity. From this, we can guess that they may turn out to be involved in "ordinary" rate-controlled processes.

The analysis presented here adopts substantially the same approach as that of WRIGHT (1934). Although his algebraic treatment would not be accepted today in view of what is now known about the kinetics of enzyme action, the essential point is the realization that sequences of chemical transformations tend to buffer the output against variation of one of them. The explanation offered by WRIGHT, namely, that under such conditions the rate is "substrate limited" is, however, a misinterpretation of the interdependence of substrates and enzymes in pathways (KACSER and BURNS 1979). This does not detract from the soundness of the general conclusion, but there is one critical difference between WRIGHT's conclusions and ours. Although he correctly suggested a hyperbolic relationship between enzyme and flux, his treatment did not explain why the majority of enzymes should lie on the plateau of the relationship. Having rejected Fisher's hypothesis of modifiers, he came down in favor of HALDANE's (1930) and PLUNKET's (1933) selection for "safety factors", that is, for increased activity of the wild type allele at the locus. The summation property eliminates the necessity of postulating selection to bring enzymes into such a position.

The observation of almost universal recessivity of Mendelian mutants, far from constituting a problem requiring an evolutionary explanation, is seen to be a necessary consequence of the interactions inherent in the kinetic organization of enzyme systems. In fact, if mutant recessivity were not general, it would throw considerable doubt on the whole of enzymology and the study of intermediary metabolism. Dominance modification and general epistasis, in turn, are seen to arise from the same interactive properties of enzyme systems. The "modifier loci" are simply all other loci controlling enzymes in the pathways, rather than loci whose sole function, in Fisher's model, is to affect the expression of the heterozygote phenotype. This eliminates a vast class of genes required by Fisher's scheme and removes the conflict with realistic estimates of the number of loci in organisms.

The evidence presented above, eliminating the necessity to invoke evolution as the origin of the phenomenon, has nevertheless evolutionary implications. In the first place, it is relevant to the Neutralist-Selectionist controversy suggesting that most variation has small or negligible effects. Second, it has implications for the mechanism of selection insofar as the sensitivity coefficient is closely related to the selection coefficient. These aspects will be discussed in another publication.

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