Algorithms for the derivation of Flux and Concentration Control Coefficients

Arthur R. SCHULZ

Department of Biochemistry and Molecular Biology, School of Medicine, Indiana University, Indianapolis, IN 46202-5122, U.S.A.

A computer program is described that derives the Flux and Concentration Control Coefficients for linear and branched metabolic pathways. The program prompts the user to enter a concise description of the metabolic pathway. From this description, the program constructs the appropriate equations in matrix form. The algorithm employed to obtain the symbolic determinants is described, and this algorithm also provides a convenient method for manual derivation of the Control Coefficients. The computer-based method will accommodate unlimited feed-forward and feed-backward loops and a maximum of two branches from each metabolite on the main pathway. The utility of the method is illustrated with a linear path with feed-forward and feed-backward loops, and with a substrate cycle as an example of a path with two branches.

INTRODUCTION

During the past two decades, a number of treatments have been developed to provide a quantitative treatment of metabolic control [1–7]. Deriving expressions for Control Coefficients and logarithmic gains requires obtaining the symbolic determinants of matrices that may be rather large. Barrett [8] has reported a matrix method, and Hofmeyr [9] and Sen [10] have reported graphical methods that simplify the process, but these methods remain laborious in the case of complex metabolic pathways.

The purpose of the present paper is to describe an algorithm that has been incorporated into a computer program for derivation of all Flux and Concentration Control Coefficients. This algorithm utilizes pointers to the non-zero elements of the matrix, and therefore takes full advantage of the fact that the matrix is usually rather sparse. Although the algorithm was developed for implementation in a computer program, it provides a relatively simple means for derivation of Control Coefficients manually.

pathway. A maximum of two branches can arise from and/or converge on each pathway in the present version of the program. As discussed below in a later section, this limit of two branches per metabolite can be extended easily to any finite number of branches per metabolite. The user is prompted to enter the number of metabolites on each branch. Regardless of whether or not there are branches in the pathway, the user is finally prompted to indicate the effect of each metabolite on each enzyme of the system. From this information, a matrix is constructed that arises from Sensitivity Theory as described by Cascante *et al.* [11,12]. The Cascante treatment was chosen because it gives rise to the appropriate equations without assuming the Summation and Connectivity Theorems of the Metabolic Control Theory, yet these theorems are verified, based on the assumption that $\pi_i' = (\partial v_i / \partial e_i) / (\partial e_i / \partial e_i) = 1$, which is an assumption inherent in Metabolic Control Theory [2].

Construction of the appropriate matrix can be demonstrated by considering a specific metabolic pathway A:

DESCRIPTION OF THE COMPUTER PROGRAM

This computer program was written in C programming language and consists of two parts. The first part is the driver portion of the program, which provides for the input of information, constructs the appropriate matrices and outputs the symbolic determinant in an appropriate form. The second portion of the program is the algorithm, which, in essence, inverts the matrix and assigns the proper sign to each term. The purpose of this paper is to describe the algorithm that executes this second portion of the program.

Only a brief description of the driver portion of the program is given here. The user is prompted to reply as to whether or not there are branches that arise or converge on the main pathway. If there are branches, the number of branches must be entered. The user is then prompted to indicate whether branches arise from or converge on each of the metabolites on the main

Pathway A is equivalent to a pathway considered by Hofmeyr [9] except that pathway A specifies feed-forward activation and feedback inhibition. In accordance with the treatment by Cascante, the velocity of each step in pathway A is:

$$v_i = f(X_0, M_1, M_2, M_3, E_1, E_2, E_3, E_4) \quad (1)$$

If the intermediate metabolites are in steady state, the flux through the system, J , is equal to the velocity of each step. Thus $J = v_i$. Since X_0 is an independent variable, the expression for the sensitivity of the flux with respect to the first enzyme is:

$$\begin{aligned} \frac{\partial J}{J} \frac{\partial E_1}{E_1} &= \left(\frac{\partial v_1}{v_1} \frac{\partial M_1}{M_1} \right) \left(\frac{\partial M_1}{M_1} \frac{\partial E_1}{E_1} \right) + \left(\frac{\partial v_1}{v_1} \frac{\partial M_2}{M_2} \right) \left(\frac{\partial M_2}{M_2} \frac{\partial E_1}{E_1} \right) + \\ &\left(\frac{\partial v_1}{v_1} \frac{\partial M_3}{M_3} \right) \left(\frac{\partial M_3}{M_3} \frac{\partial E_1}{E_1} \right) + \left(\frac{\partial v_1}{v_1} \frac{\partial E_1}{E_1} \right) + \left(\frac{\partial v_1}{v_1} \frac{\partial E_2}{E_2} \right) \left(\frac{\partial E_2}{E_2} \frac{\partial E_1}{E_1} \right) + \\ &\left(\frac{\partial v_1}{v_1} \frac{\partial E_3}{E_3} \right) \left(\frac{\partial E_3}{E_3} \frac{\partial E_1}{E_1} \right) + \left(\frac{\partial v_1}{v_1} \frac{\partial E_4}{E_4} \right) \left(\frac{\partial E_4}{E_4} \frac{\partial E_1}{E_1} \right) \quad (2) \end{aligned}$$

Similar expressions apply to the sensitivity of flux to each enzyme, and the following equations are obtained:

$$C_1 = -\bar{e}_1^1 C_1^{M_1} + \pi_1^1 \quad (3)$$

$$C_2 = \bar{e}_2^2 C_1^{M_1} - \bar{e}_2^2 C_2^{M_2} - \bar{e}_2^3 C_3^{M_3} + \pi_2^2 \quad (4)$$

$$C_3 = \bar{e}_3^3 C_1^{M_1} + \bar{e}_3^2 C_2^{M_2} - \bar{e}_3^3 C_3^{M_3} + \pi_3^3 \quad (5)$$

$$C_4 = \bar{e}_4^4 C_3^{M_3} + \pi_4^4 \quad (6)$$

where $\bar{e}_i^j = -e_i^j$ and $\pi_i^j = (\partial v_i / \partial v_j) / (\partial e_i / \partial e_j)$. The user is not required to enter eqns. (3)–(6), but rather the user is prompted to indicate the effect of each metabolite on each enzyme. Eqns. (3)–(6) give rise to the following matrices:

$$A = \begin{vmatrix} 1 & \bar{e}_1^1 & 0 & 0 \\ 1 & -\bar{e}_2^1 & \bar{e}_2^2 & \bar{e}_2^3 \\ 1 & -\bar{e}_3^1 & -\bar{e}_3^2 & \bar{e}_3^3 \\ 1 & 0 & 0 & -\bar{e}_4^3 \end{vmatrix} \quad B = \begin{vmatrix} C_1 & C_2 & C_3 & C_4 \\ C_1^{M_1} & C_2^{M_1} & C_3^{M_1} & C_4^{M_1} \\ C_1^{M_2} & C_2^{M_2} & C_3^{M_2} & C_4^{M_2} \\ C_1^{M_3} & C_2^{M_3} & C_3^{M_3} & C_4^{M_3} \end{vmatrix}$$

$$C = \begin{vmatrix} \pi_1^1 & 0 & 0 & 0 \\ 0 & \pi_2^2 & 0 & 0 \\ 0 & 0 & \pi_3^3 & 0 \\ 0 & 0 & 0 & \pi_4^4 \end{vmatrix} = \begin{vmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{vmatrix}$$

If the pathway does not contain branches, matrices **A** and **B** are the transpose of the corresponding matrices described by Fell & Sauro [4]. Matrix **C** is the product of matrices **A** and **B**, and it is the identity matrix if $\pi_i^i = 1$. Thus the order of multiplication of matrices **A** and **B** can be reversed. As pointed out by Cascante *et al.*, premultiplication of matrix **A** by matrix **B** gives the Summation and Connectivity Theorems [11]. The Flux Control Summation Theorem is given by multiplying the first row of matrix **B** by the first column of matrix **A**. The concentration control relationships are given by multiplying rows 2 to 4 of matrix **B** by column 1 of matrix **A**. The various connectivity relationships are obtained by multiplying each row of matrix **B** by each of columns 2 to 4 of matrix **A**.

ALGORITHM FOR OBTAINING THE SYMBOLIC DETERMINANT

There are a number of efficient methods for obtaining a numerical determinant, but Cramer's Rule is probably the most feasible method for obtaining a symbolic determinant, and this is not a particularly efficient method for matrix inversion. Fortunately, matrix **A** is rather sparse for most pathways, and methods employed for inversion of sparse matrices are applicable [13]. Matrix **Q** is constructed from matrix **A** such that the elements of each row of matrix **Q** identify the non-zero elements in the corresponding row of matrix **A**. For pathway A:

$$Q = \begin{vmatrix} 1 & 2 & 0 & 0 \\ 1 & 2 & 3 & 4 \\ 1 & 2 & 3 & 4 \\ 1 & 4 & 0 & 0 \end{vmatrix}$$

The terms that comprise the symbolic determinant are obtained by systematic analysis of matrix **Q**, and this can be accomplished manually by using the same algorithm employed in the computer program. Each term in the symbolic determinant is represented by a vector that is obtained by selecting one element from each row of matrix **Q**. The elements in matrix **Q** point to a column in matrix **A**, and the row in matrix **A** is identified by the position of the element in the vector. For this reason, only

the non-zero elements of matrix **Q** can be elements of the vector. Each term in the symbolic determinant must contain only one term from each column of matrix **A**, therefore each vector must contain each number only once.

The first element of the first vector is the first non-zero element of the first row of matrix **Q**, and this first element always serves as the pivot element of the vector. The subsequent elements of the first vector are the first non-zero elements of the corresponding row of matrix **Q** provided that the element is not a duplicate of a previous element. If any element is a duplicate of a previous element, that element is replaced by the next non-zero element of the corresponding row of matrix **Q**. If there is not another non-zero element on the row, the vector element is replaced by the first non-zero element on the row, and the previous vector element is replaced by the next non-zero element in the previous row. This procedure is continued until the vector is complete. Each subsequent vector must differ from the previous vectors. To assure this, the last element of the previous vector is replaced by next non-zero element of the last row of matrix **Q**. If there is not another non-zero element in the last row, or if the next non-zero element of the corresponding row of matrix **Q** is a duplicate of a previous element in the vector, the last element of the vector is replaced by the first non-zero element of the corresponding row of matrix **Q** and the preceding element of the vector is replaced by the next non-zero element of the corresponding row of matrix **Q**. This procedure is repeated until there are no further non-zero elements in the first row of matrix **Q**.

The allowable vectors for pathway A together with the symbolic terms are:

$$\begin{array}{lll} 1,2,3,4 & 0+3 & -\bar{e}_1^2 \bar{e}_2^3 \bar{e}_3^4 \\ 1,3,2,4 & 1+2 & -\bar{e}_1^3 \bar{e}_2^2 \bar{e}_3^4 \\ 2,1,3,4 & 1+2 & -\bar{e}_1^2 \bar{e}_2^3 \bar{e}_3^4 \\ 2,3,1,4 & 2+1 & -\bar{e}_1^3 \bar{e}_2^2 \bar{e}_3^4 \\ 2,3,4,1 & 3+0 & -\bar{e}_1^2 \bar{e}_2^3 \bar{e}_3^4 \\ 2,4,3,1 & 4+1 & -\bar{e}_1^3 \bar{e}_2^2 \bar{e}_3^4 \end{array}$$

After the terms have been obtained, it is necessary to assign the correct sign to each term. This is accomplished by reference to the vectors and to the elements of matrix **A** to which the elements of the vector point. Firstly, the number of deviations from sequence in the vector are counted. For example, in the first vector there are no deviations from sequence whereas in the second vector there is one deviation from sequence because 3 precedes 2. In the sixth vector there are four deviations from sequence. Secondly, the number of negative elements in each term is counted. For example, there are three negative elements represented in the first vector. The sign of the term is $(-1)^p$, where p is the total number of deviations from sequence plus the total number of negative elements pointed to. The value of p is shown above together with the vectors and terms.

The denominator of the expressions for both the Flux and Concentration Control Coefficients is the symbolic determinant of matrix **A**. The numerator of the Flux Control Coefficient [18] for the first enzyme is obtained by replacing the first column of matrix **A** with the first column of matrix **C**, and the numerator for C_2 is obtained by replacing the first column of matrix **A** by the second column of matrix **C**. This procedure is repeated to obtain the numerator of the Flux Control Coefficient for each enzyme. The numerator of the Concentration Control Coefficient for M_1 with respect to each enzyme is obtained by replacing the second column of matrix **A** by columns 1 to 4 of matrix **C**. In like manner, the numerators of Concentration Control Coefficients for M_2 and M_3 the third and fourth columns of matrix **A** are replaced by columns 1 to 4 of matrix **C**. The Flux and Concentration Control Coefficients for pathway A are given in Appendix A.

Pathway B is an example of a pathway with two branches:

Pathway B

In this example, one branch converges on M_1 and the second branch diverges from M_2 . Since the divergent and convergent paths consist of the same enzyme, pathway B represents a simple substrate cycle. The flux at any point in the pathway is identified as J_i . In the program here described, the uneven subscripts are assigned to the fluxes through the main pathway.

It is obvious in pathway B that $J_3 = J_1 + J_2$, and thus $\partial J_1 / \partial e_1 = -\partial J_2 / \partial e_1 + \partial J_3 / \partial e_1$. It follows that $J_1 C_1^1 = -J_2 C_2^1 + J_3 C_3^1$. However, $J_3 C_3^1 = J_2 C_2^1 + J_5 C_5^1$ and therefore $C_1^1 = J_5 C_5^1$, where $J_5 = J_3 / J_1$. The relevant equations for pathway B are:

$$C_1^1 = J_5 C_5^1 = -\bar{e}_1^1 C_1^{M_1} + \pi_1^1 \quad (7)$$

$$C_2^2 = J_2 C_2^2 + J_5 C_5^2 = \bar{e}_2^2 C_2^{M_2} - \bar{e}_2^2 C_2^{M_2} + \pi_2^2 \quad (8)$$

$$C_{2.1}^{2.1} = -\bar{e}_{2.1}^{2.1} C_{2.1}^{M_{2.1}} + \pi_{2.1}^{2.1} \quad (9)$$

$$C_3^3 = \bar{e}_3^3 C_3^{M_3} + \pi_3^3 \quad (10)$$

These equations can be expressed in the following matrix form:

$$\begin{bmatrix} 0 & J_5 & \bar{e}_1^1 & 0 \\ J_2 & J_5 & -\bar{e}_1^2 & \bar{e}_2^2 \\ 1 & 0 & \bar{e}_1^{2.1} & -\bar{e}_2^{2.1} \\ 0 & 1 & 0 & -\bar{e}_2^3 \end{bmatrix} \begin{bmatrix} C_1^2 & C_2^2 & C_{2.1}^2 & C_3^2 \\ C_1^5 & C_2^5 & C_{2.1}^5 & C_3^5 \\ C_1^{M_1} & C_2^{M_1} & C_{2.1}^{M_1} & C_3^{M_1} \\ C_1^{M_2} & C_2^{M_2} & C_{2.1}^{M_2} & C_3^{M_2} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

The Flux and Concentration Control Coefficients are obtained in the same manner as for the unbranched pathway, and they are given in Appendix B.

FURTHER EXTENSIONS OF THE COMPUTER PROGRAM

The present program provides for unlimited feed-forward and feedback interactions and for two branches per metabolite on the main pathway. The number of branches per metabolite can be expanded easily to any finite number. The main purpose of this paper is to describe the algorithm for obtaining a symbolic determinant. However, it is necessary to explain that the driver portion of the program includes a dimensioned variable of type structure that contains the information concerning the branches that converge on or diverge from each metabolite in the main pathway. Integer elements in this structure identify the main pathway flux that is input to the metabolite, the main pathway flux that diverges from the metabolite and the branches that converge on or diverge from the metabolite. The present program accommodates a maximum of two branches per metabolite in the main pathway, but this structure could be enlarged to provide for any finite number of branches from each metabolite.

The present program does not provide for secondary branching. Secondary branching could be accommodated by an

additional dimensioned structure for each branch in which secondary branching occurs. The C programming language provides for dynamic memory allocation, so memory for the additional variables of type structure would be allocated only as needed.

Expansion of the present program to derive Control Coefficients for moiety-conserved cycles [19] requires an additional algorithm. Pathway C is an example of a rather complex moiety-conserved cycle:

Pathway C

Following the principles of Sensitivity Theory [12], the following matrices are obtained for pathway C:

$$\begin{bmatrix} 1 & \bar{e}_1^1 + \bar{e}_4^1 M_1 / M_4 & \bar{e}_4^1 M_2 / M_4 & \bar{e}_4^1 M_3 / M_4 \\ 1 & -\bar{e}_1^2 & \bar{e}_2^2 & \bar{e}_3^2 \\ 1 & -\bar{e}_1^3 & -\bar{e}_2^3 & \bar{e}_3^3 \\ 1 & -\bar{e}_4^1 M_1 / M_4 & -\bar{e}_4^1 M_2 / M_4 & -(\bar{e}_3^4 + \bar{e}_4^4 M_3 / M_4) \end{bmatrix} \times$$

$$\begin{bmatrix} C_1 & C_2 & C_3 & C_4 \\ C_1^{M_1} & C_2^{M_1} & C_3^{M_1} & C_4^{M_1} \\ C_1^{M_2} & C_2^{M_2} & C_3^{M_2} & C_4^{M_2} \\ C_1^{M_3} & C_2^{M_3} & C_3^{M_3} & C_4^{M_3} \end{bmatrix} = \begin{bmatrix} 1 & 0 & 0 & 0 \\ 0 & 1 & 0 & 0 \\ 0 & 0 & 1 & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

The symbolic determinant of matrix A can be obtained; however, the process can be expedited by employing an heuristic approach. With reference to pathway C, one can construct a matrix that is rather analogous to the connection matrix that has been used to develop an algorithm for the computer-based derivation of rate equations for steady-state enzymic reactions in accordance with the King-Altman treatment [14]. The matrix is constructed such that the rows refer to enzymes and the columns refer to metabolites. If a metabolite does not interact with the enzyme, the element in the row corresponding to the enzyme and in the column corresponding to the metabolite is zero. If the metabolite increases the rate of the reaction catalysed by the enzyme, the element is 1. If the metabolite inhibits the enzyme, the element is -1. The matrix corresponding to pathway C is:

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

Analysis of matrix U is facilitated by construction of a Q matrix of non-zero elements:

$$Q = \begin{vmatrix} 1 & 4 & 0 \\ 1 & 2 & 3 \\ 1 & 2 & 3 \\ 3 & 4 & 0 \end{vmatrix}$$

The expression for the numerator of the Flux Control Coefficient C_i is obtained by replacing the elements in the i th row of matrix Q with zeros. A vector is constructed for each term starting with the first element of the first row. The element identifies the metabolite and the row identifies the enzyme of an Elasticity Coefficient. The next element in the term is the left-hand element in the next lower row unless that element is a duplication of a previous element. If duplication occurs, the next element to the right on the same row is selected. If there are no further elements on the right of the row, the most left-hand element on the row is selected, and the previous element is replaced by the element to the right of it on the row above. This procedure is continued until all of the possible terms have been obtained. In addition, each term is multiplied by the concentration of the metabolite not included in the term divided by the concentration of M_4 . In addition to obtaining the elements that comprise each term, the correct sign must be assigned to each term. The sign of each term will be positive if paths exist by which each of the remaining metabolites contribute to the accumulation of the metabolite in the numerator of the term. If this criterion is not satisfied, the sign of the term is negative. In this regard, it must be realized that each inhibitory Elasticity Coefficient describes the accumulation of the substrate for the inhibited enzyme. The algorithm can be illustrated by listing the vectors and numerator terms for the Flux Control Coefficient of the first enzyme in pathway C:

$$\begin{aligned} 0,1,2,3 &= e_1^2 e_2^3 e_3^4 M_4 / M_4 \\ 0,1,2,4 &= e_1^2 e_2^3 e_4^4 M_3 / M_4 \\ 0,1,3,4 &= e_1^2 e_3^3 e_4^4 M_2 / M_4 \\ 0,2,1,3 &= e_1^3 e_2^2 e_3^4 M_4 / M_4 \\ 0,2,1,4 &= e_1^3 e_2^2 e_4^4 M_3 / M_4 \\ 0,2,3,4 &= e_2^2 e_3^3 e_4^4 M_1 / M_4 \\ 0,3,1,4 &= -e_1^3 e_2^2 e_4^4 M_2 / M_4 \\ 0,3,2,4 &= e_2^3 e_3^2 e_4^4 M_1 / M_4 \end{aligned}$$

The denominator of the Control Coefficients is, of course, the sum of the numerator terms of all of the Flux Control Coefficients.

The Concentration Control Coefficients for pathway C are obtained in a similar manner. The U matrix for a Concentration Control Coefficient is derived from the original U matrix, but the elements in the column corresponding to the metabolite are replaced with zeros and the elements in the row corresponding to the enzyme are all set to zero. Thus the matrices required to obtain the numerator of $C_1^{M_2}$ are the following:

$$U = \begin{vmatrix} 0 & 0 & 0 & 0 \\ 1 & 0 & -1 & 0 \\ 1 & 0 & -1 & 0 \\ 0 & 0 & 1 & -1 \end{vmatrix} \quad Q = \begin{vmatrix} 0 & 0 \\ 1 & 3 \\ 1 & 3 \\ 3 & 4 \end{vmatrix}$$

The numerator contains all of the allowable permutations of the Elasticity Coefficients. These are obtained by first setting the elements in the second row of the foregoing Q matrix to zero and obtaining the appropriate terms, then setting the elements in the third row of matrix Q to zero and finally setting the elements in the fourth row of matrix Q to zero. Each term is multiplied by the concentration of the metabolite not included in the term divided by the concentration of M_4 . The metabolite involved

in the Control Coefficient is not considered. The sign of each numerator term in the Concentration Control Coefficient is determined in a manner similar to that employed for the Flux Control Coefficients. If a continuous path leads forward from the enzyme in the coefficient to the metabolite in the coefficient, the enzyme will increase the concentration of the metabolite and the sign of the term will be positive, for in that case the enzyme will tend to increase the concentration of the metabolite. The sign of the term will also be positive if the enzyme catalyses the removal of a metabolite that functions as an inhibitor of an enzyme that intervenes between the enzyme and the metabolite involved in the Control Coefficient. If neither of these criteria is satisfied, the enzyme will decrease the concentration of the metabolite and the sign of the term will be negative. The following terms constitute the numerator of $C_1^{M_2}$:

$$C_1^{M_2} = -e_1^3 e_3^4 M_4 / M_4 - e_1^3 e_4^4 M_3 / M_4 - e_2^3 e_4^4 M_1 / M_4 + e_1^2 e_3^4 M_4 / M_4 + e_2^2 e_4^4 M_3 / M_4 + e_2^2 e_4^4 M_1 / M_4 + e_1^2 e_3^2 M_4 / M_4 - e_1^3 e_3^2 M_4 / M_4$$

The sixth term is an example of a term that is positive because the enzyme is responsible indirectly for lowering the concentration of an inhibitor. The Flux and Concentration Control Coefficients for pathway C are presented in Appendix C. It should be noted that the Flux and Concentration Control Coefficients for pathway A listed in Appendix A are identical with the terms in Appendix C that do not contain a fractional concentration. This is as it should be, for the only difference between pathways A and C is that pathway C forms a moiety-conserved cycle. The algorithm for determining the signs of the terms involves tracing the paths that connect the nodes in the model, and for that reason the algorithm employed for the computer-based procedure for derivation of equations for steady-state enzyme kinetics [14] can be used with only minor modifications. The algorithm described here is applicable to the conserved cycles considered by Hofmeyr [9] and Cascante *et al.* [12], but would have to be modified for more extensive cycles [15]. For example, one problem is that the metabolite scaling factors only apply to reactions involving metabolites in the conservation group, so that the procedure would not be adequate for pathways with a mixture of conserved and non-conserved metabolites, or for pathways with two or more groups of conserved metabolites.

DISCUSSION

The algorithms here described provide a simple method for derivation of Flux and Concentration Control Coefficients. The appropriate equations are expressed in matrix form, and this is considerably more concise than graphical representation of the metabolic pathway. The process of derivation is further expedited by construction of vectors whose elements are pointers to the matrix elements that constitute the terms in the Control Coefficients. When the algorithms are incorporated into a computer program, they constitute a fast and efficient method for derivation of Control Coefficients, for the algorithms involve nothing more complex than integer arithmetic. The program does not require the user actually to write the appropriate equations or construct the appropriate matrices. The procedures followed in derivation of the Control Coefficients is entirely comparable with Cramer's Rule except that the procedure is expedited by employing pointers to the non-zero elements of the appropriate matrix. The present program provides for unlimited feed-forward and feed-backward loops and a maximum of two branches from each metabolite on the main pathway.

It has been emphasized that the algorithm employed for this computer-based method can be employed for manual derivation. Hence it seems appropriate to compare the method here described with graphical methods [8–10]. A major advantage of the matrix

approach is its conciseness; however, if manual derivation is employed, it is necessary to derive the appropriate equations. A major advantage of the graphical methods is that the graphs can be constructed by reference to the metabolic pathway without derivation of the equations. After the equations have been derived, construction of the matrices and their analysis following this algorithm is probably no more time-consuming than construction of and analysis of the graphs. Sen has pointed out that signal-flow-graph theory can be used to study the cause-effect relationships of the enzymes [10], and this is a significant advantage of the graph theory. However, the same generalizations can be drawn by inspection of the derived expressions for the Control Coefficients.

There are a couple of points that should be mentioned with regard to the summation relationships in the case of branched pathways. In this case the Summation Theorems are subject to additional constraints, as has been pointed out by others [12,16]. The summation constraints for control of flux 2 are given by multiplication of row one of matrix **B** by the first two columns of matrix **A**. These constraints are:

$$\hat{J}_2 C_2^2 + C_{2,1}^2 = 1 \quad (11)$$

$$\hat{J}_5 C_1^2 + \hat{J}_5 C_2^2 + C_3^2 = 0 \quad (12)$$

The summation constraints for control of flux 5 are obtained by multiplication of row two of matrix **B** by the first two columns of matrix **A**:

$$\hat{J}_2 C_2^5 + C_{2,1}^5 = 0 \quad (13)$$

$$\hat{J}_5 C_1^5 + \hat{J}_5 C_2^5 + C_3^5 = 1 \quad (14)$$

The constraint expressed in eqn. (13) is of particular interest. When the values for C_2^5 and $C_{2,1}^5$ are substituted into (13), one has:

$$\hat{J}_2 \bar{e}_1^1 \bar{e}_2^3 - \hat{J}_2 \bar{e}_1^1 \bar{e}_2^3 = 0 \quad (13a)$$

The implications of eqn. (13) have been discussed previously by others [4,17], but, in my opinion, eqns. (13) and (13a) describe the amplification of the control exerted by enzyme 2 in a most concise and clear manner. The greater the fractional flux through J_2 the greater will be the amplification of control by E_2 . However, it is clear from eqn. (13a) that amplification of C_2^5 is impossible

if either $\bar{e}_1^1$ or $\bar{e}_2^3$ equals zero, for in that case neither E_2 nor $E_{2,1}$ shares in the control of J_5 . All of the conclusions reported by Fell & Sauro [4] concerning amplification in substrate cycles are substantiated by the expressions in Appendix B. Amplification is a property of the branched pathway rather than a property of the substrate cycle. The substrate cycle is unique in that the fractional flux in the opposite direction amplifies the control exerted by the enzyme catalysing flux in the forward direction.

This work was supported in part by funds from the Grace M. Showalter Residuary Trust. I express my appreciation to Dr. A. K. Sen for helpful discussions during the course of this work.

REFERENCES

1. Savageau, M. A. (1972) *Curr. Top. Cell. Regul.* **6**, 63–130
2. Kacser, H. & Burns, J. A. (1972) *Symp. Soc. Exp. Biol.* **27**, 65–104
3. Westerhoff, H. V. & van Dam, K. (1987) *Thermodynamics and Control of Biological Free Energy Transduction*, pp. 162–186, Elsevier, Amsterdam
4. Fell, D. A. & Sauro, H. M. (1985) *Eur. J. Biochem.* **148**, 555–561
5. Crabtree, B. & Newsholme, E. A. (1985) *Curr. Top. Cell. Regul.* **25**, 21–76
6. Reder, C. (1988) *J. Theor. Biol.* **135**, 175–201
7. Welch, G. R., Keleti, T. & Vertessy, B. (1988) *J. Theor. Biol.* **130**, 407–422
8. Barrett, J. (1989) *Biochim. Biophys. Acta* **992**, 369–374
9. Hofmeyr, J.-H. S. (1989) *Eur. J. Biochem.* **186**, 343–354
10. Sen, A. K. (1990) *Biochem. J.* **269**, 141–147
11. Cascante, M., Franco, R. & Canela, E. I. (1989) *Math. Biosci.* **94**, 271–288
12. Cascante, M., Franco, R. & Canela, E. I. (1989) *Math. Biosci.* **94**, 289–309
13. Chua, L. O. & Lin, P.-M. (1975) *Computer-Aided Analysis of Electronic Circuits* pp. 616–663, Prentice/Hall, Englewood Cliffs
14. Fisher, D. D. & Schulz, A. R. (1969) *Math. Biosci.* **4**, 189–200
15. Hofmeyr, J.-H. S. (1986) *Comput. Appl. Biosci.* **2**, 5–11
16. Sorribas, A. & Savageau, M. A. (1989) *Math. Biosci.* **94**, 161–193
17. Crabtree, B. (1976) *Biochem. Soc. Trans.* **4**, 999–1002
18. Westerhoff, H. V. & Chen, Y. (1984) *Eur. J. Biochem.* **142**, 425–430
19. Hofmeyr, J.-H. S., Kacser, H. & van der Merwe, K. J. (1986) *Eur. J. Biochem.* **155**, 631–641

APPENDIX A

Flux Control Coefficients for pathway A

$$\begin{aligned} |D| &= -[e_1^2 e_2^3 e_3^4 + e_1^2 e_2^3 e_3^4 + \bar{e}_1^1 e_2^3 e_3^4 + \bar{e}_1^1 e_2^3 e_3^4 + \bar{e}_1^1 e_2^3 e_3^4 + \bar{e}_1^1 e_2^3 e_3^4] \\ C_1 &= -[\bar{e}_1^1 e_2^3 e_3^4 + \bar{e}_1^1 e_2^3 e_3^4]/|D| \\ C_2 &= -[\bar{e}_1^1 e_2^3 e_3^4]/|D| \\ C_3 &= -[\bar{e}_1^1 e_2^3 e_3^4]/|D| \\ C_4 &= -[\bar{e}_1^1 e_2^3 e_3^4 + \bar{e}_1^1 e_2^3 e_3^4]/|D| \end{aligned}$$

Concentration Control Coefficients for pathway A

$$\begin{aligned} C_1^{M_1} &= -[e_2^3 e_3^4 + \bar{e}_2^2 e_3^4 + \bar{e}_2^2 e_3^4 + e_2^3 \bar{e}_3^2]/|D| \\ C_2^{M_1} &= e_2^3 e_3^4/|D| \\ C_3^{M_1} &= \bar{e}_2^2 e_3^4/|D| \\ C_4^{M_1} &= [\bar{e}_2^2 e_3^4 + e_2^3 \bar{e}_3^2]/|D| \\ C_1^{M_2} &= -[-e_1^3 e_3^4 + e_1^2 e_3^4 + e_1^2 \bar{e}_3^2 - e_1^3 \bar{e}_3^2]/|D| \\ C_2^{M_2} &= -[e_1^3 e_3^4 + \bar{e}_1^1 e_3^4 + \bar{e}_1^1 \bar{e}_3^2]/|D| \\ C_3^{M_2} &= [e_1^3 e_3^4 + \bar{e}_1^1 e_3^4 + \bar{e}_1^1 \bar{e}_3^2]/|D| \\ C_4^{M_2} &= [e_1^3 e_3^4 - e_1^2 e_3^4 + \bar{e}_1^1 e_3^4 - \bar{e}_1^1 \bar{e}_3^2]/|D| \\ C_1^{M_3} &= -[e_1^2 e_3^4 + e_1^2 \bar{e}_3^2]/|D| \\ C_2^{M_3} &= -\bar{e}_1^1 e_3^4/|D| \\ C_3^{M_3} &= -\bar{e}_1^1 \bar{e}_3^2/|D| \\ C_4^{M_3} &= [e_1^2 e_3^4 + e_1^2 \bar{e}_3^2 + \bar{e}_1^1 e_3^4 + \bar{e}_1^1 \bar{e}_3^2]/|D| \end{aligned}$$

APPENDIX B

Flux Control Coefficients for pathway B

$$\begin{aligned}
 |D| &= J_2 J_5 \bar{e}_1^{2,1} \bar{e}_2^3 + J_2 \bar{e}_1^{2,1} \bar{e}_2^{2,1} + J_5 \bar{e}_1^2 \bar{e}_2^3 + J_5 \bar{e}_1^{2,1} \bar{e}_2^3 + \bar{e}_1^2 \bar{e}_2^3 \\
 C_1^2 &= [-J_5 \bar{e}_1^{2,1} \bar{e}_2^3 + \bar{e}_1^2 \bar{e}_2^{2,1} - \bar{e}_1^{2,1} \bar{e}_2^3]/|D| \\
 C_2^2 &= [J_5 \bar{e}_1^{2,1} \bar{e}_2^3 + \bar{e}_1^2 \bar{e}_2^{2,1}]/|D| \\
 C_{2,1}^2 &= [J_5 \bar{e}_1^2 \bar{e}_2^3 + J_5 \bar{e}_1^{2,1} \bar{e}_2^3 + \bar{e}_1^2 \bar{e}_2^3]/|D| \\
 C_3^2 &= [-J_5 \bar{e}_1^2 \bar{e}_2^{2,1} + J_5 \bar{e}_1^{2,1} \bar{e}_2^3 - J_5 \bar{e}_1^2 \bar{e}_2^{2,1}]/|D| \\
 C_1^6 &= [J_2 \bar{e}_1^{2,1} \bar{e}_2^3 + \bar{e}_1^{2,1} \bar{e}_2^3]/|D| \\
 C_2^6 &= \bar{e}_1^2 \bar{e}_2^3/|D| \\
 C_{2,1}^6 &= -J_2 \bar{e}_1^{2,1} \bar{e}_2^3/|D| \\
 C_3^6 &= [J_2 \bar{e}_1^{2,1} \bar{e}_2^{2,1} + \bar{e}_1^2 \bar{e}_2^3]/|D|
 \end{aligned}$$

Concentration Control Coefficients for pathway B

$$\begin{aligned}
 C_1^{M_1} &= [J_2 \bar{e}_1^{2,1} + J_5 \bar{e}_2^3 + \bar{e}_2^3]/|D| \\
 C_2^{M_1} &= -J_5 \bar{e}_2^3/|D| \\
 C_{2,1}^{M_1} &= J_2 J_5 \bar{e}_2^3/|D| \\
 C_3^{M_1} &= -[J_2 J_5 \bar{e}_2^{2,1} + J_5 \bar{e}_2^3]/|D| \\
 C_1^{M_2} &= [J_2 \bar{e}_1^{2,1} + \bar{e}_1^2]/|D| \\
 C_2^{M_2} &= \bar{e}_1^2/|D| \\
 C_{2,1}^{M_2} &= -J_2 \bar{e}_1^{2,1}/|D| \\
 C_3^{M_2} &= -[J_2 J_5 \bar{e}_1^{2,1} + J_5 \bar{e}_1^2 + J_5 \bar{e}_1^{2,1}]/|D|
 \end{aligned}$$

APPENDIX C

Flux Control Coefficients for pathway C

$$\begin{aligned}
 |D| &= \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 \\
 &\quad + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 \\
 &\quad + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4/|D| \\
 C_1 &= [\bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_2 &= [\bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_3 &= [\bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_4 &= [\bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_3/M_4 - \bar{e}_1^2 \bar{e}_3^4 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 \bar{e}_4^3 M_1/M_4]/|D|
 \end{aligned}$$

Concentration Control Coefficients for pathway C

$$\begin{aligned}
 C_1^{M_1} &= [\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_2^3 \bar{e}_3^4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_4^3 + \bar{e}_2^3 \bar{e}_3^4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_2/M_4]/|D| \\
 C_2^{M_1} &= -[\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_2^3 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4]/|D| \\
 C_3^{M_1} &= [-\bar{e}_2^3 \bar{e}_3^4 - \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4]/|D| \\
 C_4^{M_1} &= [-\bar{e}_2^3 \bar{e}_3^4 - \bar{e}_2^3 \bar{e}_4^3 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_2/M_4]/|D| \\
 C_1^{M_2} &= [-\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 - \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_2^{M_2} &= [\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_3^{M_2} &= -[\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_4^{M_2} &= [-\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 - \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_3/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_1^{M_3} &= [\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 M_3/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_2^{M_3} &= [\bar{e}_1^2 \bar{e}_3^4 - \bar{e}_1^2 \bar{e}_3^4 M_2/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 - \bar{e}_1^2 \bar{e}_3^4 M_2/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_3^{M_3} &= [\bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 M_2/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_1^2 \bar{e}_3^4 M_2/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 + \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D| \\
 C_4^{M_3} &= [-\bar{e}_1^2 \bar{e}_3^4 - \bar{e}_1^2 \bar{e}_3^4 - \bar{e}_1^2 \bar{e}_3^4 - \bar{e}_1^2 \bar{e}_3^4 + \bar{e}_1^2 \bar{e}_3^4 M_2/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_1/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_2/M_4 - \bar{e}_2^3 \bar{e}_4^3 M_1/M_4]/|D|
 \end{aligned}$$

Received 22 August 1990/11 February 1991; accepted 13 March 1991