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Metabolic Regulation: A Control Analytic Perspective[1]

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A possible basis for a quantitative theory of metabolic regulation is outlined. Regulation is defined here as the alteration of reaction properties to augment or counteract the mass-action trend in a network reactions. In living systems the enzymes that catalyze these reactions are the "handles" through which such alteration is effected. It is shown how the elasticity coefficients of an enzyme-catalyzed reaction with respect to substrates and products are the sum of a mass-action term and a regulatory kinetic term; these coefficients therefore distinguish between mass-action effects and regulatory effects and are recognized as the key to quantifying regulation. As elasticity coefficients are also basic ingredients of metabolic control analysis, it is possible to relate regulation to such concepts as control, signalling, stability, and homeostasis. The need for care in the choice of relative or absolute changes when considering questions of metabolic regulation is stressed. Although the concepts are illustrated in terms of a simple coupled reaction system, they apply equally to more complex systems. When such systems are divided into reaction blocks, co-response coefficients can be used to measure the elasticities of these blocks.

KEY WORDS: Metabolic regulation; metabolic control analysis; signals; homeostasis; co-response analysis.

INTRODUCTION

This is a review of some established concepts of metabolic regulation. It is also a fresh view in the sense that these concepts are examined through the spectacles of metabolic control analysis, the quantitative framework for describing metabolic behavior originated by Kacser and Burns (1973) and Heinrich and Rapoport (1974), and refined by numerous other workers [see Fell (1992) and Cornish-Bowden (1995) for recent reviews]. I make no attempt to review the extensive literature of either of the two fields. The reference list is highly selective and reflects those publications that were most influential in laying the foundation for my own understanding of metabolic regulation. The main inspiration for the definition of metabolic regulation formulated here came from the work of Reich and Sel'kov (1981); to a large extent, this review is a summary of their thinking. The other major source of inspiration was Atkinson (1977), a landmark in the development of our understanding of metabolic regulation.

I shall first argue for a broad definition of metabolic regulation as a set of strategies to either augment or counteract the intrinsic mass-action trend in open reaction networks. Then I illustrate how the elasticity coefficient (which biochemists will recognize as the familiar kinetic order of a reaction) is the sum of mass-action and regulatory effects of enzymes and signals. Elasticity is also the basic ingredient of metabolic control analysis and thus forms the link between the concepts of control, regulation, and stability. The last section shows how the overall elasticities of reaction blocks can be measured in terms of co-response coefficients, thus allowing the type of analysis described here to be extended to systems of arbitrary complexity.

[1] I dedicate this paper to Henrik Kacser, co-founder of and guiding light in the field of metabolic control analysis. His recent death leaves us bereft of a fount of wisdom and kindness, but his work remains as a monument along the path of our search for an understanding of metabolic behavior.

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METABOLIC REGULATION: AUGMENTING OR COUNTERACTING MASS-ACTION

A metabolic system is an open reaction network of enzyme-catalyzed reactions linked by common intermediates; the network spreads across membrane-bounded compartments so that transport systems are included. The idea that in living systems such networks are "regulated" has become so entrenched that it is surprising that a general and useful definition of regulation is so hard to come by. Usually, the question "What do you mean by regulation?" is answered in terms of some "regulatory mechanism(s)" central to the respondent's sphere of interest, be it allosteric end-product inhibition, covalent modification cycles, induction and repression of enzyme synthesis, etc. It seems that the majority of biochemists and molecular biologists see regulation as a collection of phenomena, but exactly what criterion binds these phenomena into a distinct class is not clear. Nevertheless, if such a criterion exists it must be rather broad to accommodate mechanistically disparate phenomena.

What we study as metabolic systems are reaction networks of which the intrinsic properties have been altered and moulded by evolution to fulfill specific functions essential to the processes of life. As so eloquently argued by Atkinson (1977), our understanding of metabolic systems must be, as is an engineer's understanding of technological systems, in terms of a product designed by evolution, the "Blind Watchmaker" (Dawkins, 1986). As a corollary to this, when our understanding is deep enough, we should be able to function as effective "metabolic designers," even if only in theory and not in practice (although with modern recombinant DNA techniques of molecular biology we may already have an adequate metabolic designer's toolbox). Nevertheless, even without evolution's tinkering (Jacob, 1983), all open nonequilibrium reaction networks spontaneously organize themselves into some state such as the steady state (Nicolis and Prigogine, 1977; Kauffman, 1993). This is one of Reich and Sel'kov's (1981) main messages when they argue that much of metabolic behavior is "a spontaneous coordination and expediency of motion like that of droplets in a stream," instead of the sole result of "collections of interactive regulatory feedback systems" without which all will be chaos. This is not to deny the importance of these regulatory mechanisms for tuning or for creating novel responses, but to stress the importance of the evolved stoichiometry (Atkinson, 1977) of the metabolic network.

To understand metabolism as a designed system, we must study the properties of the raw materials and processes, and how these properties have been moulded via enzymes to fulfill a specific function. The intrinsic processes are of course the stoichiometric chemical reactions found on the metabolic map. Why these specific reactions have been selected for life processes is a complicated question and one that will not be addressed here. It has, however, been the subject of a series of recent studies (Melendez-Hevia *et al.*, 1994). I shall regard the revealed metabolic structure as a given.

Taking open uncatalyzed reaction networks as a starting point we see that mass-action is the main driving force for self-organization. This is not to say that the behavior of these systems is necessarily simple; for instance, nonenzymic networks can contain autocatalytic cycles that cause oscillations or deterministic chaos, multiple steady states, trigger behavior. However, by identifying mass-action as the basic driving force, we can broadly define metabolic regulation as *the alteration of reaction properties to augment or counteract the mass-action trend in a network of reactions.* On the basis of our current knowledge, living systems have achieved this on two levels: *first,* by evolving (i) enzymes with high catalytic and binding specificity, (ii) mechanisms for altering enzyme activity, concentration and binding properties, and (iii) allosteric and other signals; *second,* by evolving special stoichiometric network structures such as moiety-conserved and autocatalytic cycles. Without the evolution of enzymes this moulding process would have been impossible: on the one hand, enzymes lift the metabolic network from the underlying network of thermodynamically feasible reactions onto a different timescale; on the other, enzymes add controllability and regulability to metabolism—without enzymes evolution would have had no "handles" to work with. This review focuses on enzymes and signals; for a discussion of stoichiometric aspects of regulation see Reich and Sel'kov (1981) and Atkinson (1977).

The prototype system that will be used as a basis for further discussion is extremely simple, but nevertheless important as the simplest example of one of the two ways in which reactions are coupled in metabolic systems (Fig. 1). The moiety-conserved cycle (Fig. 1B), being the principle component in Reich and Sel'kov's (1981) treatment, has been amply discussed; I shall consider the chain (Fig. 1A).

ELASTICITY IS THE SUM OF MASS-ACTION AND REGULATION

In Fig. 1A substrate S is converted to a product P by two reactions that are coupled by a common intermediate X. Both reactions are in principle reversible; the equilibrium constant K_{12} of the sequence is the product of the two individual equilibrium constants $K_{12} = K_1K_2$. If external influences keep S and P in a nonequilibrium ratio, the system is *open* and attains a steady state in which the concentration x of X does not change with time (it attains a steady-state value $\bar{x}$) and in which there is a constant flux of matter J through the system; the value of the flux is equal to the rates through the individual reactions, $J = v_1 = v_2$, and the direction of flux is from S to P when $\Gamma_{12}/K_{12} < 1$, where Γ_{12} is the mass-action ratio p/s, where p and s are the concentrations of P and S.

The dependence of each reaction rate on the concentration x can be visualized as a *rate characteristic* (Chance, 1961; Higgins, 1967; Reich and Sel'kov, 1981). A graph containing the rate characteristics of both reactions is an informative way of visualizing the steady state and the behavior of the system around the steady state. The solid lines in Fig. 2A are the rate characteristics of the two reactions in Fig. 1A when catalyzed by enzymes obeying simple Michaelis–Menten kinetics (kinetic details are given in the legend). The steady state is the point at which the rate curves intersect. A number of features are immediately apparent. For this constellation of parameters (s, p, kinetic constants) there is only one possible steady state (unique steady state); this steady state is stable, both dynamically (stable to perturbations in $\bar{x}$) and structurally (stable to perturbations in any of the parameters). That the system is dynamically stable is easy to see. If x is increased from its steady-state value, its rate of consumption (v_2) is greater that its rate of synthesis (v_1) so that x is driven back to the original

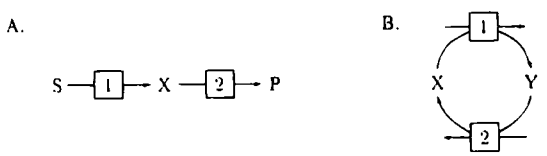

Fig. 1. The two ways in which reactions are coupled. (A) Reaction 2 takes the linking metabolite, X, on to form P, a new substance. (B) Reaction 2 reverses the action of reaction 1 as far as the linking metabolite is concerned. Whereas in (A) the concentration of X is in principle free to assume any value, the sum of X and Y concentrations in (B) is constant (a moiety-conserved cycle, e.g., the NAD^+/NADH cycle).

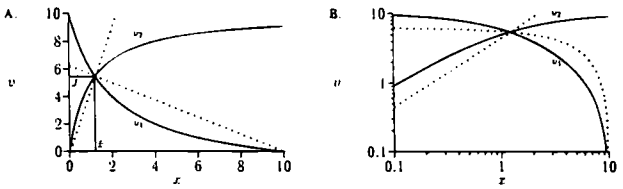

Fig. 2. Rate characteristics of the reactions in Fig. 1A with respect to the linking metabolite X. (A) Unscaled rate characteristics; (B) log-log form of the rate characteristics. The point of intersection is the steady state (J, $\bar{x}$). The solid lines were calculated from the rate equations $v_1 = V_{1f}/K_{1s}(s - x/K_1)/(1 + s/K_{1s} + x/K_{1x})$ and $v_2 = V_{2x}x/(K_{2x} + x)$. Parameter settings: $s = 1$, $V_{1f} = 20$, $K_1 = 10$, $K_{1s} = 1$, $K_{1x} = 1$, $V_2 = 10$, $K_{2x} = 1$. The dotted lines were calculated from simple mass-action rate equations: $v_1 = k_1(s - x/K_1)$ and $v_2 = k_2x$. Parameter settings: $s = 1$, $k_1 = 6.23$, $K_1 = 10$, $k_2 = 4.524$. As discussed later in the text, the differences between the solid and the dotted curves express how the saturation terms in the kinetic equations of the catalyzed system allow a greater range of regulatory behavior than would be possible in an uncatalyzed system.

steady state. If x is decreased, $v_1 > v_2$ and again the steady state is re-established. One can even get an idea of the magnitude of dynamic stability from the value of $\partial(v_1 - v_2)/\partial x$, the difference between the slopes at the steady state; the more negative, the faster the system returns to the steady state and the more stable it is. Structural stability means that the new steady state that is approached after perturbation in any of the parameters is very close to the original one [see Reich and Sel'kov (1981) and Westerhoff and van Dam (1987) for discussions of stability: the first from a classical dynamical systems point of view, the second from a nonequilibrium thermodynamic point of view that incorporates metabolic control analysis].

The response of the system to perturbations of any kind must depend on both the intrinsic mass-action trend and the kinetic properties of the enzymes; our purpose is to distinguish between the two. First we must find a quantitative measure of mass-action trend. For the general catalyzed reaction S $\rightleftharpoons$ X any physically realizable rate equation must be the product of a rate constant k, a saturation term Θ, and a thermodynamic term (Reich and Sel'kov, 1981):

$$v = k \cdot \Theta \cdot \left(s - \frac{x}{K_{eq}}\right) \qquad (1)$$

The saturation term Θ is a function of kinetic constants and the concentrations of substrates (s), products (x), effectors, and enzyme. For a simple uncatalyzed reaction, Θ would of course be 1. The following rate equation for a reversible Michaelis–Menten catal-

ysis of S ⇌ X illustrates typical expressions for k and Θ:

$$v = \frac{V_f}{K_s} \cdot \frac{1}{1 + s/K_s + x/K_x} \cdot \left(s - \frac{x}{K_{eq}}\right) \qquad (2)$$

where V_f is the limiting forward rate, and K_s and K_x the Michaelis constants for substrate and product.

The *kinetic order* of a reaction with respect to one of its substrates, products, or effectors is defined as $\partial \ln v / \partial \ln c$, where c is the appropriate concentration. This reaction property distinguishes between the contribution of mass-action and the contribution of kinetic properties in an additive, rather than multiplicative, way. In metabolic control analysis, this measure is symbolized by ϵ_c^v and called the *elasticity coefficient*, or just *elasticity* (Kacser and Burns, 1973). A simple way of obtaining analytical expressions for elasticity coefficients is given in the Appendix. If this procedure is applied to Eq. (2) the following elasticity expressions are obtained:

$$\epsilon_s^v = \frac{\partial \ln v}{\partial \ln s} = \frac{-s/K_s}{1 + s/K_s + x/K_x} + \frac{1}{1 - \Gamma/K_{eq}} \qquad (3)$$

$$\epsilon_x^v = \frac{\partial \ln v}{\partial \ln x} = \frac{-x/K_x}{1 + s/K_s + x/K_x} - \frac{\Gamma/K_{eq}}{1 - \Gamma/K_{eq}} \qquad (4)$$

where $\Gamma = x/s$, the mass-action ratio under the prevailing conditions. The second right-hand terms are *thermodynamic* as they depend only on Γ/K_{eq} [remember that $\Delta G = RT \ln (\Gamma/K_{eq})$]; they quantify the mass-action contribution to the sensitivity of reaction rate towards changes in a substrate or product. The denominator $(1 - \Gamma/K_{eq})$ gives the direction of the reaction and its distance from equilibrium: > 0, net reaction forward; < 0, net reaction backward; $= 0$, equilibrium; $= 1$, unidirectional. The first right-hand terms are *kinetic* in that they depend on reaction mechanism; they augment or counteract the mass-action term and are therefore the key to quantifying regulation. If the reaction were uncatalyzed its rate would be governed by mass-action only and the reaction would be unregulated; the kinetic terms would vanish, leaving only the thermodynamic terms in the elasticity expressions. Note that this applies to a single reaction; uncatalyzed, purely mass-action driven reaction *networks* can be regulated by stoichiometric structures such as moiety-conserved and autocatalytic cycles.

For this particular rate equation, the first terms are clearly saturation terms that vary between 0 and 1. Near equilibrium the second mass-action terms approach $+\infty$ and $-\infty$ respectively and dominate the elasticity. Far from equilibrium the mass-action terms approach 1 and 0 respectively so that the degree of saturation has a significant effect on the elasticity; *the further from equilibrium, the greater the potential for regulation.* The elasticity coefficients can be visualized as the slopes of the tangents to the rate-curves at the steady-state point when the rate characteristics are plotted in log-log space (solid curves in Fig. 2B). The slopes of the dotted curves quantify the mass-action contributions to the elasticities at the steady state; the regulatory terms account for the differences between the slopes of the solid and dotted curves.

In the system analyzed in Fig. 2 the only variable concentration is x, and it must be the elasticities of v_1 and v_2 with respect to x at the steady state that determine the response of the system to perturbations at fixed s and p. It is clear that enzyme binding of product augments the product mass-action effect in reaction 1 (making ϵ_x^{v1} more negative), and therefore increases the sensitivity of v_1 to changes in x. Binding of substrate, on the other hand, counteracts the substrate mass-action effect in reaction 2 (making ϵ_x^{v2} less positive), and therefore desensitizes v_2 to changes in x. In a sense, the desensitization to substrate and sensitization to product is a price that is paid when an enzyme is added to a reaction; nevertheless, the magnitude of both effects, even their sign, can be changed, as we shall see in later sections.

SUBSTRATE AND PRODUCT BINDING CAN BE REGULATORY

We have seen that the potential for regulation by substrates and products becomes greater the further the reaction is from equilibrium. Although all reactions are in principle reversible, some are unidirectional under virtually all conditions (when K_{eq} is extremely large so that even when $\Gamma \gg 1$, Γ/K_{eq} still approaches zero; this is called an irreversible reaction) or only under certain conditions (when $\Gamma \ll K_{eq}$). Although enzymes cannot alter the position of equilibrium, their catalytic and binding properties can be altered within the constraints of the Haldane relationship, which for the reversible Michaelis–Menten catalysis of S ⇌ X is

$$K_{eq} = \frac{k_f}{k_r} \frac{K_x}{K_s} \qquad (5)$$

where k_f and k_r are the forward and reverse turnover

numbers, and K_s and K_x the Michaelis constants for substrate and product.

One example serves to show how changes in enzyme properties affect the regulatory roles of substrates or products. Figure 3 depicts the effects of changes in K_x of the enzyme catalyzing reaction 1 in Fig. 1A. Enzyme 1 is functioning at a substrate concentration equal to K_s. The reference curve *a* is calculated for $K_s = K_x$, i.e., equal binding affinities for substrate and product; this situation is compared to one in which both k_r and K_x are increased by a factor of 10 (curve b) and one in which they are decreased by a factor of 10 (curve c). As K_x is increased (curve b), the regulatory saturation term in the elasticity coefficient becomes smaller at all values of x so that the elasticity is determined mostly by mass-action (curve d shows the theoretical limit where $K_x = \infty$ so that only mass-action effects obtain). When K_x is decreased (curve c), the regulatory term dominates at low values of x where mass-action is negligible (see the log-log plot in Fig. 3B where around $x = 1$ the slope approaches -1, the value of the regulatory term at a high degree of saturation by X); only at higher x does mass-action begin to take over the response.

The effect of such changes on the steady state depend on the properties of the enzyme that catalyzes the reaction that consumes X. We examine the situation where enzyme 2 is a Michaelis–Menten enzyme (curve e). It is clear that a decrease in both k_r and K_x results in a lower J and $\bar{x}$, while an increase in both k_r and K_x has the opposite effect.

This rather limited analysis does indicate that, although the regulatory properties of substrates and products can be altered by changes in binding affinities, other more sophisticated strategies are needed to enrich metabolic behavior. However, before discussing some of these, we need to clarify the question of control and controllability, concepts that we regard as distinct from regulation and regulability.

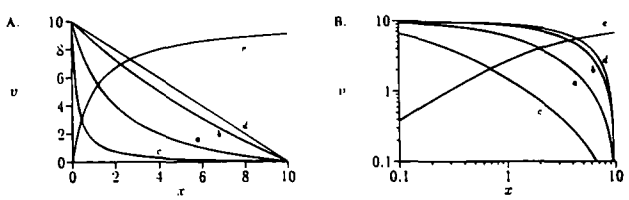

Fig. 3. Rate characteristics of the reactions in Fig. 1A with respect to the linking metabolite X. (A) Unscaled rate characteristics; (B) log-log form of the rate characteristics. The rate equation and parameters for v_1 (curves a, b, c) are the same as that in Fig. 2, except for (a) $V_{1r} = 2$, $K_{1x} = 1$; (b) $V_{1r} = 20$, $K_{1x} = 10$; (c) $V_{1r} = 0.2$, $K_{1x} = 0.1$; (d) $v_1 = 10 \cdot (1 - x/10)$. The rate equation for v_2 (curve e) is $v_2 = 10x/(1 + x)$.

ENZYMES ADD CONTROLLABILITY TO A REACTION NETWORK

Steady-state control, as used in metabolic control analysis, is a concept that can in principle be applied to any reaction network, catalyzed or uncatalyzed. What control is can perhaps be most easily understood as follows: Consider a reaction network in a steady state determined by a set of parameters, one of which specifically and directly affects the rate of reaction *i*. What happens when this parameter P_j is perturbed? The immediate effect is a change in the local reaction rate v_i to a degree given by the elasticity coefficient $\epsilon_{P_j}^{v_i}$. This local rate change is then propagated through the system and eventually leads to a new steady state. The overall response to the change in P_j is therefore a combination of a local response and a systemic response. For any steady-state variable $\bar{y}$ (flux or concentration) we could write

$$\frac{\partial \ln \bar{y}}{\partial \ln P_j} = \frac{\partial \ln \bar{y}}{\partial \ln v_i} \cdot \frac{\partial \ln v_i}{\partial \ln P_j} \tag{6}$$

Each of these partial derivatives has a distinct operational meaning and is given its own symbol:

$$R_{P_j}^{\bar{y}} = C_{v_i}^{\bar{y}} \epsilon_{P_j}^{v_i} \tag{7}$$

where $R_{P_j}^{\bar{y}}$ is a *response coefficient* quantifying the overall effect of a change in P_j on $\bar{y}$, and $C_{v_i}^{\bar{y}}$ is a *control coefficient* quantifying the effect of a change in v_i on $\bar{y}$. This equation embodies the *combined response property* (Kacser and Burns, 1973) and is one of the pillars on which metabolic control analysis is built.

From Eq. (6) it is clear that a control coefficient is defined in a parameter-independent way (Kacser and Burns, 1973; Heinrich *et al.*, 1977; Schuster and Heinrich, 1992). In other words, any reaction step has control coefficients, whether there is a specific parameter that can be tweaked or not. However, the measurement or modification of control coefficients is difficult, if not impossible, if reactions are not equipped with enzyme "control knobs" that can be adjusted experimentally or by evolution. It is in this sense that individual reactions in enzyme-catalyzed networks are controllable, while those in uncatalyzed networks are not. The enzyme "control knob" can be turned in a number of ways: altering enzyme concentration, adding specific inhibitors or activators, altering binding

affinities, to mention a few. As the elasticity of a reaction to its own enzyme is usually 1 (enzyme concentration being a multiplier in the rate equation), any response coefficient with respect to enzyme concentration is numerically equal to the corresponding control coefficient of that step, i.e., $R^{y}_{e_i} = C^{y}_{v_i}$ [this is the basis of the widely-used definition of a control coefficient in terms of enzyme concentration instead of local rate (Burns *et al.*, 1985)].

From the above, there is still no logical necessity that any step should have nonzero control coefficients. That this must be so for at least some of the steps follows from the other theorems of metabolic control analysis, the so-called summation and connectivity properties [reviewed in Fell (1992)]. Important for an understanding of how control and regulation fit together, is that, when combined, these properties allow the expression of control coefficients in terms of elasticity coefficients. As an example, the J and $\bar{x}$-control coefficients of the two enzymes in Fig. 1A can be expressed as

$$C^{J}_{1} = \frac{\epsilon^{v2}_{x}}{\epsilon^{v2}_{x} - \epsilon^{v1}_{x}}, \qquad C^{J}_{2} = \frac{-\epsilon^{v1}_{x}}{\epsilon^{v2}_{x} - \epsilon^{v1}_{x}},$$

$$C^{\bar{x}}_{1} = \frac{1}{\epsilon^{v2}_{x} - \epsilon^{v1}_{x}}, \qquad C^{\bar{x}}_{2} = \frac{-1}{\epsilon^{v2}_{x} - \epsilon^{v1}_{x}} \qquad (8)$$

These equations also illustrate the summation properties (Kacser and Burns, 1973; Chen and Westerhoff, 1986): the control coefficients of all steps with respect to a flux sum to one (i.e., flux-control is shared between the steps in a system); the control coefficients of all steps with respect to a particular concentration sum to zero (i.e., whereas some steps increase a metabolite concentration, others must decrease it).

We can now begin to appreciate that although control and regulation are distinct concepts, they are inextricably interwoven in any understanding of metabolic behavior. Control can be expressed in terms of elasticity; regulation forms part of elasticity.

Up to now arguments have been couched in terms of relative changes ($\partial \ln \bar{y} = \partial \bar{y}/\bar{y}$), but is there any logical reason for preferring relative changes to absolute changes? The usual answer is that whereas the value of an absolute change depends on the dimensions used, a relative change is dimensionless (Kacser and Burns, 1973). True enough, but why not use a standard state to get rid of dimensions, instead of the prevailing state, which varies? Let us attempt to find a more satisfying answer by looking at a specific example: how a change in the enzyme concentration of reaction 2 affects the steady-state behavior of the system described in Fig. 2.

Figure 4A depicts two steady states: one at $e_2 = 2$ and one at $e_2 = 10$. The dotted curves around these two v_2-characteristics were calculated after increasing and decreasing the two e_2-values by 1 (i.e., equal absolute changes). The boxes show the magnitudes of the concomitant changes in J and $\bar{x}$. The problem with using absolute changes is apparent in Fig. 4A. The obvious difference in the size of the boxes is possibly a reflection of the relative insensitivity of the steady state at $e_2 = 10$ to changes in e_2, but in part it must also be a reflection of the fact that increasing $e_2 = 10$ to 11 represents a 1.1-fold change, while increasing $e_2 = 2$ to 3 represents a 1.5-fold change. These two changes, although equal in absolute value, cannot be regarded as having the "same" effect on the enzyme concentration. Only when the same fold-change in e_2 is considered at both steady states (Fig. 4B), can one be sure that the effects on the steady state are not partly due to a difference in scale. Figure 4B shows

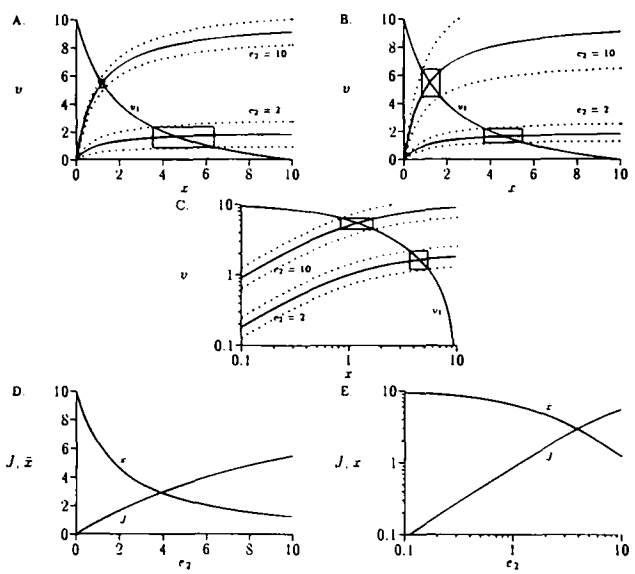

Fig. 4. Rate characteristics of the reactions in Fig. 1A with respect to the linking metabolite X (A, B, and C) and parameter portraits of steady-state variables with respect to variation in e_2 (C and D). (A, B) Unscaled rate characteristics; (C) log-log form of the rate characteristics. The rate equation and parameters for v_1 and v_2 are the same as that in Fig. 2; v_2 was calculated at $e_2 = 2$ and $e_2 = 10$ (solid lines). In (A) the dotted lines were calculated for a decrease and increase of 1.0 in the two e_2-values. In (B) and (C) the dotted lines were calculated for a 1.4-fold decrease and increase of the two e_2-values. (D) A portrait of the steady-state response in J and $\bar{x}$ when parameter e_2 is varied continuously, as calculated with the metabolic simulation program MetaModel (Cornish-Bowden and Hofmeyr, 1991). (E) Log-log form of the parameter portrait in (D).

that a 1.4-fold change around $e_2 = 10$ has a greater effect on the flux and a smaller effect on $\bar{x}$ than a 1.4-fold change around $e_2 = 2$. However, the above argument that the context in which any change is observed must be taken into account holds equally for these changes in J and $\bar{x}$. This point is made by Cornish-Bowden (1981) in relation to the need for considering pH changes instead of changes in the hydrogen-ion concentration: "For example, in the mammalian stomach at a hydrogen-ion concentration of 10^{-3} mol liter^{-1} an increase of 10^{-5} mol liter^{-1} would be virtually imperceptible and would be expected to have only slight consequences; on the other hand, the same increase in a cell at a hydrogen-ion concentration of 10^{-7} mol liter^{-1} might well be devastating." Here the comparison is between 1.01-fold and 100-fold changes, whereas if equal pH changes had been considered the fold-changes would have been equal. The crucial point is that in most circumstances we want to compare *changes* in state, rather than the states themselves, and a particular change can have very different effects in different contexts. Scaling the changes to a standard state does not get rid of this problem; standard states do not eliminate units, they just sweep them under the carpet.

The more appropriate representation of the rate characteristics is therefore in log-log space (Fig. 4C); the dotted curves were calculated for a 1.4-fold decrease and increase in each of the two concentrations of E_2 (i.e., equal relative changes). From this graph it is clear that the flux is more sensitive to changes in e_2 at 2 than at 10; just the opposite holds for $\bar{x}$, which is less sensitive at the lower e_2 (note that consideration of Fig. 4B led to the opposite conclusion). As before, the slopes of the log-rate characteristics at the two steady states are equal to the elasticities or the two rates with respect to x.

A complete picture of the steady-state response to changes in e_2 is given in a *parameter portrait* (Fig. 4D). For the same reasons as above, the log-log representation of this graph (Fig. 4E) is the one to consider. The fact that e_2 is now varied continuously does not change the argument; we are still comparing different steady states, albeit series that lie next to each other. At any point, the slope of the curve gives the control coefficient. For values of e_2 up to about 2, reaction 2 has a flux-control coefficient of 1; at higher e_2 the flux control of reaction 2 decreases (the summation property of flux-control coefficients requires that here C_1^J starts to increase from 0).

With regard to $\bar{x}$, the difference between the unscaled and the scaled curves is more striking than with regard to the flux. If one assumes the definition of homeostasis given by Reich and Sel'kov (1981): "*Homeostasis* means that the change in level of a stationary metabolite is very low after parameter perturbation," one would not deduce from Fig. 4D that $\bar{x}$ is homeostatically maintained, except perhaps at high values of e_2. However, the above argument compels us to consider relative rather than absolute changes. From Fig. 4E it is clear that the relative change in $\bar{x}$ is small at low e_2, while at higher e_2 the relative change in $\bar{x}$ becomes inversely proportional to the relative change in e_2 ($C_2^{\bar{x}} = -1$). If a definition of homeostasis in terms of small relative change is accepted, then it is clear that $\bar{x}$ is homeostatically maintained at low e_2. However, at low e_2 reaction 1 is near equilibrium, so that the observed homeostasis in $\bar{x}$ is *thermodynamic* rather than kinetic (the elasticity is dominated by mass-action), with the concomitant disadvantage that near-equilibrium concentrations can be disastrously high for reactions or reaction blocks with large equilibrium constants. One of the major regulatory strategies is to maintain kinetic homeostasis in key metabolites that are far from equilibrium. In the next section we shall discuss the role of signals in this context, but here we note that stoichiometric structures such as moiety-conserved cycles show built-in kinetic homeostasis without signals [see Reich and Sel'kov (1981) for a detailed discussion of this phenomenon].

KINETIC METABOLIC SIGNALS AUGMENT OR COUNTERACT MASS-ACTION

Metabolic signals influence the rate of a reaction by modifying either the enzyme concentration (adaptive signals) or the kinetic properties of existing enzyme molecules (kinetic signals) (Reich and Sel'kov, 1981). The subject of metabolic signals is a vast one, and the reader is referred to Reich and Sel'kov (1981) for a detailed discussion. The aim here is to show how kinetic signals augment or counteract mass-action through elasticity. We shall again use rate characteristics for explanatory purposes.

Signals are mediated by metabolites that influence reaction rate by binding to the enzyme. Such metabolites can be substrates, products, or effectors. For substrates and products the difference between reaction partner influence and signal influence is not always clear and depends on the catalytic mechanism;

effectors have only signal influence. Neve.theless, for many mechanisms, the expression for elasticity demarcates clearly between mass-action, reaction partner influence, and signal influence. The Monod–Wyman–Changeux (MWC) model for cooperative and allosteric interactions (Monod *et al.*, 1965) illustrates this. For an MWC-enzyme catalyzing the irreversible reaction $S \rightarrow X$ that is allosterically activated by A and inhibited by I, the rate equation can be written in the following form (Popova and Sel'kov, 1975), assuming that the substrate does not bind to the *T*-form of the enzyme:

$$v = V \cdot \sigma \cdot \frac{1}{1+\sigma} \cdot \frac{1}{1+q},$$

$$\text{where } q = L_0 \cdot \frac{(1+\iota)^n}{(1+\sigma)^n(1+\alpha)^n} \qquad (9)$$

L_0 is the equilibrium constant for the $R_0 \rightleftharpoons T_0$ transition in the absence of ligands, n the number of subunits, $\sigma = s/K_R$, $\iota = i/K_I$, and $\alpha = a/K_A$. K_R, K_I, and K_A are the intrinsic dissociation constants for single subunit complexes with substrate, inhibitor, and activator respectively.

The elasticity coefficients with respect to S, I, and A can be obtained from Eq. (9) as before:

$$\epsilon_s^v = 1 - \frac{\sigma}{1+\sigma} + \frac{n\sigma}{1+\sigma} \cdot \frac{q}{1+q},$$

$$\epsilon_i^v = \frac{-n\iota}{1+\iota} \cdot \frac{q}{1+q},$$

$$\epsilon_a^v = \frac{n\alpha}{1+\alpha} \cdot \frac{q}{1+q} \qquad (10)$$

In the expression for ϵ_s^v the first term of 1 quantifies the mass-action contribution, while the other terms are regulatory: the second term describes single-site substrate binding in the absence of signals (reaction partner influence), the third term the effect of cooperative binding (signal influence). Figure 5A shows how, in the absence of inhibitor or activator, the elasticity with respect to substrate varies with σ and how the mass-action, reaction partner, and signal terms contribute to the elasticity. When $\sigma \ll 1$, the elasticity approaches a value of 1 (pure mass-action); when $\sigma \gg 1$, the elasticity approaches zero (saturation). Figure 5B shows that the elasticity approaches a value of n (here equal to 4) with increasing L_0.

The expressions for ϵ_i^v and ϵ_a^v contain only signal terms, i.e., their mass-action contributions are zero because they are not reactants. These elasticities also depend, through q, on the prevailing value of σ (this

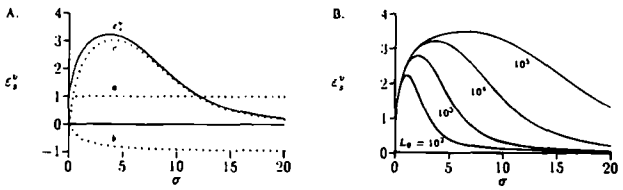

Fig. 5. The elasticity coefficient ϵ_s^v of an enzyme with a Monod–Wyman–Changeux mechanism. (A) The value of ϵ_s^v and the contribution of the various terms (curves a, b, and c show mass-action, reaction partner influence, and signal influence) were calculated from Eq. (10) for parameter settings: $K_I = K_A = \infty$, $n = 4$, $L_0 = 10^4$. (B) The value of ϵ_s^v was calculated with the same parameter settings as in (A) with L_0-values given on the graph.

will not be explored here). More general MWC rate expressions for reversible reactions with more than one substrate and product have been described by Popova and Sel'kov (1978), and can be used to obtain elasticity expressions.

Kinetic signals can be classified in different ways (Reich and Sel'kov, 1981). Appropriate descriptive categories are *intensity, action scale,* and *time scale.* The maximum absolute value of the elasticity with respect to signal concentration measures the intensity, while the half-effect concentration $x_{0.5}$ measures the action scale. Kinetic signals can also be be classified according to their influence on steady-state stability, being either *stabilizing* or *destabilizing* (Tyson, 1975). As Reich and Sel'kov (1981) have shown, such a classification has pitfalls, as a signal that may normally be destabilizing can become stabilizing, and vice versa, when stoichiometric autocatalysis occurs in the system. In simple networks, stabilizing signals augment mass-action, while destabilizing signals counteract mass-action. Lastly, a kinetic signal can be *homeostatic* when it promotes (relative) constancy of its own concentration in the face of parameter perturbations [negative feedback and positive feedforward signals are of this type, although some long-armed signals can produce oscillations (Higgins, 1967)], or *productive* when it can produce multiple steady states with potential trigger behavior, or oscillatory behavior (certain ranges of positive feedback and negative feedforward signals).

We shall consider an example of a homeostatic signal (Fig. 6) to show how the flux and the steady-state concentration $\bar{x}$ of the system in Fig. 1A change in response to changes in demand for the signalling metabolite (reaction 2). We consider the introduction of (i) a cooperative product inhibition signal affecting the supply step (reaction 1), and (ii) a cooperative substrate activation signal affecting the demand step. To keep matters simple, both supply and demand reac-

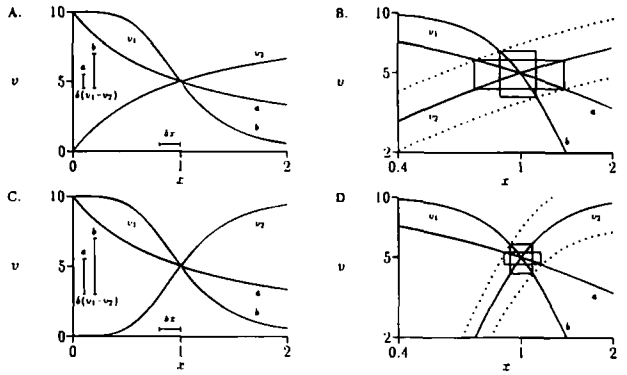

Fig. 6. Rate characteristics of the reactions in Fig. 1A with respect to the linking metabolite X. (A, C) Unscaled rate characteristics; (B, D) log-log form of the rate characteristics. Curve a was calculated from $v_1 = 20/(1 + 1 + 2x)$; curve b from $v_1 = 20/(1 + 1 + 2x^4)$. In (A) and (B) the rate equation for reaction 2 was $v_2 = 10x/(1 + x)$, while in (C) and (D) it was $v_2 = 10x^4/(1 + x^4)$. The dotted lines are for a 1.4-fold increase and decrease in V_2. The indicated magnitudes of $\delta(v_1 - v_2)$ are for $|\delta x| = 0.2$.

tions are far from equilibrium, so that the mass-action contributions of x in the elasticities ϵ_x^{v1} and ϵ_x^{v2} are respectively 0 and 1; product inhibition effects, whatever the Hill coefficient, are therefore solely regulatory.

Two effects become clear when curve a (noncooperative product inhibition by x with Hill coefficient of 1) is compared with curve b (cooperative product inhibition by x with Hill coefficient of 4).

First, the boxed responses in Fig. 6B show that increasing steepness in the feedback signal transfers the response to a change in demand from the concentration scale to the flux scale, i.e., flux-control by demand is increased (at the expense of flux-control by supply), while $\bar{x}$-control by demand is decreased (the response in $\bar{x}$ becomes more homeostatic). In terms of control coefficients, C_2^J increases from 0.5 to 0.8, while $C_2^{\bar{x}}$ changes from -1 to -0.4, i.e., its absolute value decreases. When substrate cooperativity is introduced into the demand step (Fig. 6D), less flux-control resides in the demand step, as C_2^J-values are lower than in the corresponding cases with no demand cooperativity. Nevertheless, increasing the strength of feedback on the supply still increases flux control (C_2^J increases from 0.2 to 0.5). Introduction of cooperativity into the demand also decreases $\bar{x}$-control by demand, while retaining the reduction caused by increasing the feedback on supply ($C_2^{\bar{x}}$ goes from -0.4 to -0.25). Here it is imperative to compare Figs. 6B and D, not A and C, as we have defined control and homeostasis in relative, not absolute, terms. It must be stressed that, because reaction 1 is unidirectional, the type of homeostasis we are discussing here is *kinetic* homeostasis, not thermodynamic homeostasis.

Second, in Fig. 6A, increasing steepness of the feedback signal stabilizes the steady state by making the eigenvalue $\partial(v_1 - v_2)/\partial x$ more negative (incidentally, this function is equal to the difference in the unscaled elasticity coefficients: $\partial v_1/\partial x - \partial v_2/\partial x = \bar{\epsilon}_x^{v1} - \bar{\epsilon}_x^{v2}$). This effect on dynamic stability is amplified when substrate cooperativity is introduced into the demand step 2 (Fig. 6C). Dynamic stability is a matter of absolute changes in the sense that it depends on the eigenvalues of the system, and the eigenvalues are composed of unscaled elasticity coefficients. On the other hand, as argued above, structural stability (homeostasis) is a matter of relative changes. Nevertheless, this analysis confirms the point made by Reich and Sel'kov (1981) that homeostasis with respect to changes in demand for signal, and dynamic stability with respect to perturbations in signal concentration, are two sides of the same coin.

The central message of this analysis is again that scaled and unscaled elasticity is the key to regulation. Although, for the sake of simplicity, a very simple system has been used as an example, the analysis can be extended to systems of arbitrary complexity, especially if the powerful matrix formulation of control analysis [see references in Hofmeyr *et al.* (1993)] is brought into play. In the next section the above analysis of feedback signals is extended to a more typical system, namely that of allosteric end-product inhibition in biosynthesis.

CO-RESPONSE COEFFICIENTS MEASURE ELASTICITIES OF REACTION BLOCKS

Figure 7A shows a typical biosynthetic structure where the so-called "end-product" of biosynthesis X_3 is consumed by a demand step (or steps). For example, X_3 could be an amino acid, in which case P could be protein. Many systems of this type are equipped with

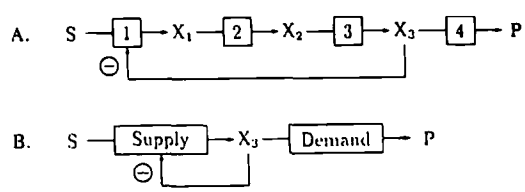

Fig. 7. A linear pathway with a feedback loop caused by X_3-inhibition of E_1. In (B) the first three enzymes are grouped to form the biosynthetic supply block for X_3, while E_4 is the demand for X_3.

a long-armed inhibitory feedback signal from X_3 to the committing step of the biosynthetic sequence (Stadtman, 1970). If the biosynthetic sequence is regarded as a supply block for X_3, the system simplifies to that in Fig. 7B.

The analogy between the systems in Fig. 1A and Fig. 7B is clear, but, in general, analytical expressions for the rates of the supply and demand blocks as functions of X_3 are not available, so that the powerful explanatory properties of rate characteristics seem to be unavailable (although numerical steady-state simulation can supply the rate characteristics if appropriate rate equations for the individual enzyme reactions are available—see Hofmeyr and Cornish-Bowden (1991) for a numerical analysis of the system in Fig. 7A).

It is in such situations that metabolic control analysis comes to the rescue, especially the recently-developed concept of co-response coefficient (Hofmeyr *et al.*, 1993) and the top-down approach to control analysis (Brown *et al.*, 1990; Brown, 1993). Imagine that the system is in steady state, and that we are interested in the behavior around that steady state, i.e., we want to construct the area around the point where the rate characteristics of the supply and demand block intersect. For this we need to vary the activity of the demand step around its prevailing value, while keeping the supply block parameters unchanged [this can be done by varying enzyme concentration, specific inhibitors, etc.—see Fell (1992) for a review of experimental strategies in control analysis]. At each demand activity the new steady-state must be established and the value of J and $\bar{x}_3$ measured. The same procedure is repeated for varying supply at constant demand. The two sets of $(J, \bar{x}_3)$-values are then plotted on a log-log scale to yield partial rate characteristics around the steady-state intersection (similar to Fig. 2B). The slopes of the two curves at the steady-state point equal the elasticities of the supply and demand blocks with respect to x_3. If the equilibrium constants of the supply and demand blocks are known, the mass-action contribution to the supply and demand elasticities can be calculated, and, therefore, how much regulation there is.

It has recently been shown that for systems where a metabolite clearly separates two blocks, the block elasticities with respect to the separating metabolite are equivalent to co-response coefficients, which relate the concomitant change in two independent steady-state variables in response to a perturbation (Hofmeyr *et al.*, 1993; Cornish-Bowden and Hofmeyr, 1994). For example, the $(J, \bar{x}_3)$ co-response with respect to a perturbation in v_4 is defined as

$$ {}^4O^J_{x_3} = \frac{\partial \ln J}{\partial \ln x_3} = \frac{R^J_p}{R^{x_3}_p} = \frac{C^J_4}{C^{x_3}_4} \qquad (11) $$

Here it is assumed that parameter p only affects v_4, so that it cancels from the R-ratio to give the C-ratio.

In our system $\epsilon^{supply}_{x_3} = {}^4O^J_{x_3}$ and $\epsilon^{demand}_{x_3} = {}^1O^J_{x_3} = {}^2O^J_{x_3} = {}^3O^J_{x_3}$. To assess the effectiveness of feedback by x_3 we must obtain ${}^4O^J_{x_3}$ by varying the activity of E_4. We can also use the formalism of metabolic control analysis to obtain an expression for ${}^4O^J_{x_3}$ in terms of individual elasticity coefficients (Hofmeyr and Cornish-Bowden, 1991):

$$ {}^4O^J_{x_3} = \frac{-\epsilon^{v_1}_{x_1}\epsilon^{v_2}_{x_2}\epsilon^{v_3}_{x_3} - \epsilon^{v_2}_{x_1}\epsilon^{v_3}_{x_2}\epsilon^{v_1}_{x_3}}{-\epsilon^{v_2}_{x_1}\epsilon^{v_3}_{x_2} + \epsilon^{v_1}_{x_1}\epsilon^{v_3}_{x_2} - \epsilon^{v_1}_{x_1}\epsilon^{v_2}_{x_2}} \qquad (12) $$

If, as is often the case in biosynthetic systems, the first reaction is unidirectional and insensitive to its immediate product ($\epsilon^{v_1}_{x_1} = 0$), the expression in Eq. (12) simplifies to ${}^4O^J_{x_3} = \epsilon^{supply}_{x_3} = \epsilon^{v_1}_{x_3}$. This means that the supply elasticity depends only on the regulatory effect of x_3 on v_1; if a rate-equation for v_1 is available, the supply elasticity can be calculated analytically.

This is just one example of the use of co-response coefficients. They can also form the basis of a control and elasticity analysis (Hofmeyr *et al.*, 1993). A fuller treatment to be published elsewhere will show that the principles illustrated by the simple example in this review are quite general. David Fell (personal communication) has kindly pointed out a useful link between the type of analysis of Figs. 4B, 6B, and 6D and co-response analysis. The absolute value of co-response coefficient ${}^2O^J_x$ specifies the aspect ratio of the response box, i.e., vertical side/horizontal side, while its sign specifies whether the two variables change in the same (+) or opposite (−) direction. Strictly speaking this is of course true only for small parameter changes. The actual size of the box depends on the values of the control coefficients. For example, in Fig. 6B an increase in the steepness of feedback inhibition changes ${}^2O^J_x$ from $0.5/(-1.0) = -0.5$ to $0.8/(-0.4) = -2$, while in the presence of substrate cooperativity in the demand step (Fig. 6D) it changes ${}^2O^J_x$ from $0.2/(-0.4) = -0.5$ to $0.5/(-0.25) = -2$. So, the box sizes differ although the values of the aspect ratios, and the way they change when the steepness of feedback is increased, are the same in the presence or absence of substrate cooperativity.

Nevertheless, although a co-response analysis can be of great help in obtaining insight into regulation, it must be stressed that it is of much less use for

studying system stability. Simplifying the system by grouping reactions together is of little use for assessing dynamic stability. Only under very special conditions is the function $\partial(v_{supply} - v_{demand})/\partial x_3$ (which is the difference between two unscaled co-response coefficients) an eigenvalue of the system.

CONCLUSION

This control analytic perspective of metabolic regulation has outlined a possible basis for a quantitative theory of regulation. In the context of a definition of regulation as a battle with mass-action, I have attempted to show the advantages of elasticity, control, and co-response analysis, the use of rate characteristics as an explanatory tool, and the need for care in the choice of relative or absolute changes when considering questions of metabolic regulation.

APPENDIX: OBTAINING ELASTICITY EXPRESSIONS

Consider the general rate equation [Eq. (1)]. A simple way of obtaining analytical expressions for elasticity coefficients is to write the rate equation in logarithmic form as a function of logarithmic concentrations using the equality $y = e^{\ln y}$:

$$\ln v = \ln k + \ln \Theta + \ln\left(e^{\ln s} - \frac{e^{\ln x}}{K_{eq}}\right) \quad \text{(A1)}$$

and then partially differentiate with respect to the logarithm of a substrate or product. The elasticity coefficients with respect to substrate S and product X would be expressed by

$$\epsilon_s^v = \frac{\partial \ln v}{\partial \ln s} = \frac{\partial \ln \Theta}{\partial \ln s} + \frac{1}{1 - \Gamma/K_{eq}} \quad \text{(A2)}$$

$$\epsilon_x^v = \frac{\partial \ln v}{\partial \ln x} = \frac{\partial \ln \Theta}{\partial \ln x} - \frac{\Gamma/K_{eq}}{1 - \Gamma/K_{eq}} \quad \text{(A3)}$$

The form of the saturation terms depends on the kinetic mechanism; the form of the thermodynamic terms is fixed: for substrates as in Eq. (A2) and for products as in Eq. (A3).

For example, writing Eq. (2) in logarithmic form gives

$$\ln v = \ln \frac{V_f}{K_s} - \ln\left(1 + \frac{e^{\ln s}}{K_s} + \frac{e^{\ln x}}{K_x}\right) + \ln\left(\epsilon^{\ln s} - \frac{e^{\ln x}}{K_{eq}}\right) \quad \text{(A4)}$$

Partial differentiation with respect to $\ln s$ and $\ln x$ gives Eqs. (3) and (4) in the main text.

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