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Posted Date: 27 March 2023

doi: 10.20944/preprints202303.0446.v1

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Summation Laws in Biological Systems

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Abstract: Dynamic variables in the non-equilibrium systems of life are determined by catalytic activities. These relate to the expression of the genome. The extent to which such a variable depends on the catalytic activity defined by a gene has become more and more important in view of the possibilities to modulate gene expression or intervene with enzyme function through the use of medicinal drugs. With all the complexity of cellular systems biology, there are still some very simple principles that guide the control of variables such as fluxes, concentrations, and half-times. Using time-unit invariance we here derive a multitude of laws governing the sums of the control coefficients that quantify the control of multiple variables by all the catalytic activities. We show that the sum of the control coefficients of any dynamic variable over all catalytic activities is determined by the control of the same property by time. When the variable is at a maximum, minimum or steady, this limits the sums to simple integers like 0, -1, 1, and -2, depending on the variable under consideration. Some of the implications for biological control are discussed as is the dependence of these results on the precise definition of control.

Keywords: control coefficients; metabolic control analysis; systems biology; genomics; pharmacokinetic principles; systems biology and PBPK; time-dependent control analysis

Introduction

The sequencing of whole genomes, with the subsequent mapping of the roles that the subset of metabolic genes play in human metabolism (Thiele et al. 2013), has led to increased attention for how biological function may be adjusted by modulating gene expression or enzyme activities. With his background in both genetics and theoretical biochemistry, Henrik Kacser, together with James Burns, realized how gene copy number could determine biological function quantitatively. They identified the molecular basis of dominance (Kacser and Burns 1981). The explanation is that the sum over all the enzymes in a network of their control coefficients on a flux must equal 1 (Kacser and Burns 1973; Heinrich and Rapoport 1974) and that there are many enzymes in the usual network, so that the average control must be small (Kacser and Burns 1981). The fact that the sum of the flux control coefficients must equal 1 at steady state inspired Kacser and others to ask whether that control would then be distributed homogeneously among the enzymes or reside in a single key factor. For most biochemical networks, the answer appears to be: distributed but not such that all enzymes have the same small control on that flux e.g. (Flint et al. 1981; Groen et al. 1982; Maeda et al. 2019).

At first the summation laws were limited to metabolic fluxes and concentrations at steady state, with 1 and 0 for their sums. Subsequently, the laws have been extended to incude time dependent metabolite concentrations and fluxes (Acerenza, Sauro, and Kacser 1989), as the time dependencies themselves (Bier et al. 1996; Teusink, Bakker, and Westerhoff 1996; Reijenga et al. 2005; Kholodenko, Demin, and Westerhoff 1997; Hornberg, Binder, et al. 2005). Proofs of these laws have been based on implicit differentiation (Heinrich and Rapoport 1974; Reder 1988), or on the theory of homogeneous functions (Giersch 1988; Westerhoff 1987), but the number properties for which the laws have been derived remain limited. Here we develop a novel mathematical proof of the summation laws, now

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for a multitude of new variables reflecting the dynamics of biochemical networks. We base this proof on the concept of time-invariance.

Results

The system

We will consider biochemical networks away from equilibrium in which various substances at well-defined concentrations (or mole numbers; we shall consider the volume of the well-stirred compartment to be fixed) are connected by (mostly) enzyme-catalyzed reactions, which may be chemical conversion or transmembrane transport processes (Westerhoff 1987). Each reaction i between a number of reactant molecules ('substrates') forms a number of products, and occurs at a certain reaction rate v_i. The boundaries of the system of the networks are set by fixed concentrations, fixed fluxes, or combinations of these. The concentrations and reaction rates within the network are variables that depend on and reflect the state of the system. The latter is determined by all the enzyme activities and other parameters, such as the rate constants of the enzymes, their k_{cat}, their Michaelis and product inhibition constants, as well as by the fixed external concentrations or fluxes. We assume that the time evolution of the state variables is stable in the sense of Lyapunoff (Glansdorff and Prigogine 1971; Keizer 1987). If the system parameters are left unaltered, the systems considered here will evolve into a stationary state, but the analysis in this paper is not limited to such steady states: it also addresses the time dependence of concentrations and of other state variables. In particular we shall discuss the extent to which, at any point in time, the state variables change magnitude when any of the enzyme activities has undergone a permanent infinitesimal modulation at time zero. We shall prove a number of laws that constrain the magnitudes of these, so-called (Burns et al. 1985), control coefficients.

Control Coefficients

A control coefficient quantifies the extent to which a catalytic process determines a system variable. Originally control coefficients of metabolite concentrations and metabolic fluxes were defined only for systems at steady state (Kacser and Burns 1973) (Heinrich and Rapoport 1974) (Burns et al. 1985). Their definition has since been generalized to time dependent metabolite concentrations and fluxes(Acerenza, Sauro, and Kacser 1989; Conradie et al. 2006) and to properties characterizing time dependencies such as cycling times, oscillation frequencies, half times and transit times (Demin, Kholodenko, and Westerhoff 1996; Bier et al. 1996) (Hornberg, Bruggeman, et al. 2005; Westerhoff 2008; Kholodenko, Demin, and Westerhoff 1997). Here we write the definition of the control coefficient of any dependent state variable x(t) in a biochemical network, as:

$$C_{e_j}^{x(t)} \stackrel{\text{def}}{=} \left(\frac{\partial \ln(x(t))}{\partial \ln(e_j)} \right)_{de_k=0 \; for \; all \; k \neq j}$$

e_j refers to the catalytic activity of enzyme j (or of any other process if not enzyme catalysed; sometimes written as v_j or as $V_{max,j}$ This definition applies to any variable x, which may be a concentration, a reaction rate, an electric potential, a time change of a concentration, or the area under the curve (AUC) of an intracellular toxin concentration, etc. All these together will populate the vector $\vec{x}(t)$, which we here abbreviate by $x(t)$, with t referring to time.

The vector $\vec{e}$ (which will be denoted by e below) represents the *complete* set of catalytic activities, each with a specificity j for a chemical or transport reaction that can be formulated explicitly and does not duplicate others: the e's represent *all* parameters with time dimensionality -1 (see below).

For more complex systems, for instance with metabolite channeling (Kholodenko, Cascante, and Westerhoff 1995) or reaction steps involving multiple proteins (Rohwer et al. 2000), e_j may need to be replaced by a vector of the corresponding rate constants (Kholodenko, Cascante, and Westerhoff 1995), but we shall not deal explicitly with such complications here.

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The definition should be interpreted in the sense of an agent p_j specifically affecting enzyme activity e_j. The concentration of that agent is altered instantaneously at time zero and the effect on the variable x(t) is determined. Therefore, more precisely (Kholodenko et al. 1995):

$$C_j^{x(t)} = \left(\frac{\frac{\partial \ln(x(t))}{\partial p_j}}{\frac{\partial \ln(e_j)}{\partial p_j}} \right)_{de_k=0 \; for \; all \; k \neq j}$$

The time coefficient is defined as (Acerenza, Sauro, and Kacser 1989):

$$C_t^{x(t)} = \left(\frac{\partial \ln(x(t))}{\partial ln(t)} \right)_{de_k=0 \; for \; all \; k}$$

Setting the time

The time coefficient may be rewritten as:

$$C_t^{x(t)} = \frac{t}{|x(t)|} \cdot \left(\frac{\partial |x(t)|}{\partial t} \right)_{de_k=0 \; for \; all \; k}$$

This shows that for any actual development over time of the property x, $\frac{\partial |x(t)|}{\partial t}$ is well defined, but the time coefficient defined above is not yet uniquely determined. This is because t depends on the choice made by the observer of when t should be called zero. Choosing that time point at 50 rather than 2 minutes before the time point t would change the time coefficient of x by a factor of 25 (through the factor t in the above equation). A similar uncertainty exists for the property x. In order to remove these uncertainties we consider the type of system that we address more closely: deterministic Markovian systems. These are systems of which the development over time after any given time point is unique, and fully determined by the magnitudes of a number of state variables (called y) of that system at that time point, plus two types of parameters. For these state variables y:

$$dy = f(k, q, y) \cdot dt$$

We shall call the magnitudes of time and state variables at the initial time point t_0 and y_0, respectively (both may be set to zero later, see below). In a metabolic system (biochemical network) at given temperature and pressure, the properties y are the concentrations of all the molecules, indicated by the vector y. One type of parameters is represented by the vector k, which contains all parameters with dimension 1/time, including the rate constants and the enzyme activities. The vector q represents all other parameters, which do not have a time dimension, such as equilibrium and Michaelis-Menten constants, standard chemical potentials and reaction stoichiometries. We assume that there are no parameters with other time dimensionalities than -1 or 0. All these parameter values are here considered to be fixed over the time span considered. When enzyme activities do depend on time, e.g. because of gene expression changes, this is dealt with by Hierarchical Control Analysis (Snoep et al. 2002), or can remain part of the present analysis by describing them within y. The above expression is a generalization of the time dependence of metabolite concentrations in metabolic networks, which is described by:

$$\frac{d\vec{y}}{dt} = \mathbf{N} \cdot \vec{v} = \mathbf{N} \cdot \overrightarrow{E_\iota \cdot \varphi_\iota(\vec{y}, \vec{k}, \vec{K})} >$$

with φ a vector of enzyme rate laws, k a vector of rate constants and K a vector of Michaelis-Menten and equilibrium constants (Goldberg et al. 2023), E a corresponding vector of enzyme concentrations, N the matrix of reaction stoichiometries (Westerhoff 1987). N and K are now subsumed in q and the vectors will be further represented by the corresponding scalars. Integration of the generalized equation then leads to:

$$y(t) = y_0 + \int_{t_0}^{t} f(k, q, y(t)) \cdot dt$$

The state the deterministic system is in, is fully defined by y, k and q and thereby by y_0, k, q and t-t_0. Consequently, also all other state functions x are also determined by y_0, k, q and t-t_0.

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We will here further focus on what we call 'ideal biochemical networks'(Kholodenko et al. 1995). Every catalytic activity (including transport activities) in these is catalyzed by a specific protein (enzyme) and all these proteins function independently of each other. This means that there is neither substrate channeling nor group transfer between proteins (when there are such processes, the treatment becomes more complex but remains essentially the same (Kholodenko et al. 1998)). In such networks, the set of rate constants k, can be replaced by the smaller set of enzyme activities e. The initial concentrations can be added to the q parameters to constitute the parameter set p, all without time dimension. Consequently, the change since time zero of any state variable x is a function of the enzyme activity vector e, the vector of parameters without time dimension p, and the time t-t_0:

$$x - x_0 = x(e, p, t - t_0)$$

The parameter (independent variable) t (and t_0 though not their difference) is ambiguous as its value at any occurrence of interest depends on the moment in history at which t is taken to equal zero. Multiplying dln(t) by $\frac{t}{t-t_0}$ one obtains the fully defined property $\frac{dt}{t-t_0}$, which will not change when a different moment in time is taken as zero for the time axis:

$$dln(\tau) \stackrel{\text{def}}{=} \frac{t}{t - t_0} \cdot dln(t) = dln(t - t_0) = \frac{dt}{t - t_0}$$

Consequently, one should substitute time parameter $\tau \stackrel{\text{def}}{=} t - t_0$ for time t in the definition of the time coefficient, or (Acerenza, Sauro, and Kacser 1989) take t_0=0. This defines t_0 as the time at which the integration that was mentioned above, starts and x_0 as the corresponding 'initial' magnitude of x: $x_0 \stackrel{\text{def}}{=} x(t_0)$. In summary, for the control coefficients to be unambiguously defined one should either resort to the definitions

$$C_j^{\chi(\tau)} = \left(\frac{\partial \ln(\chi(\tau))}{\partial \ln(e_j)}\right)_{de_k=0 \; for \; all \; k \neq j, d\tau=0}$$

$$C_\tau^{\chi(\tau)} = \left(\frac{\partial \ln(\chi(\tau))}{\partial ln(\tau)}\right)_{de_k=0 \; for \; all \; k}$$

with:

$$\chi(\tau) \stackrel{\text{def}}{=} x - x_0$$

and

$$\tau \stackrel{\text{def}}{=} t - t_0$$

, or to the simpler definitions:

$$C_j^{x(t)} = C_{e_j}^{x(t)-x_0} \cdot \frac{x(t) - x_0}{x(t)}$$

$$C_t^{x(t)} = C_\tau^{x(t)-x_0} \cdot \frac{x(t) - x_0}{x(t)} \cdot \frac{t}{t - t_0}$$

with the *proviso* that t refers to the time elapsed since x equalled x_0 and x refers to the value of x minus x_0. The simplest approach is then to set both x_0 and t_0 to zero as was done by (Acerenza, Sauro, and Kacser 1989).

Summation laws

The observed magnitude of a system property that does not have the dimension of time should not depend on the unit that the observer uses to measure time. The observed magnitude of a property that does have a time dimension must depend on the time unit to the extent that is precisely in accordance with that dimensionality. In order to illustrate this we consider two observers of the same natural phenomena. One observer measures the time τ in hours and the other, referred to by ', measures it in minutes: τ' is 60 times larger than τ numerically, although the two times actually refer to the same physical moment of time:

$$\tau' = \lambda \cdot \tau$$

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with $\lambda = 60\,min/h$ in the example. System properties x may partially have a time dimension. Reaction rates, for instance, become 60 times smaller numerically when expressed in moles per minute than in moles per hour, although physically they remain the same. More in general for x' expressed in the time unit of t' and x expressed in time units of t:

$$\chi' = \lambda^{\rho_x} \cdot \chi$$

ρ_x represents the time dimensionality of x. For concentrations, thermodynamic efficiency, growth yield (as flux ratio), electric potentials, and chemical potentials $\rho = 0$. For reaction rates, transport rates and fluxes $\rho = -1$. For the half times, area under the curve (AUC), and mean residence time (MRT) much used in pharmacology $\rho = +1$. For 'area under the moment curve' (AUMC) $\rho = +2$. Some parameters also have time dimensionality: enzyme activities and rate constants have time dimensionality $\rho = -1$. The other parameters lacktime dimensionality and are represented by p.

The second observer will find:

$$\chi' = f(e', p', t' - t_0')$$

This will compare as follows with the observations made by the first observer:

$$\lambda^{\rho_x} \cdot \chi(t, e_j, p_k) = \chi' = \chi(\lambda \cdot \tau, e_j/\lambda, p_k)$$

Since this should be true for any value of λ>0, we can equate the logarithmic derivative with respect to ln(λ) of the left hand side to that of the right-hand side:

$$\rho_x = \frac{\partial \ln(\chi)}{\partial \ln(\lambda \cdot t)} \cdot \frac{\partial \ln(\lambda \cdot t)}{\partial \ln(\lambda)} + \sum_j \frac{\partial \ln(\chi)}{\partial \ln\left(\frac{e_j}{\lambda}\right)} \cdot \frac{\partial \ln\left(\frac{e_j}{\lambda}\right)}{\partial \ln(\lambda)} + \sum_k \frac{\partial \ln(\chi)}{\partial \ln(p_k)} \cdot \frac{\partial \ln(p_k)}{\partial \ln(\lambda)}$$
$$= \frac{\partial \ln(\chi)}{\partial \ln(\lambda \cdot \tau)} - \sum_j \frac{\partial \ln(\chi)}{\partial \ln\left(\frac{e_j}{\lambda}\right)}$$

For $\lambda = 1$, and after multiplication by $\frac{x - x_0}{x}$ (if $x_0 \neq 0$; see above) this becomes the generalized summation law for time dependent control coefficients C_j^{x} (we write x for x(τ)):

$$\rho_x \cdot \frac{x - x_0}{x} + \sum_j C_j^{\mathrm{x}} = C_\tau^x$$

For concentrations y this implies that at any time point (Acerenza, Sauro, and Kacser 1989):

$$\sum_j C_j^y = C_\tau^y$$

The same should be true for the control of the thermodynamic efficiency, the control of flux and concentration ratios, and the control of electric and chemical potentials. The sum over all enzymes (catalytic activities) in the network of the concentration control coefficients of any substance should herewith be positive when its concentration is on the increase with time, negative when it is on the decrease, and zero when it is at its maximum or just steady:

$$\sum_j C_j^{[y]} = C_\tau^{[y]} =_{min,max,or\ steady\ state} 0$$

The formulation for steady state is the classical summation law for concentration control coefficients (Kacser and Burns 1973; Heinrich and Rapoport 1974). To the control of transmembrane electric potentials, of phosphorylation potential and of DNA supercoiling the same law applies (Westerhoff 1987; Snoep et al. 2002). For any reaction rate at time point τ:

$$\sum_j C_j^{\mathrm{v}} = C_\tau^v + \frac{v}{v - v_0}$$

Assuming (see above) that at $t=t_0=0$ the system was started from a state at zero flux so that $v_0=0$, this becomes:

$$\sum_j C_j^{\mathrm{v}} = C_\tau^v + 1$$

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This implies that when the flux is at a maximum (or minimum), or the system is at steady state, increasing all activities in proportion will increase the flux in the same proportion, as then $C_\tau^v = 0$. Only when the flux is decreasing with time, such a collective increase of process activities may leave the flux unaffected. The steady state case is the classical flux-control summation law (Kacser and Burns 1973; Heinrich and Rapoport 1974).

Let the "area under the curve up to time point t" (AUCt) be the time integral of the variable concentration of a substance in the compartment of interest:

$$AUCt \stackrel{\text{def}}{=} \int_0^t y \cdot dt$$

As this has the dimension of time, the summation law predicts:

$$\sum_j C_j^{\text{AUCt}} = C_\tau^{AUCt} - 1$$

When all xenobiotic has left the body, time no longer affects the AUCt so that $C_\tau^{AUCt} = 0$. AUCt then becomes the AUC known in pharmacology, for which the sum of the control coefficients should equal -1:

$$\sum_j C_j^{\text{AUC}} = -1$$

The "area under the first moment curve up to time point t" is defined by:

$$AUMCt \stackrel{\text{def}}{=} \int_0^t y \cdot t \cdot dt$$

and has a time dimensionality of +2. Accordingly its summation law reads:

$$\sum_j C_j^{\text{AUMCt}} = C_\tau^{AUCt} - 2$$

As the xenobiotic leaves the system, also the AUMCt becomes constant in time and the summation law reads:

$$\sum_j C_j^{\text{AUMC}} = -2$$

The mean residence time up to time point t is defined by:

$$MRTt \stackrel{\text{def}}{=} \frac{\int_0^t y \cdot t \cdot dt}{\int_0^t y \cdot dt}$$

and thereby has a time dimensionality of 1. Consequently:

$$\sum_j C_j^{\text{MRTt}} = C_\tau^{MRTt} - 1$$

When all xenobiotic has left the system, the MRTt becomes the mean residence time MRT and the total control exercised by the enzymes on this equals -1.

Considering the concentration versus time curve of a xenobiotic after its injection into the body one may wonder at what time a certain concentration is reached. One then sees the time as a function of that concentration (and of all the enzyme activities). We consider a modulation of all enzyme activities by the same factor, so that for all i's $dlne_i = dlne_1$. We allow for a simultaneous modulation of time to such an extent that there is no change in y. This leads us to:

$$0 = \left(\frac{\partial \ln(y)}{\partial \ln(\tau)}\right)_{de=0} \cdot \frac{\left(dln(\tau_y)\right)_{dy=0}}{\left(dln(e_1)\right)_{dy=0}} + \sum_j \left(\frac{\partial ln(y)}{\partial \ln(e_i)}\right)_{dy=0} \cdot \frac{\left(dln(e_i)\right)_{dy=0}}{\left(dln(e_1)\right)_{dy=0}}$$

$$= \left(\frac{\partial \ln(y)}{\partial \ln(\tau)}\right)_{de=0} \cdot \left(\sum_j C_j^{\tau y} + 1\right)$$

where we have used the corresponding summation law as well as the expression:

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$$\left(dln(\tau)\right)_{dy=0} = \sum_j \left(\frac{\partial ln(\tau_y)}{\partial \ln(e_j)}\right)_{dy=0} \cdot \left(dln(e_j)\right)_{dy=0}$$

and the definition of the control coefficients of the time at which the curve reaches y, i.e.:

$$C_j^{\tau_y} \stackrel{\text{def}}{=} \left(\frac{\partial ln(\tau_y)}{\partial \ln(e_j)}\right)_{dy=0}$$

The general solution is that the sum of the control coefficients of the specific time point (τ_y) at which the curve reaches the concentration y equals -1:

$$\sum_j C_j^{\tau_y} = -1$$

This will be true for any concentration of the substance, and therefore also for where it is half the maximum value, either on the increasing or on the decreasing slope, and where it is at the maximum.

Discussion

We have here derived a number of summation laws constraining the control of time dependent properties of metabolic networks. For control with respect to concentrations, these summation laws had been derived before, by using different methodologies. (Acerenza, Sauro, and Kacser 1989) used the phenomenon that an acceleration of all processes by a factor should make everything happen in the same way but at a time point earlier by that factor. Our approach of a changes in the time unit in which the system is observed, is similar to this. For the steady state summation laws, others and us have used the property that steady state fluxes and concentrations are homogeneous functions of enzyme activities (Giersch 1988; Westerhoff 1987), performed the corresponding thought experiments (Kacser and Burns 1973), or have taken derivatives of the balance equation at steady state (Heinrich and Rapoport 1974; Reder 1988; Kahn and Westerhoff 1991).

In the present paper we have found summation laws for many more properties than the previous works had found, such as for read-outs of pharmacokinetics (AUMC), results of microbial growth experiments (yields), non-equilibrium thermodynamic properties (efficiencies), chemical potentials and Gibbs energy differences of reactions, and half times of dynamic changes in concentrations (cf. (Hornberg, Bruggeman, et al. 2005)). In fact the summation laws we proved here are valid for state variables of any time dimensionality ρ.

The summation laws have multiple implications for the control of dynamic phenomena. We here mention only a few examples: When a concentration is at its time maximum, the corresponding summation law implies that this maximum concentration cannot just be determined by a single reaction activity in the system; there must be at least one additional controlling activity, with opposite sign. The steady state thermodynamic efficiency of microbial growth cannot be determined by a single process activity either. For the half times the summation law implies that if one activity controls that time, then there must at least be one other activity in control, unless a factor increase of the former causes a reduction of the half time by the same factor. When some enzyme activity is limiting for a biotechnological or medical process, one often tries to activate it. This will then reduce its control coefficient. The summation law has the implication that this automatically makes some other process more limiting, suggesting a second candidate for optimization.

Of course, the existence of the summation laws depends on the way 'control' is defined. It hinges on taking the double logarithmic derivatives, which correspond to the percentage increase for a 1 % activation of a process. It also depends on limiting the controlling factors to the set of catalytic activities: the control coefficients are but a subset of all possible sensitivity coefficients. Examining systems in terms of more sensitivity coefficients than the control coefficients can lead to more insight in the why's and how's of their design and functioning (Savageau 1976; Mondeel et al. 2020), but not to these summation laws. Formulation of the control in terms of straight rather than log-log derivatives (Reder 1988), changes the summations laws to equations that are so complex that their meaning may elude the reader.

For the summations to lead to the fixed numbers found here, i.e. for the properties to become laws, the set of control coefficients (over which the sum is taken) should be complete. This completeness means that all parameters with time dimension should be subsumed in the set, either directly, or indirectly because their effects can be represented by a modulation of the enzyme activities (Cornish-Bowden 2012). The completeness is much served by the advent of both genomics and systems biology with their interest in producing the genome and proteome of complete organisms (Uhlén et al. 2015) and with the vision of making genome wide metabolic maps (Thiele et al. 2013).

It is occasionally suggested that summation laws pertain to sums over all enzymes in the same pathway as the one that is under consideration in terms of the controlled flux or concentration. This is not so: the sum is over all the reaction activities in the entire network. Indeed, steps with major control may reside outside the pathway proper (Hoefnagel et al. 2002). That the control may be distributed over the entire genome wide network, explains why so many genes exert so little control in biology, notwithstanding the ubiquitous myth that every biological function is determined by a single 'key' gene or enzyme. Where Kacer and Bursn noted this for the control of fluxes, we may now generalize to all functional properties of complex Life if not complex society.

Acknowledgements: This paper is dedicated to the late Reinhart Heinrich and the late Henrik Kacser who have done so much for the understanding of biology and life, with their Metabolic Control Analysis that turns 50 this year.

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