Metabolic Control Theory: A Structural Approach

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In the general framework of metabolic control theory, we describe a method of mathematical modelling that provides a way of analysing the sensitivity of a metabolic system to perturbations of the environment or of the internal state of this system. The method can be applied to any metabolic system, involving for instance conservation relationships, non-specific external parameters, etc., and leads in particular to a characterization of the control matrices and to a generalization of the summation and connectivity theorems. In this paper, we emphasize the *structural* characterizations and properties of the systems which depend only on the structure of the metabolic network, and not on the reaction kinetics. The advantage of this approach lies of course in the fact that the structure of the metabolic network is an *invariant* of the system which depends neither on the environment nor on the internal state of this system. The aim of this paper is to show the efficiency of such a structural approach.

Introduction

In the study of a biochemical system, or of any scientific object, it is interesting to emphasize the *invariants* of this system, i.e. its characteristics or properties that depend neither on the state of its environment nor on its internal state, but only on its *structure*. To construct and study mathematical representations of this system, it is natural to follow the same approach.

In this paper, we work in the general framework of biochemical kinetic theory, and more precisely of *metabolic control theory* (Kacser & Burns, 1973; Heinrich & Rapoport, 1974). We shall underline the structural characteristics and properties of the models, and will show that these structural properties alone provide much information about the behaviour of the system—for instance in terms of relationships between the control coefficients.

We must first define what we decide to call the structure of the biochemical kinetic models. This occupies the first section of this paper, where we reconstruct these models. But let us give a preliminary idea of our choice: the structure of the model will be defined as the stoichiometric reaction scheme, which describes how the metabolites of the system combine. No hypothesis on the expression of the reaction rates will be held as structural.

We now try to justify this "minimalist" attitude. First of all, the kinetic data become meaningful only after the reaction scheme has been defined; they only provide supplementary information about the system. But this difference in level is not the only reason for separating the kinetic properties from the stoichiometric

characteristics: contrary to the stoichiometric characteristics, the kinetic properties depend on the environment of the system as well as on its internal state; they are not invariants of the system.

Besides, it appears that either the hypotheses or the questions on a biochemical system *at first* concern the underlying, or postulated, reaction scheme. Even in the ideal case when all the reactions with their stoichiometry and their rates are known, the problem would remain to define a subset of these reactions in order to provide a model that explains the observed behaviour. With respect to this aim, it would be incoherent to work with detailed kinetic expressions before analysing the structure of the model and its consequences. Moreover, it would lead to a huge and useless intricacy; it could cause also illusive disappointments, (a model could "work" if one had chosen a slightly different kinetic expression or parameter), or illusive convictions, (due for instance to a too blind use of identification programs).

So, our choice is *a priori* justified by two reasons: on the one hand our modelling approach is in accord with biochemical practice; on the other hand it aims at simplicity.

The second section of the present paper describes a first structural property of the model systems: the temporal invariance of some linear combinations of metabolite concentrations. We also define there some objects related to this conservation property, which will be used in the later sections.

In the third section, we recall definitions and results concerning metabolic control theory, and in particular the definitions and characterizations of steady state and flux control matrices. This theory was initiated by Higgins (1965), but was developed into a more coherent and complete state by Kacser & Burns (1973), and independently by Heinrich & Rapoport (1974), (see also Heinrich *et al.*, 1977). It provides a way to describe and study the response of a biochemical or model system to perturbations. This response is evaluated through the means of the estimation of the sensitivity of steady states and associated fluxes to small perturbations of parameters. Many papers approach biochemical questions using this theoretical framework. Among the more recent ones, let us mention papers in which a mathematical modelling approach is proposed: Castante *et al.* (1988), Fell & Sauro (1985), Hofmeyr *et al.* (1986), Kell & Westerhoff (1986), Kholodenko (1988), Mazat & Reder (1988), Reder (1986), Sauro *et al.* (1987), Westerhoff & Chen (1984), Westerhoff *et al.* (1984).

In the fourth section, we give the structural properties of the control matrices, and show that the use of these properties alone allows a substantial reduction in the number of unknown and independent coefficients of these matrices.

To close this introduction, let us notice that the present paper is based on the first two chapters of Reder (1986). We refer to this paper for more details.

1. Construction of the Mathematical Model and Definition of its Structure

This section describes a classical way to represent a biochemical system as a system of differential equations. It introduces also part of the notation used later.

Consider the biochemical system, whose mathematical representation we wish to construct, and suppose that its state is entirely described by the data of several quantities: metabolite concentrations, pressure, temperature, etc.

We classify those quantities into two classes: *internal variables* and *external parameters*. The parameters are the quantities whose values are kept constant during an experiment, whereas the variable values are not.

We assume that the biochemical system variables are metabolite concentrations. Let:

$$X_1, X_2, \ldots, X_m$$

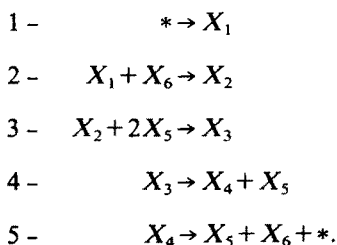denote these metabolites, and let:

$$x_1, x_2, \ldots, x_m$$

be their respective concentrations. Define the concentration vector x as:

$$\mathbf{x} = \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_m \end{bmatrix}.$$

In order to construct the model, we at first write the stoichiometric reaction scheme that describes how the metabolites X_i combine. In this scheme, we ignore the "external" metabolites whose concentrations are fixed external parameters (shown below as *); we arbitrarily number the r reactions that constitute the scheme and assign them directions.

Let us give an example of reaction scheme for which m is equal to 6 and r to 5:

$$1 - \qquad * \rightarrow X_1$$

$$2 - \quad X_1 + X_6 \rightarrow X_2$$

$$3 - \quad X_2 + 2X_5 \rightarrow X_3$$

$$4 - \qquad X_3 \rightarrow X_4 + X_5$$

$$5 - \qquad X_4 \rightarrow X_5 + X_6 + *.$$

It will be convenient to associate to the reaction scheme the matrix N of m rows and r columns constructed as follows: the column j of N represents the reaction j, and we write in this column at row i:

$+\alpha$ if the reaction j produces α molecules of X_i

$-\alpha$ if the reaction j consumes α molecules of X_i

0 if the reaction j neither produces nor consumes X_i,

that is to say the stoichiometric coefficient of X_i in reaction j.

For the example, the scheme matrix is:

$$N = \begin{bmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & -2 & 1 & 1 \\ 0 & -1 & 0 & 0 & 1 \end{bmatrix}.$$

As we are working in the framework of biochemical kinetic theory, we assume that the rate of change of the concentration x_i of a metabolite X_i is the sum of the r reaction rates, each weighted by the corresponding stoichiometric coefficient of X_i.

Using v_j to denote the rate of the reaction j, and:

$$v = \begin{bmatrix} v_1 \\ v_2 \\ \vdots \\ v_r \end{bmatrix}$$

to denote the rate vector, this hypothesis can be expressed as:

$$\frac{dx}{dt} = Nv.$$

For the example this equation can be written as follows:

$$\frac{d}{dt} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \\ x_6 \end{bmatrix} = \begin{bmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & -2 & 1 & 1 \\ 0 & -1 & 0 & 0 & 1 \end{bmatrix} \begin{bmatrix} v_1 \\ v_2 \\ v_3 \\ v_4 \\ v_5 \end{bmatrix}$$

or equivalently as follows:

$$\frac{d}{dt} \begin{bmatrix} x_1 \\ x_2 \\ x_3 \\ x_4 \\ x_5 \\ x_6 \end{bmatrix} = \begin{bmatrix} v_1 - v_2 \\ v_2 - v_3 \\ v_3 - v_4 \\ v_4 - v_5 \\ -2v_3 + v_4 + v_5 \\ -v_2 + v_5 \end{bmatrix}.$$

According to kinetic theory, the rates v_j are functions both of the m concentrations x_i and of the p external parameters μ_k.

For a given value μ of the external parameter vector, the evolution of the internal state of the system is therefore described by the *model differential system*:

$$\mathcal{S}(\mu): \quad \frac{d\mathbf{x}}{dt} = \mathbf{N}\mathbf{v}(\mathbf{x}; \mu).$$

From now on we shall study this model system.

We define the scheme matrix $\mathbf{N}$ as being structural; in an abbreviated way we say that $\mathbf{N}$ is the *structure* of the model. On the other hand, information about the expression of $\mathbf{v}$ in function of $\mathbf{x}$ and μ will not be considered as structural.

By extension, a property of an object related to the model will be called *structural* if it only depends on the structural data. However this extension can lead to a misuse of the word. Indeed we shall speak of structural properties of objects which are not defined or the existence of which is not guaranteed by the structural data alone; for instance, we shall describe structural properties of a steady state of a system $\mathcal{S}(\mu)$ though the existence and the value of such a state depend on the kinetic data $\mathbf{v}$.

2. First Structural Properties: The Conservation Relationships

Let us consider again the example of the model differential system given in the previous section. We remark that for any choice of the parameter μ and of the expression of the rate vector $\mathbf{v}$ in function of $\mathbf{x}$ and μ, we have:

$$\frac{d}{dt}(x_2 + x_3 + x_4 + x_6) = 0$$

which means that the quantity $(x_2 + x_3 + x_4 + x_6)$ remains constant. This linear conservation relationship is structural, as a consequence of a property of the scheme matrix $\mathbf{N}$: the sum of its second, third, fourth and sixth rows is indeed a null row.

In this conservation relationship, one recognizes a linear "first invariant" of the model system $\mathcal{S}(\mu)$. The concept of first invariant is one of the most classical in the theory of dynamical systems. In the present section, we will give a method to construct all the linear structural first invariants of a model system $\mathcal{S}(\mu)$. This method is based on a decomposition of the scheme matrix $\mathbf{N}$, we will now explain.

Let us extract from $\mathbf{N}$ a subset of its rows which constitutes a *basis* of the whole set of its rows. For such a subset:

—the rows are independent, which means that there is no null linear combination of these rows, except the trivial one, where all the coefficients are zero.

—every row of $\mathbf{N}$ is a linear combination of the rows of this subset.

Whatever extraction method is used, the number of rows of such a subset must be the same. By definition, this number is called the *rank* of the matrix $\mathbf{N}$, and we shall use m_0 to denote it.

As it is always possible to change the order of the rows of $\mathbf{N}$—that is to renumber the metabolites X_i—we shall assume that its first m_0 rows satisfy the above properties. Let $\mathbf{N_R}$ denote the matrix composed of those m_0 rows. The properties we have

imposed to them allow us to decompose N as the product:

$$N = LN_R$$

where the m-row and m_0-column matrix L has the form:

$$L = \begin{bmatrix} 1 & 0 & \ldots & \ldots & \ldots & 0 \\ 0 & 1 & 0 & \ldots & \ldots & 0 \\ \ldots & \ldots & \ldots & \ldots & \ldots & \ldots \\ 0 & \ldots & \ldots & 0 & 1 & 0 \\ 0 & \ldots & \ldots & \ldots & 0 & 1 \\ \hline & & & L_0 & & \end{bmatrix}$$

and L_0 is a $(m - m_0)$-row and m_0-column matrix.

We shall call N_R the *reduced* matrix of N and L its *link* matrix.

Notice that if the rows of N are independent, i.e. the rank m_0 of N is equal to m, this decomposition is trivial: N_R is equal to N and L is the m-dimensional identity matrix.

For the example given in the first section, the four first rows of the scheme matrix N are independent, the rank m_0 of N is equal to 4, and the following decomposition can be easily worked out by hand:

$$N = \begin{bmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \\ 0 & 0 & -2 & 1 & 1 \\ 0 & -1 & 0 & 0 & 1 \end{bmatrix} = \underbrace{\begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \\ \hline 0 & 0 & -2 & -1 \\ 0 & -1 & -1 & -1 \end{bmatrix}}_{L} \underbrace{\begin{bmatrix} 1 & -1 & 0 & 0 & 0 \\ 0 & 1 & -1 & 0 & 0 \\ 0 & 0 & 1 & -1 & 0 \\ 0 & 0 & 0 & 1 & -1 \end{bmatrix}}_{N_R}.$$

The matrix L_0 is constituted by the two last rows of L:

$$L_0 = \begin{bmatrix} 0 & 0 & -2 & -1 \\ 0 & -1 & -1 & -1 \end{bmatrix}.$$

The reduced matrix N_R is obtained by extracting from N its m_0 first rows. As the rows of N are associated with the components of the concentration vector x, it is natural to decompose also the concentration vector x into its first m_0 components x_R and its $(m - m_0)$ last components x'_R:

$$x = \begin{bmatrix} x_R \\ x'_R \end{bmatrix}.$$

The following result is obtained:

Theorem 1: *Every structural conservation relationship is a linear combination of the* $(m - m_0)$ *independent conservation relationships described by the equality*:

$$\frac{d}{dt}(x'_R - L_0 x_R) = 0.$$

In the previous example, this vector equality can be developed as:

$$\frac{d}{dt}(2x_3 + x_4 + x_5) = 0$$

$$\frac{d}{dt}(x_2 + x_3 + x_4 + x_6) = 0.$$

In other words, the two quantities $(2x_3 + x_4 + x_5)$ and $(x_2 + x_3 + x_4 + x_6)$ remain constant.

Let us give a last definition: for any concentration vector χ decomposed as:

$$\chi = \begin{bmatrix} \chi_R \\ \chi'_R \end{bmatrix}$$

we define the *link subspace* of χ as the m_0 dimensional affine subspace:

$$\mathscr{L}(\chi) = \{x \in \mathbb{R}^m / x'_R - L_0 x_R = \chi'_R - L_0 \chi_R\}.$$

(If N has maximum rank m, for every χ, $\mathscr{L}(\chi)$ is the whole space $\mathbb{R}^m$.)

Using this definition, Theorem 1 can be expressed equivalently as follows: *if the concentration vector of a system is χ at time zero, it remains in the link subspace $\mathscr{L}(\chi)$ of this initial state* (the statement is obviously empty if the rows of N are independent). Notice that the definition and the invariance of the link subspaces $\mathscr{L}(\chi)$ do not depend on the kinetic properties of the system, but only on the matrix N, i.e. on the structure of the reaction scheme.

3. Metabolic Control Theory

This section is based on ideas already expressed by people working on metabolic control theory (Kacser & Burns, 1973; Heinrich & Rapoport, 1974, etc.). Nevertheless, we work here in a general framework, without hypotheses about the structure of the metabolic network, nor about the kinetics of the reactions, nor about the way the external parameters act on them. Hence our definitions are, in their form, different from the definitions traditionally used in metabolic control theory. In particular we choose to work with "simple" derivatives instead of logarithmic derivatives—one of the reasons for this choice is detailed in Appendix 3, and the relations between our definitions or notations and the traditional ones is given in Appendix 2; this appendix is referred to by (A2.1), (A2.2) etc. in the text—.

A) STEADY STATES OF THE MODEL SYSTEM

Let us consider a model differential system $\mathscr{S}(\mu^0)$, and suppose that σ^0 is a *steady state* of this system, i.e. a concentration vector such that:

$$Nv(\sigma^0; \mu^0) = 0.$$

Metabolic control theory describes the variations of this steady state and of the associated fluxes induced by small perturbations of the system. These perturbations can be of two types: perturbation of the external parameter μ^0 or perturbation of the internal state σ^0.

In order to make the problem well posed in the mathematical framework, we have to assume that one and only one steady state is associated with such a perturbation. Using the implicit function theorem one can easily prove the following:

Proposition 2: Assume the mathematical hypothesis:

(MH):   The matrix $(\mathbf{N_R} \mathbf{D_x v} \mathbf{L})$ is invertible.

Then, for every state χ and every parameter μ sufficiently close to σ^0 and μ^0 respectively, there exists a single state $\sigma(\chi; \mu)$ in a neighbourhood of σ^0, which is a steady state of the system $\mathscr{S}(\mu)$ and which belongs to the link subspace $\mathscr{L}(\chi)$.

In the expression of (MH), and from now on, $\mathbf{D_x v}$ denotes the r-row and m-column matrix of the partial derivatives of $\mathbf{v}$ with respect to $\mathbf{x}$, calculated at the point $(\sigma^0; \mu^0)$: the coefficient at the intersection of its row i and column j is:

$$\frac{\partial v_i}{\partial x_j} (\sigma^0; \mu^0)$$

(the traditional elasticity matrix is related to this matrix $\mathbf{D_x v}$; see (A2.1)).

If $\mathbf{N}$ has maximum rank m, the (MH) expression is simpler: in that case, $\mathbf{N_R}$ is equal to $\mathbf{N}$ and $\mathbf{L}$ is the identity matrix. Then:

$$(\mathbf{N_R} \mathbf{D_x v} \mathbf{L}) = (\mathbf{N} \mathbf{D_x v}),$$

and the (MH) hypothesis is the classical regularity hypothesis of the steady state σ^0: at this point, the matrix $(\mathbf{N} \mathbf{D_x v})$ is actually the Jacobian matrix of the system $\mathscr{S}(\mu^0)$. Notice also that if $\mathbf{N}$ has maximum rank $\mathscr{L}(\chi)$ is the whole space $\mathbb{R}^m$; hence, the steady state $\sigma(\chi; \mu)$ does not depend on χ, but only on μ.

In order to give a physical interpretation to Proposition 2, we have to comment on the notion of observability of a steady state (a more detailed discussion is given in Reder, 1986). First of all, one can observe that $(\mathbf{N_R} \mathbf{D_x v} \mathbf{L})$ is the Jacobian matrix of the system $\mathscr{S}(\mu^0)$ restricted to the link subspace $\mathscr{L}(\sigma^0)$ (if this restricted system is expressed with the components of $\mathbf{x_R}$ as variables).

Hence, one knows that if the matrix $(\mathbf{N_R} \mathbf{D_x v} \mathbf{L})$ has an eigenvalue with positive real part, the steady state σ^0 of $\mathscr{S}(\mu^0)$ is unstable: almost every state close to σ^0 at time zero will move away. Therefore, a necessary condition to the physical observability of this steady state σ^0, translated into mathematical terms, is that the eigenvalues of $(\mathbf{N_R} \mathbf{D_x v} \mathbf{L})$ have non-positive real parts.

This condition is not, however, sufficient to guarantee the stability of σ^0, but if the real parts of all the eigenvalues of $(\mathbf{N_R} \mathbf{D_x v} \mathbf{L})$ are negative, σ^0 is a stable steady state of $\mathscr{S}(\mu^0)$. Moreover, under this strong hypothesis, for any state χ and any external parameter μ, sufficiently close to σ^0 and μ^0, the solution of the system $\mathscr{S}(\mu)$ initiating from χ reaches asymptotically a steady state of $\mathscr{S}(\mu)$ belonging to the link subspace of χ; this steady state is (locally) unique, stable, and is of course the state $\sigma(\chi; \mu)$ of Proposition 2.

This result provides a clear physical interpretation of the conclusion of Proposition 2. One could also notice that the hypothesis (MH)—zero is not an eigenvalue of ($N_R\, D_x v\, L$)—is weaker than the strong hypothesis that guarantees the physical observability of the steady state σ^0 of $\mathscr{S}(\mu^0)$, and is not very demanding when compared to the necessary condition for this observability.

B) CONTROL COEFFICIENTS: DEFINITION AND CHARACTERIZATION

We assume from now on that σ^0 is a steady state of the model system $\mathscr{S}(\mu^0)$, and that the hypothesis (MH) is satisfied.

Using Proposition 2, we can define a function σ:

$$(\chi; \mu) \to \sigma(\chi; \mu)$$

that associates with every couple $(\chi; \mu)$ sufficiently close to $(\sigma^0; \mu^0)$ the unique steady state of $\mathscr{S}(\mu)$ belonging to the link subspace $\mathscr{L}(\chi)$.

We can now define the *steady state flux* function J as:

$$(\chi; \mu) \to J(\chi; \mu) = v(\sigma(\chi; \mu); \mu).$$

It associates with every couple $(\chi; \mu)$ sufficiently close to $(\sigma^0; \mu^0)$ the rate vector which corresponds to the steady state $\sigma(\chi; \mu)$ of the system $\mathscr{S}(\mu)$. In the following J^0 will denote the flux which corresponds to the steady state σ^0 of $\mathscr{S}(\mu^0)$, i.e.:

$$J^0 = v(\sigma^0; \mu^0).$$

Let $\mathscr{V}(\chi; \mu)$ denote the set of rate vectors associated to the value μ of the external parameter and to the link subspace $\mathscr{L}(\chi)$, i.e.:

$$\mathscr{V}(\chi; \mu) = \{v(x; \mu); \quad x \in \mathscr{L}(\chi)\}.$$

The following theorem, the first part of which reproduces the result of Proposition 2, provides a characterization of the functions σ and J:

Theorem 3: The function σ is the unique function that takes its values in a neighbourhood of σ^0 and has the two properties:

$$\sigma(\chi; \mu) \in \mathscr{L}(\chi) \quad and \quad Nv(\sigma(\chi; \mu); \mu) = 0.$$

The function J is the unique function that takes its values in a neighbourhood of J^0 and has the two properties:

$$J(\chi; \mu) \in \mathscr{V}(\chi; \mu) \quad and \quad J(\chi; \mu) \in \mathrm{Ker}\,(N).$$

In this statement, Ker (N) denotes the kernel of N, that is the $(r - m_0)$-dimensional vector space:

$$\mathrm{Ker}\,(N) = \{w \in \mathbb{R}^r / Nw = 0\}.$$

Both properties of J are an immediate consequence of the definition of this function and of the first part of Theorem 3. The fact that they provide a characterization of J allows us to use them instead of the definition of J, and is a consequence of the hypothesis (MH): under this hypothesis, $\mathscr{V}(\chi; \mu)$ is indeed a m_0-dimensional

manifold which intersects transversally the vector space Ker (N), the dimension of which is the complementary $(r - m_0)$. The intersection point of these two manifolds is hence locally unique.

The central idea of metabolic control theory is to describe the linear approxima- tions of the variations of the functions σ and J around the point $(\sigma^0; \mu^0)$. Let:

$D_\chi\sigma$ denote the m-row m-column matrix, the coefficient at row i and column j of which is:

$$\frac{\partial \sigma_i}{\partial \chi_j}(\sigma^0; \mu^0).$$

$D_\mu\sigma$ denote the m-row p-column matrix, the coefficient at row i and column j of which is:

$$\frac{\partial \sigma_i}{\partial \mu_j}(\sigma^0; \mu^0).$$

$D_\chi J$ denote the r-row m-column matrix, the coefficient at row i and column j of which is:

$$\frac{\partial J_i}{\partial \chi_j}(\sigma^0; \mu^0).$$

$D_\mu J$ denote the r-row p-column matrix, the coefficient at row i and column j of which is:

$$\frac{\partial J_i}{\partial \mu_j}(\sigma^0; \mu^0).$$

The matrices $D_\chi\sigma$ and $D_\chi J$ represent the sensitivity of the steady state and of the steady-state fluxes to perturbations of the internal state of the metabolic system (A2.2), whereas the matrices $D_\mu\sigma$ and $D_\mu J$ represent the sensitivity of the steady state and of the fluxes to perturbations of the external parameters (A2.3).

The proof of the following result is given in Appendix 1:

Proposition 4: There exists a matrix Γ such that:

$$D_\mu\sigma = \Gamma \, D_\mu v$$

$$D_\chi\sigma = Id_m + \Gamma \, D_\chi v$$

and a matrix C such that:

$$D_\mu J = C \, D_\mu v$$

$$D_\chi J = C \, D_\chi v.$$

These two matrices only depend on the system $\mathcal{S}(\mu^0)$, and not on the variations of the rate vector v in function of μ. They can be expressed by:

$$\Gamma = -L \, (N_R \, D_\chi v \, L)^{-1} \, N_R$$

$$C = Id_r - D_\chi v \, L \, (N_R \, D_\chi v \, L)^{-1} \, N_R.$$

In this statement, Id_m and Id_r denote the identity matrices of dimensions m and r respectively, and $D_\mu v$ denotes the r-row p-column matrix of the partial derivatives of v with respect to μ, calculated at point $(\sigma^0; \mu^0)$ (see (A2.4)).

One can give the following interpretation of the coefficients of the matrices Γ and C: suppose that for every index j between 1 and r there exists a parameter λ_j that acts specifically on the rate v_j at point $(\sigma^0; \lambda^0)$, i.e.:

$$\frac{\partial v_j}{\partial \lambda_j}(\sigma^0; \lambda^0) \neq 0 \quad \text{and} \quad \frac{\partial v_i}{\partial \lambda_j}(\sigma^0; \lambda^0) = 0 \quad \text{if} \quad i \neq j.$$

Then, from first part of Proposition 4, the coefficient of Γ at row i and column j appears to be:

$$\left[\frac{\partial \sigma_i}{\partial \lambda_j}(\sigma^0; \lambda^0)\right]\left[\frac{\partial v_j}{\partial \lambda_j}(\sigma^0; \lambda^0)\right]^{-1}$$

and the coefficient of C at row i and column j is:

$$\left[\frac{\partial J_i}{\partial \lambda_j}(\sigma^0; \lambda^0)\right]\left[\frac{\partial v_j}{\partial \lambda_j}(\sigma^0; \lambda^0)\right]^{-1}.$$

These two coefficients appear to represent the control of the step j on the concentration σ_i and the flux J_i (associated to the steady state σ^0 of $\mathscr{S}(\lambda^0)$): indeed, they do not depend on the way the specific parameter λ_j acts on the kinetic v_j. They are related to the traditional concentration control coefficients and flux control coefficients (A2.5), but their coefficients are not normalized by the use of logarithmic derivatives. We shall call Γ the *simple steady state control matrix*, and C the *simple steady state flux control matrix*. (One of the reasons for not using logarithmic normalizations is given in Appendix 3.)

The matrices Γ and C play a leading part in the description of the sensitivity of the steady state σ and flux J to perturbations of the external parameters. Indeed, if the matrix Γ, (or C), is known, and if the matrix $D_\mu v$ is also known, (i.e. the external parameters μ act in a known way on the kinetics v), one can deduce the matrix $D_\mu \sigma$, (or $D_\mu J$), from the equality:

$$D_\mu \sigma = \Gamma D_\mu v \quad (\text{or} \quad D_\mu J = C D_\mu v)$$

(i.e. one can deduce how the parameter μ acts on the steady state σ (or the flux J)). Conversely, if μ is a family of external parameters rich enough for the matrix $D_\mu v$ to have maximum rank r, one can use the previous equality to deduce from the knowledge of $D_\mu v$ and $D_\mu \sigma$ (or $D_\mu J$) the coefficients of Γ (or C), even if each of the parameters is not specific of one of the reactions.

On the other hand, the occurrence of the matrices Γ and C in the equalities:

$$D_x \sigma = Id_m + \Gamma D_x v \quad \text{and} \quad D_x J = C D_x v$$

do not have a fundamental importance in the description of the sensitivity of the steady state σ or flux J to perturbations of the internal state of the system. Indeed, the knowledge of $D_x v$ and $D_x \sigma$ (or $D_x J$) is almost never sufficient to give the matrix Γ (or C). In other words, the coefficients of Γ and C have no direct interpretation

in the framework of perturbations of the internal state of the system. With respect to the sensitivity of the steady state σ or flux J to such internal perturbations, the central objects are the matrices $D_x\sigma$ and D_xJ.

The importance of these four matrices Γ, C, $D_x\sigma$ and D_xJ leads us to study them more closely. Observe that Proposition 4 gives expressions of these matrices, which do not distinguish their structural properties from those that depend on the kinetic data. In order to get a better characterization of, first of all, the simple control matrices, let us choose a matrix K of r rows and $(r - m_0)$ columns, whose columns constitute a basis of the kernel $\text{Ker}(N)$ or the matrix N. We then have:

Theorem 5: The simple steady state control matrix Γ is the unique matrix satisfying simultaneously:

$$\Gamma K = 0 \quad and \quad \Gamma(D_x v L) = -L.$$

The simple steady state flux control matrix C is the unique matrix satisfying simultaneously:

$$C K = K \quad and \quad C(D_x v L) = 0.$$

Observe that the two last equalities satisfied by Γ and C depend on the kinetic data of the system $\mathscr{S}(\mu^0)$, whereas the first equalities are consequences of the structural data alone: they neither depend on the value of the external parameter μ^0, nor on the choice of the steady state σ^0 of $\mathscr{S}(\mu^0)$.

These first structural equalities define linear relationships between the columns of the simple control matrices Γ and C. We call them *simple summation relationships*; their number is equal to $(r - m_0)$ (the classical summation relationship between the columns of the normalized control matrices can be derived from these simple summation relationships, as mentioned in Appendix 3).

The second equalities, which depend on the kinetic data, also define linear relationships between the columns of Γ and C. We call them *simple connectivity relationships*: their number is equal to m_0 (they are analogous to the known connectivity relationships between the columns of the normalized control matrices).

A short analytical proof of these connectivity and summation relationships is given in Appendix 1. But let us comment on them here (for more details, see Mazat & Reder, 1988). Remember that, if μ is a scalar parameter that acts on the kinetic vector v, one has, from Proposition 4:

$$D_\mu\sigma = \Gamma D_\mu v \quad and \quad D_\mu J = C D_\mu v.$$

Hence, the equalities of Theorem 5 can be understood by thinking of each column of the matrices K or $(D_x v L)$ as a vector $D_\mu v$ corresponding to a particular choice of a scalar parameter μ:

Let us at first suppose that the perturbation of v is such that $D_\mu v$ is in the kernel of N. It means that, in first order approximation, $v(\sigma^0; \mu)$ is in the kernel of N. Moreover, this vector belongs obviously to the manifold $\mathscr{V}(\sigma^0; \mu)$, and σ^0 belongs to its own link subspace $\mathscr{L}(\sigma^0)$. One deduces directly from Theorem 3 that, in first-order approximation:

$$\sigma(\sigma^0; \mu) \simeq \sigma^0 \quad and \quad J(\sigma^0; \mu) \simeq v(\sigma^0; \mu)$$

and then, using the properties of Γ and C:

$$\Gamma \, D_\mu v = D_\mu \sigma = 0 \quad \text{and} \quad C \, D_\mu v = D_\mu J = D_\mu v.$$

Therefore, and as these equalities can be obtained for any vector $D_\mu v$ in Ker (N), one has proved the simple summation relationships:

$$\Gamma \, K = 0 \quad \text{and} \quad C \, K = K.$$

The simple connectivity relationships could be explained in the same way: remark at first that the image of $(D_x v \, L)$ is the tangent vector space to the manifold $\mathcal{V}(\sigma^0; \mu^0)$. Let us suppose that the perturbation of v is such that $D_\mu v$ is a vector, denoted by $(D_x v \, L)z$, of the image of $(D_x v \, L)$. It means that, in first order approximation, $v(\sigma^0; \mu)$ belongs to the manifold $\mathcal{V}(\sigma^0; \mu^0)$ but also, conversely, that $v(\sigma^0; \mu^0)$ belongs to $\mathcal{V}(\sigma^0; \mu)$. Hence, using second part of Theorem 3:

$$J(\sigma^0; \mu) \simeq v(\sigma^0; \mu^0) = J^0$$

and therefore, and from the property of C:

$$C \, (D_x v \, L)z = C \, D_\mu v = D_\mu J = 0.$$

To interpret the equality, which concerns Γ, we have to convince ourselves that, in first order approximation:

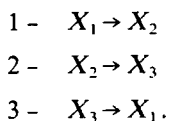$$v(\sigma^0 - Lz(\mu - \mu^0); \mu) \simeq v(\sigma^0; \mu^0) = J^0.$$

Because $\sigma^0 - Lz(\mu - \mu^0)$ belongs to the link space $\mathscr{L}(\sigma^0)$ and J^0 belongs to Ker (N), it follows that:

$$\sigma(\sigma^0; \mu) \simeq \sigma^0 - Lz(\mu - \mu^0)$$

and hence:

$$\Gamma \, (D_x v \, L)z = \Gamma \, D_\mu v = D_\mu \sigma = -Lz.$$

Let us show how to use Theorem 5 by applying it to an example. Suppose that the reaction scheme is cyclic, as follows:

$$1 - \quad X_1 \rightarrow X_2$$
$$2 - \quad X_2 \rightarrow X_3$$
$$3 - \quad X_3 \rightarrow X_1.$$

The scheme matrix N is:

$$N = \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \\ 0 & 1 & -1 \end{bmatrix}.$$

The first two rows of N are independent, and the third row is a linear combination of these two rows; one can therefore define the matrix N_R as composed by the two first rows of N:

$$N_R = \begin{bmatrix} -1 & 0 & 1 \\ 1 & -1 & 0 \end{bmatrix}$$

and then, calculate L to satisfy the relationship $N = LN_R$; one gets:

$$L = \begin{bmatrix} 1 & 0 \\ 0 & 1 \\ -1 & -1 \end{bmatrix}.$$

The kernel of N is the set of vectors v such that $Nv = 0$, i.e. such that:

$$v_1 = v_2 = v_3.$$

It is therefore the one dimensional vector space generated by the vector with components equal to 1. Hence, the matrix K can be defined by:

$$K = \begin{bmatrix} 1 \\ 1 \\ 1 \end{bmatrix}.$$

According to Theorem 5, the matrix Γ is the unique three-dimensional matrix solution of:

$$\Gamma [K \quad (D_x vL)] = \begin{bmatrix} 0 & -1 & 0 \\ 0 & 0 & -1 \\ 0 & 1 & 1 \end{bmatrix}$$

where $[K \quad (D_x vL)]$ is the (invertible) matrix:

$$[K \quad (D_x vL)] = \begin{bmatrix} 1 & \left(\dfrac{\partial v_1}{\partial x_1} - \dfrac{\partial v_1}{\partial x_3}\right) & \left(\dfrac{\partial v_1}{\partial x_2} - \dfrac{\partial v_1}{\partial x_3}\right) \\ 1 & \left(\dfrac{\partial v_2}{\partial x_1} - \dfrac{\partial v_2}{\partial x_3}\right) & \left(\dfrac{\partial v_2}{\partial x_2} - \dfrac{\partial v_2}{\partial x_3}\right) \\ 1 & \left(\dfrac{\partial v_3}{\partial x_1} - \dfrac{\partial v_3}{\partial x_3}\right) & \left(\dfrac{\partial v_3}{\partial x_2} - \dfrac{\partial v_3}{\partial x_3}\right) \end{bmatrix}$$

(the partial derivatives being calculated at the point $(\sigma^0; \mu^0)$).

The matrix C is the unique three dimensional matrix solution of:

$$C [K \quad (D_x vL)] = \begin{bmatrix} 1 & 0 & 0 \\ 1 & 0 & 0 \\ 1 & 0 & 0 \end{bmatrix}.$$

We now go back to the general discussion. To determine the matrices $D_x \sigma$ and $D_x J$, it is, of course, possible to calculate Γ and C first and then to use the first part of Proposition 4; but, following the same ideas as in Theorem 5, one may also use the direct characterization:

Proposition 6: *The matrix $D_x \sigma$ is the unique matrix solution of the two equalities:*

$$D_x \sigma L = 0 \quad and \quad D_x \sigma Y = Y.$$

The matrix $D_x J$ is the unique matrix solution of the two equalities:

$$D_x J L = 0 \quad and \quad D_x J Y = D_x v Y.$$

In this statement, Y denotes a matrix with m rows and $(m - m_0)$ columns, whose columns constitute a basis of the kernel of the matrix $(N_R D_x v)$.

Notice that, here again, the first equalities satisfied by $D_x \sigma$ and $D_x J$ are structural properties of these matrices, whereas the second such equalities depend on the kinetic characteristics of the system $\mathscr{S}(\mu^0)$.

We find again that if the matrix N has maximum rank m the functions σ and J do not depend on χ: in that case, as L is the identity matrix, the first structural relationships lead to:

$$D_\chi \sigma = D_\chi J = 0.$$

A short analytic proof of Proposition 6 is given in Appendix 1. But this result can be commented on in the same way as Theorem 5: observe first that if y denotes a m-dimensional vector, then $(D_\chi \sigma)y$ and $(D_\chi J)y$ are the derivatives with respect to the scalar variable ξ of $\sigma(\sigma^0 + \xi y; \mu^0)$ and $J(\sigma^0 + \xi y; \mu^0)$, calculated for ξ equal to zero. Suppose first that y is a vector that belongs to the image of L, i.e. that y is a linear combination of the columns of L. Then, the "initial condition" $(\sigma^0 + \xi y)$ and σ^0 belong to the same link space. Hence, from Theorem 3:

$$\sigma(\sigma^0 + \xi y; \mu^0) = \sigma^0 \quad \text{and} \quad J(\sigma^0 + \xi y; \mu^0) = J^0$$

and therefore:

$$(D_\chi \sigma)y = 0 \quad \text{and} \quad (D_\chi J)y = 0.$$

One deduces from these equalities:

$$D_\chi \sigma\, L = 0 \quad \text{and} \quad D_\chi J\, L = 0.$$

Suppose now that y is in the kernel of $(N_R D_x v)$. It means that, in first-order approximation, $v(\sigma^0 + \xi y; \mu^0)$ is in $\text{Ker}(N)$. Then, from Theorem 3:

$$\sigma(\sigma^0 + \xi y; \mu^0) \simeq \sigma^0 + \xi y \quad \text{and} \quad J(\sigma^0 + \xi y; \mu^0) \simeq v(\sigma^0 + \xi y; \mu^0)$$

and therefore:

$$(D_\chi \sigma)y = y \quad \text{and} \quad (D_\chi J)y = (D_\chi v)y.$$

One deduces from these equalities:

$$D_\chi \sigma\, Y = Y \quad \text{and} \quad D_\chi J\, Y = D_x v\, Y.$$

In practice, this proposition is not so easy to use as Theorem 5, because of the necessity of estimating a basis of the kernel of $(N_R D_x v)$. In the case of the previous example, we thus have at first to find a vector y solution of:

$$
\begin{bmatrix}
\left(-\dfrac{\partial v_1}{\partial x_1} + \dfrac{\partial v_3}{\partial x_1}\right) & \left(-\dfrac{\partial v_1}{\partial x_2} + \dfrac{\partial v_3}{\partial x_2}\right) & \left(-\dfrac{\partial v_1}{\partial x_3} + \dfrac{\partial v_3}{\partial x_3}\right) \\[2ex]
\left(\dfrac{\partial v_1}{\partial x_1} - \dfrac{\partial v_2}{\partial x_1}\right) & \left(\dfrac{\partial v_1}{\partial x_2} - \dfrac{\partial v_2}{\partial x_2}\right) & \left(\dfrac{\partial v_1}{\partial x_3} - \dfrac{\partial v_2}{\partial x_3}\right)
\end{bmatrix}
\begin{bmatrix} y_1 \\ y_2 \\ y_3 \end{bmatrix}
=
\begin{bmatrix} 0 \\ 0 \\ 0 \end{bmatrix}
$$

(all the derivatives being calculated at the point $(\sigma^0; \mu^0)$).

Then, we can apply Proposition 6: $\mathbf{D}_x\boldsymbol{\sigma}$ is the unique three-dimensional matrix solution of:

$$\mathbf{D}_x\boldsymbol{\sigma}\begin{bmatrix} 1 & 0 & y_1 \\ 0 & 1 & y_2 \\ -1 & -1 & y_3 \end{bmatrix} = \begin{bmatrix} 0 & 0 & y_1 \\ 0 & 0 & y_2 \\ 0 & 0 & y_3 \end{bmatrix}$$

and $\mathbf{D}_x\mathbf{J}$ is the unique three-dimensional matrix solution of:

$$\mathbf{D}_x\mathbf{J}\begin{bmatrix} 1 & 0 & y_1 \\ 0 & 1 & y_2 \\ -1 & -1 & y_3 \end{bmatrix} = \begin{bmatrix} 0 & 0 & \mathbf{D}_x v_1\, \mathbf{y} \\ 0 & 0 & \mathbf{D}_x v_2\, \mathbf{y} \\ 0 & 0 & \mathbf{D}_x v_3\, \mathbf{y} \end{bmatrix}.$$

—In this equality, $(\mathbf{D}_x v_i\, \mathbf{y})$ denotes the component i of the vector $(\mathbf{D}_x v\, \mathbf{y})$. These three components are necessarily equal: indeed the vector $\mathbf{y}$ belongs to the kernel of $(\mathbf{N}_R\, \mathbf{D}_x v)$, and hence the vector $(\mathbf{D}_x v\, \mathbf{y})$ belongs to the kernel of $\mathbf{N}$.—

4. Structural Properties of the Control Coefficients

Suppose now that we know the structure of a model system, that is its scheme matrix $\mathbf{N}$, but nothing about the rate functions v. We assume only that $\boldsymbol{\sigma}^0$ is a steady state of $\mathscr{S}(\boldsymbol{\mu}^0)$, and that the corresponding hypothesis (MH) is satisfied.

We have already seen in the preceding section that part of the results expressed in Theorem 5 provide structural properties of the simple control matrices $\boldsymbol{\Gamma}$ and $\mathbf{C}$, which can be expressed as the following linear relationships between the columns of these matrices:

$$\boldsymbol{\Gamma}\,\mathbf{K} = 0, \qquad \mathbf{C}\,\mathbf{K} = \mathbf{K}.$$

Other structural properties of these matrices can be found. For instance, we can prove, using Proposition 4, that the following relationships between their rows are satisfied:

$$[-\mathbf{L}_0 \quad \mathbf{Id}_{m-m_0}]\,\boldsymbol{\Gamma} = 0, \qquad \mathbf{N}_R\,\mathbf{C} = 0.$$

They can be interpreted following the same idea as in the comments made on Theorem 5: from the definitions of the functions σ and $\mathbf{J}$, $\sigma(\boldsymbol{\sigma}^0; \boldsymbol{\mu})$ belongs to $\mathscr{L}(\boldsymbol{\sigma}^0)$ and $\mathbf{J}(\boldsymbol{\sigma}^0; \boldsymbol{\mu})$ belongs to $\mathrm{Ker}\,(\mathbf{N})$. Therefore, one has:

$$[-\mathbf{L}_0 \quad \mathbf{Id}_{m-m_0}]\,(\sigma(\boldsymbol{\sigma}^0; \boldsymbol{\mu}) - \boldsymbol{\sigma}^0) = 0, \qquad \mathbf{N}_R\,\mathbf{J}(\boldsymbol{\sigma}^0; \boldsymbol{\mu}) = 0$$

and then:

$$[-\mathbf{L}_0 \quad \mathbf{Id}_{m-m_0}]\,\mathbf{D}_\mu\boldsymbol{\sigma} = 0, \qquad \mathbf{N}_R\,\mathbf{D}_\mu\mathbf{J} = 0.$$

Using now the properties of the simple control matrices, one gets:

$$[-\mathbf{L}_0 \quad \mathbf{Id}_{m-m_0}]\,\boldsymbol{\Gamma}\,\mathbf{D}_\mu v = 0, \qquad \mathbf{N}_R\,\mathbf{C}\,\mathbf{D}_\mu v = 0.$$

As these equalities are satisfied for any choice of parameter $\boldsymbol{\mu}$, one may suppose that $\mathbf{D}_\mu v$ is invertible. Hence, one gets:

$$[-\mathbf{L}_0 \quad \mathbf{Id}_{m-m_0}]\,\boldsymbol{\Gamma} = 0, \qquad \mathbf{N}_R\,\mathbf{C} = 0.$$

In Proposition 6 we also gave structural properties of the matrices $D_X \sigma$ and $D_X J$. (Recall that these matrices describe the linear approximations of the variations of the steady states of $\mathscr{S}(\mu^0)$ and their associated fluxes induced by a perturbation of the steady state σ^0.) These structural properties of $D_X \sigma$ and $D_X J$ are the following linear relationships between their columns:

$$D_X \sigma \, L = 0, \qquad D_X J \, L = 0.$$

But the following equalities are also satisfied:

$$[-L_0 \quad Id_{m-m_0}] D_X \sigma = [-L_0 \quad Id_{m-m_0}], \qquad N_R \, D_X J = 0.$$

They can be proved directly using Proposition 4, or interpreted following the same idea as in the comments on Proposition 6: using the same notations as in these comments, as $\sigma(\sigma^0 + \xi y; \mu^0)$ belongs to $\mathscr{L}(\sigma^0 + \xi y)$ and $J(\sigma^0 + \xi y; \mu^0)$ belongs to Ker (N), one has:

$$[-L_0 \quad Id_{m-m_0}] \sigma(\sigma^0 + \xi y; \mu^0) = [-L_0 \quad Id_{m-m_0}] (\sigma^0 + \xi y), \qquad N_R \, J(\sigma^0 + \xi y; \mu^0) = 0$$

and therefore:

$$[-L_0 \quad Id_{m-m_0}] D_X \sigma \, y = [-L_0 \quad Id_{m-m_0}] y, \qquad (N_R \, D_X J) y = 0.$$

As y can be chosen arbitrarily, these equalities prove that:

$$[-L_0 \quad Id_{m-m_0}] D_X \sigma = [-L_0 \quad Id_{m-m_0}], \qquad N_R \, D_X J = 0.$$

Actually, one can prove the following theorem:

Theorem 7: All the structural properties of Γ are consequences of the following:

$$[-L_0 \quad Id_{m-m_0}] \Gamma = 0, \qquad \Gamma K = 0,$$

$$\mathrm{rank} \, (\Gamma) = m_0.$$

All the structural properties of C are consequences of the following:

$$N_R \, C = 0, \qquad C \, K = K.$$

All the structural properties of $D_X \sigma$ are consequences of the following:

$$[-L_0 \quad Id_{m-m_0}] D_X \sigma = [-L_0 \quad Id_{m-m_0}], \qquad D_X \sigma \, L = 0.$$

All the structural properties of $D_X J$ are consequences of the following:

$$N_R \, D_X J = 0, \qquad D_X J \, L = 0.$$

Let us make a digression to explain the expression "all the structural properties" which appears in this statement. It has to be interpreted as follows: let N be a scheme matrix, σ^0 a concentration vector, μ^0 an external parameter vector, and, for instance, C a matrix for which the structural properties of the statement are satisfied. Then, there exists a rate function v, which completes the construction of the model, such that σ^0 is a steady state of the system $\mathscr{S}(\mu^0)$, such that the hypothesis (MH)

is satisfied, and finally such that **C** is the simple flux control matrix at the point $(\sigma^0; \mu^0)$. The same interpretation is valid for each of the three other matrices.

In fact one can prove a stronger result:

Proposition 8: Let **N** *be the matrix which defines the structure of a model system, and* **LN**$_R$ *its decomposition. Let* σ^0 *be a m-dimensional vector, and* μ^0 *an external parameter. Finally, let us consider*:

Γ, *a matrix of m rows and r columns*

C, *a matrix of r rows and r columns*

$\mathbf{D}_x\sigma$, *a matrix of m rows and m columns*

$\mathbf{D}_x\mathbf{J}$, *a matrix of r rows and m columns.*

Assume that each of those matrices verifies its structural properties expressed in Theorem 7.

Then there exist kinetic data v—*function of the variables* x *and* μ—*which complete the construction of the model systems* $\mathscr{S}(\mu)$, *such that* σ^0 *is a steady state of the system* $\mathscr{S}(\mu^0)$ *for which the hypothesis (MH) is satisfied, and such that the simple steady state control matrix, the flux control matrix, etc., at the point* $(\sigma^0; \mu^0)$ *are precisely the matrices* Γ, **C**, $\mathbf{D}_x\sigma$, *and* $\mathbf{D}_x\mathbf{J}$.

Furthermore, if a rate function verifies the above properties, the associated matrix $\mathbf{D}_x$v *is entirely determined by the four matrices* Γ, **C**, $\mathbf{D}_x\sigma$, *and* $\mathbf{D}_x\mathbf{J}$.

Hence, locally around a steady state of a metabolic system, the variations of the rates v_j of the isolated steps in function of the concentrations x_i can be determined if one knows properties of the whole system and if one knows how the rates v_j vary in function of the external parameters of a sufficiently rich family. Indeed, the matrices $\mathbf{D}_x\sigma$, $\mathbf{D}_x\mathbf{J}$, $\mathbf{D}_\mu\sigma$ and $\mathbf{D}_\mu\mathbf{J}$ represent properties of the whole system. And if μ is an external parameter vector such that $\mathbf{D}_\mu$v is invertible, one can deduce the control matrices Γ and **C** from the knowledge of $\mathbf{D}_\mu\sigma$, $\mathbf{D}_\mu\mathbf{J}$ and $\mathbf{D}_\mu$v (the proof of the proposition is a little technical; we therefore will not write it down in the present paper, but only mention how the matrix $\mathbf{D}_x$v can be deduced from the four matrices Γ, **C**, $\mathbf{D}_x\sigma$ and $\mathbf{D}_x\mathbf{J}$: $\mathbf{D}_x$v is the unique matrix solution of:

$$\mathbf{D}_x\text{v}\,\Gamma = -\mathbf{Id}_r + \mathbf{C} \quad \text{and} \quad \mathbf{D}_x\text{v}\,\mathbf{D}_x\sigma = \mathbf{D}_x\mathbf{J}\,\mathbf{D}_x\sigma).$$

We now return to Theorem 7, and especially to the part that concerns the simple steady state flux control matrix **C**. The first structural equality provides m_0 linear independent relationships between the rows of **C**, and hence its utilization reduces to $(r - m_0)$ the number of independent rows of this matrix that remain to be determined. In the same way, the second structural equality provides $(r - m_0)$ linear independent relationships between the columns of **C**, and hence its use reduces to m_0 the number of independent columns of this matrix that remain to be determined.

After the structural properties of **C** have been used, the proportion of independent coefficients that remain to be determined, with respect to the number r^2 of coefficients of **C**, is therefore equal to:

$$\frac{m_0}{r}\left(1 - \frac{m_0}{r}\right).$$

This number can be considered as a measurement of the efficiency of the structural study of the simple steady state flux control matrix. Observe that this number cannot be greater than $\frac{1}{4}$, i.e. after the structural properties of C have been used, there remains at most a quarter of the coefficients of C undetermined.

Using the same arguments on the simple steady state control matrix Γ, we could see that the proportion of coefficients which remain to be determined after the two structural equalities expressed in Theorem 7 have been used, with respect to the number (mr) of coefficients of Γ, is equal to:

$$\frac{m_0}{m} \frac{m_0}{r}.$$

Let us close this section with a very simple example to illustrate Theorem 7, in which X_1 is a unique metabolite that occurs at a branch point, i.e.:

$$1 - \quad * \to X_1$$
$$2 - \quad X_1 \to *$$
$$3 - \quad X_1 \to *.$$
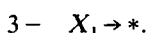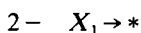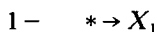
The scheme matrix N is then:

$$N = [1 \quad -1 \quad -1].$$

Its rank is obviously 1, and is maximum. The matrix N_R is therefore N, and L is the one-dimensional identity matrix.

The elements of the kernel of N are the vectors κ, the components of which κ_1, κ_2, κ_3, are the solution of:

$$\kappa_1 - \kappa_2 - \kappa_3 = 0.$$

We can then define the matrix K, for instance, as:

$$K = \begin{bmatrix} 1 & 1 \\ 1 & 0 \\ 0 & 1 \end{bmatrix}.$$

Assume that σ^0 is a steady state of a model system $\mathscr{S}(\mu^0)$ associated to the structural matrix N, and that the hypothesis (MH) is satisfied. Let:

$$C = \begin{bmatrix} c_{11} & c_{12} & c_{13} \\ c_{21} & c_{22} & c_{23} \\ c_{31} & c_{32} & c_{33} \end{bmatrix}$$

denote the associated simple flux control matrix.

The first structural property of C in Theorem 7:

$$N_R C = 0$$

shows that the first row of C is the sum of the two last rows. Hence:

$$c_{11} = c_{21} + c_{31}$$

$$c_{12} = c_{22} + c_{32}$$

$$c_{13} = c_{23} + c_{33}.$$

The second structural property:

$$\mathbf{C}\,\mathbf{K} = \mathbf{K}$$

allows us to express the two last columns of C with the help of the first one. Indeed, this equality can be written in the form:

$$c_{11} + c_{12} = 1 \qquad c_{11} + c_{13} = 1$$

$$c_{21} + c_{22} = 1 \qquad c_{21} + c_{23} = 0$$

$$c_{31} + c_{32} = 0 \qquad c_{31} + c_{33} = 1.$$

Using the two structural properties of C, we can express all the coefficients of C in function of c_{21} and c_{31}, which for notational convenience we note a and b. We finally obtain the expression of C:

$$\mathbf{C} = \begin{bmatrix} a+b & 1-(a+b) & 1-(a+b) \\ a & 1-a & -a \\ b & -b & 1-b \end{bmatrix}.$$

It is also easy to prove, using its structural properties, that the simple steady state control matrix Γ has the form:

$$\Gamma = \begin{bmatrix} \alpha & -\alpha & -\alpha \end{bmatrix} \qquad (\alpha \neq 0).$$

Finally, the second structural properties of $\mathbf{D_x\sigma}$ and $\mathbf{D_xJ}$ prove that these matrices vanish. This is not a surprise, because there is no structural conservation relationship for the model system considered.

Let us emphasize again the fact that the above properties of the matrices $\mathbf{C}$, Γ, $\mathbf{D_x\sigma}$ and $\mathbf{D_xJ}$ are only deduced from the scheme matrix $\mathbf{N}$; they neither depend on the expression of the three rates v_i as a function of x_1 and of the external parameter μ, nor on the value of this parameter, nor on the choosen steady state σ^0.

Proposition 8 can be illustrated on this simple example. Suppose that the coefficients a, b, and α are known. Using for instance the connectivity relationships, one can prove that $\mathbf{D_xv}$ is necessarily defined by:

$$\mathbf{D_xv} = \begin{bmatrix} (a+b-1)/\alpha \\ a/\alpha \\ b/\alpha \end{bmatrix}.$$

Conclusion

In Sections 1 to 3, we gave a method, already described in Reder (1986), which provides a way to define and compute from the data of the model, the control matrices and more generally the linear approximations of the variations of steady states and steady-state fluxes induced by perturbations of the system.

Our method generalizes the previously known results. It can be applied to any metabolic system: for instance, there can be conservation relationships, and the external parameters may act in any manner on the reaction rates (in particular, the parameters do not have to act specifically on one reaction rate only). It also provides a way to describe and compute the steady state variations induced by a small change of the constants appearing in the conservation relationships, that is, by a perturbation of the internal state of the system.

The aim of this paper was to exhibit the advantage of a structural approach to biochemical systems. The results announced in the present paper, and especially those of Section 4, show that, in the framework of the metabolic control theory, much information can be derived from the study of the metabolic network structure alone, represented by the scheme matrix N.

In the introduction we gave *a priori* arguments to justify both our decision to study the structural properties of the biochemical models and the choice of the structure. The proof we gave of the efficiency of a structural approach to the metabolic control theory provides an *a posteriori* justification to this approach [*Note*: A computer program written for PC compatible is now being developed by Mazat *et al.* (IBCN-CNRS, F-33077 Bordeaux CEDEX, France): this program uses the methods of this paper to the determination of the control coefficients].

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APPENDIX 1

Proof of Proposition 4, Theorem 5, and Proposition 6

Proof of Proposition 4: Let $\alpha(\chi; \mu)$ denote the m_0-dimensional vector composed by the m_0 first components of the vector:

$$\sigma(\chi; \mu) - \chi.$$

Using this notation, the characterization of σ given in Theorem 3 can be expressed as:

$$\sigma(\chi; \mu) = \chi + L\alpha(\chi; \mu)$$

$$N_R v(\chi + L\alpha(\chi; \mu); \mu) = 0.$$

Differentiating the second equality with respect to χ and μ at the point $(\sigma^0; \mu^0)$, one gets the two equalities:

$$N_R D_x v + (N_R D_x v L) D_x \alpha = 0 \quad \text{and} \quad (N_R D_x v L) D_\mu \alpha + N_R D_\mu v = 0.$$

(All the partial derivatives which appear in this proof are implicitly supposed to be calculated at point $(\sigma^0; \mu^0)$.)

Hence, using the invertibility of the matrix $(N_R D_x v L)$:

$$D_x \alpha = -(N_R D_x v L)^{-1} N_R D_x v \quad \text{and} \quad D_\mu \alpha = -(N_R D_x v L)^{-1} N_R D_\mu v.$$

Differentiating now the first equality, we obtain:

$$D_x \sigma = Id_m + L D_x \alpha \quad \text{and} \quad D_\mu \sigma = L D_\mu \alpha.$$

Hence:

$$D_x \sigma = Id_m - L (N_R D_x v L)^{-1} N_R D_x v \quad \text{and} \quad D_\mu \sigma = -L (N_R D_x v L)^{-1} N_R D_\mu v.$$

Let Γ be defined by:

$$\Gamma = -L (N_R D_x v L)^{-1} N_R.$$

We have proved the two first equalities expressed in Proposition 4:

$$D_\mu \sigma = \Gamma D_\mu v \quad \text{and} \quad D_x \sigma = Id_m + \Gamma D_x v.$$

Differentiating now the equality:

$$J(\chi; \mu) = v(\sigma(\chi; \mu); \mu)$$

with respect to χ and μ one gets:

$$D_x J = D_x v D_x \sigma \quad \text{and} \quad D_\mu J = D_x v D_\mu \sigma + D_\mu v$$

and then, using the already proved properties of $\mathbf{D_x\sigma}$ and $\mathbf{D_\mu\sigma}$:

$$\mathbf{D_xJ} = (\mathbf{Id_r} - \mathbf{D_xv\,L}\,(\mathbf{N_R\,D_xv\,L})^{-1}\,\mathbf{N_R})\,\mathbf{D_xv}$$

$$\mathbf{D_\mu J} = (\mathbf{Id_r} - \mathbf{D_xv\,L}\,(\mathbf{N_R\,D_xv\,L})^{-1}\,\mathbf{N_R})\,\mathbf{D_\mu v}.$$

Let $\mathbf{C}$ be defined by:

$$\mathbf{C} = \mathbf{Id_r} - \mathbf{D_xv\,L}\,(\mathbf{N_R\,D_xv\,L})^{-1}\,\mathbf{N_R}.$$

We have proved the two second equalities expressed in Proposition 4:

$$\mathbf{D_\mu J} = \mathbf{C\,D_\mu v} \quad \text{and} \quad \mathbf{D_xJ} = \mathbf{C\,D_xv}.$$

Proof of Theorem 5: One can easily verify, using the expression of Γ and $\mathbf{C}$ given in Proposition 4:

$$\mathbf{\Gamma\,K} = 0, \qquad \mathbf{\Gamma}\,(\mathbf{D_xv\,L}) = -\mathbf{L}$$

and

$$\mathbf{C\,K} = \mathbf{K}, \qquad \mathbf{C}\,(\mathbf{D_xv\,L}) = 0.$$

The uniqueness of the solutions Γ and $\mathbf{C}$ of these two systems is an immediate consequence of the (MH) hypothesis: under this hypothesis, the rate space $\mathbb{R}^r$ is indeed the direct sum of the kernel of $\mathbf{N}$ and the image of $(\mathbf{D_xv\,L})$. (This property is actually equivalent to (MH).)

Proof of Proposition 6: Using Proposition 4, we easily verify the equalities:

$$\mathbf{D_xJ\,L} = 0, \qquad \mathbf{D_xJ\,Y} = \mathbf{D_xv\,Y}$$

and

$$\mathbf{D_xJ\,L} = 0, \qquad \mathbf{D_xJ\,Y} = \mathbf{D_xv\,Y}.$$

The uniqueness of the solutions of these two systems is a consequence of the (MH) hypothesis: under this hypothesis, the state space $\mathbb{R}^m$ is indeed the direct sum of the image of $\mathbf{L}$ and the kernel of $(\mathbf{N_R\,D_xv})$. (This property is actually equivalent to (MH).)

APPENDIX 2

Relations Between the Present Definitions and the Traditional Ones

In this appendix:

—if $\mathbf{M}$ is a matrix, $[\mathbf{M}]_{ij}$ denotes its coefficient at row i and column j

—if $\mathbf{B}$ is a vector, $(\delta\mathbf{B})$ denotes the diagonal matrix, the diagonal coefficients of which are the components of $\mathbf{B}$

—all the partial derivatives are implicitly supposed to be calculated for the value σ^0 of the concentration vector and the value μ^0 of the external parameter.

(A2.1) *Matrix* $\mathbf{D_xv}$:

$$[\mathbf{D_xv}]_{ij} = \frac{\partial v_i}{\partial x_j}.$$

The *elasticity matrix* $\mathbf{\varepsilon}_x^v$, defined by:

$$[\mathbf{\varepsilon}_x^v]_{ij} = \varepsilon_{x_i}^{v_i} = \frac{\sigma_j^0}{J_i^0} \frac{\partial v_i}{\partial x_j}$$

is therefore a normalized form of $\mathbf{D}_x v$.

The relationship between these two matrices is given by the equality:

$$\mathbf{\varepsilon}_x^v = (\delta \mathbf{J}^0)^{-1} (\mathbf{D}_x v)(\delta \sigma^0).$$

(A2.2) *Matrices* $\mathbf{D}_x \sigma$ *and* $\mathbf{D}_x \mathbf{I}$:

$$[\mathbf{D}_x \sigma]_{ij} = \frac{\partial \sigma_i}{\partial \chi_j}$$

$$[\mathbf{D}_x \mathbf{J}]_{ij} = \frac{\partial J_i}{\partial \chi_j}.$$

One could define the normalized forms of these coefficients and interpret them as generalized control coefficients of the initial concentration χ_j on σ_i and J_i:

$$C_{\chi_j}^{\sigma_i} = \frac{\sigma_j^0}{\sigma_i^0} \frac{\partial \sigma_i}{\partial \chi_j}$$

$$C_{\chi_j}^{J_i} = \frac{\sigma_j^0}{J_i^0} \frac{\partial J_i}{\partial \chi_j}.$$

If $\mathbf{C}_x^{\sigma}$ and $\mathbf{C}_x^{J}$ denote the matrices composed by these coefficients, they are linked with $\mathbf{D}_x \sigma$ and $\mathbf{D}_x \mathbf{J}$ by the equalities:

$$\mathbf{C}_x^{\sigma} = (\delta \sigma^0)^{-1} (\mathbf{D}_x \sigma)(\delta \sigma^0)$$

$$\mathbf{C}_x^{J} = (\delta \mathbf{J}^0)^{-1} (\mathbf{D}_x \mathbf{J})(\delta \sigma^0).$$

Observe that we prefer to define the sensitivity of σ and $\mathbf{J}$ to perturbations of the initial concentration vector, instead of the conservation constants (as done for instance in a particular case in Hofmeyr *et al.*, 1986). The reason for doing this choice is that, for general metabolic networks, there are different manners for expressing the conservation relationships and not a unique one that can be considered as "natural".

(A2.3) *Matrices* $\mathbf{D}_\mu \sigma$ *and* $\mathbf{D}_\mu \mathbf{J}$:

$$[\mathbf{D}_\mu \sigma]_{ij} = \frac{\partial \sigma_i}{\partial \mu_j}$$

$$[\mathbf{D}_\mu \mathbf{J})_{ij} = \frac{\partial J_i}{\partial \mu_j}.$$

The normalized forms of these coefficients are the control coefficients of the external parameter μ_j on σ_i and J_i (see e.g. Kholodenko, 1988):

$$C_{\mu_j}^{\sigma_i} = \frac{\mu_j^0}{\sigma_i^0} \frac{\partial \sigma_i}{\partial \mu_j}$$

$$C_{\mu_j}^{J_i} = \frac{\mu_j^0}{J_i^0} \frac{\partial J_i}{\partial \mu_j}.$$

If C_μ^σ and C_μ^J denote the two matrices composed by these coefficients, they are linked with $D_\mu\sigma$ and $D_\mu J$ by the equalities:

$$C_\mu^\sigma = (\delta\sigma^0)^{-1}(D_\mu\sigma)(\delta\mu^0)$$
$$C_\mu^J = (\delta J^0)^{-1}(D_\mu J)(\delta\mu^0).$$

(A2.4) *Matrix* $D_\mu v$:

$$[D_\mu v]_{ij} = \frac{\partial v_i}{\partial \mu_j}.$$

The normalized form of this coefficient can be considered as the coefficient of elasticity of v_i with respect to μ_j:

$$\varepsilon^{v_i}_{\mu_j} = \frac{\mu_j^0}{J_i^0} \frac{\partial v_i}{\partial \mu_j}.$$

If ε_μ^v denotes the matrix composed by these coefficients, the link between ε_μ^v and $D_\mu v$ is given by:

$$\varepsilon_\mu^v = (\delta J^0)^{-1}(D_\mu v)(\delta\mu^0).$$

(A2.5) *Simple control matrices* Γ *and* C: If for all indice j between 1 and r, λ_j is an external parameter which acts specifically on the rate v_j at point $(\sigma^0; \lambda^0)$, one has:

$$[\Gamma]_{ij} = \left[\frac{\partial\sigma_i}{\partial\lambda_j}\right]\left[\frac{\partial v_j}{\partial\lambda_j}\right]^{-1}$$

$$[C]_{ij} = \left[\frac{\partial J_i}{\partial\lambda_j}\right]\left[\frac{\partial v_j}{\partial\lambda_j}\right]^{-1}.$$

The normalized forms of these coefficients are:

$$[\Gamma^{norm}]_{ij} = \frac{J_j^0}{\sigma_i^0}\left[\frac{\partial\sigma_i}{\partial\lambda_j}\right]\left[\frac{\partial v_j}{\partial\lambda_j}\right]^{-1}$$

$$[C^{norm}]_{ij} = \frac{J_j^0}{J_i^0}\left[\frac{\partial J_i}{\partial\lambda_j}\right]\left[\frac{\partial v_j}{\partial\lambda_j}\right]^{-1}.$$

They are the control coefficients of the step j on σ_i and J_i (as defined for instance in Heinrich *et al.*, 1977). The control matrices, which they compose, are related to the simple control matrices by the equalities:

$$\Gamma^{norm} = (\delta\sigma^0)^{-1}\Gamma(\delta J^0)$$
$$C^{norm} = (\delta J^0)^{-1}C(\delta J^0)$$

(observe that in the particular case where λ_j acts linearly on the rate v_j, the normalized control coefficients become:

$$[\Gamma^{norm}]_{ij} = \frac{\lambda_j^0}{\sigma_i^0}\left[\frac{\partial\sigma_i}{\partial\lambda_j}\right]$$

$$[C^{norm}]_{ij} = \frac{\lambda_j^0}{J_i^0}\left[\frac{\partial J_i}{\partial\lambda_j}\right]$$

and are the control coefficients of λ_j on σ_i and J_i, as defined in (A2.3)).

APPENDIX 3

A Reason for not using Logarithmic Derivatives

The definitions of the control matrices are usually given in terms of logarithmic derivatives. In this appendix we give a reason for preferring "normal" derivatives.

For simplicity, we restrict our attention to the structural properties of the steady state flux control matrix. To use logarithmic normalizations, we have to assume the positivity of the components J_i^0 of the flux vector $\mathbf{J}^0$ associated with the considered steady state $\boldsymbol{\sigma}^0$ of the system $\mathscr{S}(\boldsymbol{\mu}^0)$. Then, the usual normalized flux control matrix $\mathbf{C}^{\text{norm}}$ is, as mentioned in Appendix 2, defined by:

$$\mathbf{C}^{\text{norm}} = (\delta \mathbf{J}^0)^{-1} \mathbf{C} (\delta \mathbf{J}^0)$$

where $(\delta \mathbf{J}^0)$ denotes the diagonal matrix whose diagonal coefficients are the components of $\mathbf{J}^0$.

We want to express the structural properties of $\mathbf{C}^{\text{norm}}$, i.e. the properties that do not depend on the kinetic $\mathbf{v}$, and in particular, which do not depend on the value of $\mathbf{J}^0$.

We claim the following three statements:

The only structural property of $\mathbf{C}^{\text{norm}}$ which is easy to establish is the well known "summation relationship", which says that the sum of the columns of $\mathbf{C}^{\text{norm}}$ is equal to the column whose components are 1. Indeed, the vector $\mathbf{J}^0$ is a particular vector of the kernel of $\mathbf{N}$, and from Theorem 5 we see that:

$$\mathbf{C}\,\mathbf{J}^0 = \mathbf{J}^0.$$

Hence:

$$\mathbf{C}^{\text{norm}} \begin{bmatrix} 1 \\ 1 \\ \vdots \\ 1 \end{bmatrix} = (\delta \mathbf{J}^0)^{-1} \mathbf{C}\,\mathbf{J}^0 = (\delta \mathbf{J}^0)^{-1} \mathbf{J}^0 = \begin{bmatrix} 1 \\ 1 \\ \vdots \\ 1 \end{bmatrix}.$$

In some situations, it is the unique structural linear relationship between the coefficients of $\mathbf{C}^{\text{norm}}$.

In the general case, there can be structural linear relationships between the rows or the columns of $\mathbf{C}^{\text{norm}}$, but their number is always less than or equal to the number of structural relationships between the rows or the columns of our simple control matrix $\mathbf{C}$, and their determination requires a quite complicated study of the matrix $\mathbf{N}$, which we do not want to describe here.

For instance, for the matrix $\mathbf{C}^{\text{norm}}$ associated with the example in Section 4, there is no structural relationship between its rows. Moreover, the unique structural relationship between its columns is the "summation relationship": the sum of the three columns of $\mathbf{C}^{\text{norm}}$ is a column of 1. After the structural properties of $\mathbf{C}^{\text{norm}}$ have been used, the number of its independent coefficients which remain to be determined is six (instead of two, if logarithmic normalizations are not used).

In my opinion, these remarks are sufficient to justify the choice not to use the logarithmic normalizations in the study of the model system.

Nevertheless, with the help of Appendix 2, it is possible to derive easily from the statements 3 to 6 of the present paper all the corresponding statements in terms of the normalized control matrices, the elasticity matrix, etc. However, the structural character of some of these statements are lost by this translation. Moreover, we are not convinced of the usefulness of such translations. Indeed, in order to get dimensionless expressions, instead of using logarithmic normalizations—that is, "variable" normalizations which depend on the internal state of the system—it could be preferable to use a fixed normalization associated to the region in which the external parameters, internal states and reaction rates vary during the experiments.