Control analysis applied to the whole body: control by body organs over plasma concentrations and organ fluxes of substances in the blood

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Metabolic control analysis is adapted as a method for describing and analysing the control by organs in the body over the fluxes and concentrations of substances carried in the blood. This physiological control analysis can most usefully be applied to substances with fluxes into and out of organs that are uniquely dependent only on their plasma concentrations. The organ flux of a substance is defined as the steady-state net flux of a substance into a particular organ. The organ flux control coefficients quantify the extent to which a particular organ controls the flux of a substance into the same or another

particular organ. Organ concentration control coefficients quantify the extent to which an organ controls the steady-state concentration of a substance in the blood. The control coefficients are additive and obey summation, connectivity and branching theorems. Thus the control coefficients can be determined experimentally by measuring the sensitivities (elasticities) of organ fluxes to the plasma concentration of the substance. As an example of the application of these concepts, the control of ketone-body metabolism *in vivo* is analysed using data from the literature.

INTRODUCTION

Blood contains many substances, including metabolites, ions, dissolved gases, drugs, hormones, transport proteins and cells. These 'substances' are produced in some organs and consumed in others, as a result of either metabolism or transport. The control of the plasma levels of a substance and the control of the rates of entry to and exit from the blood have conventionally been described in terms of regulation of individual organs. However, there are now considerable data available, obtained from *in vivo* arteriovenous concentration differences and tracer fluxes, on the rates of production and utilization of metabolites, hormones and drugs *in vivo* [see for example Soling and Seufert (1978), Girard et al. (1992), Sacca et al. (1992) and Pye et al. (1993)]. These data and techniques could be used to examine the interaction amongst different organs in determining fluxes and plasma concentrations *in vivo*. However, there is as yet no theoretical framework for examining the extent to which different organs affect fluxes or plasma concentrations. Different organs have been claimed to be 'rate-limiting' for various whole-body processes [such as ketone-body synthesis (Foster, 1984) and maximal metabolic rate (Taylor, 1987; Weibel, 1987)], but the concept of a 'rate-limiting' organ has not been defined or quantified, although Crabtree and Newsholme (1985) have discussed in a general way the interactions amongst organs via common metabolites.

Metabolic control analysis is a method of analysing the control of steady-state rates and concentrations in a metabolic pathway. This type of analysis was initiated by Kacser and Burns (1973) and Heinrich and Rapoport (1974) and has since undergone a number of revisions and extensions (reviewed in Kacser and Porteous, 1987; Cornish-Bowden and Cardenas, 1990; Fell, 1992; Quant, 1993). It has recently been adapted to analyse control within single enzymes by rate constants (Brown and Cooper, 1993). Here I adapt the 'top-down' (Brown et al., 1990) and 'modular' approaches (Kahn and Westerhoff, 1991; Schuster et al., 1993) to metabolic control analysis to describe how the different organs of the body control the concentration of a substance in the blood and the rates at which that substance is used by the organs.

A number of conditions and assumptions are necessary in

order to apply control analysis to the body, and these conditions limit the application of the analysis. There must be a steady state for the fluxes and concentrations analysed. The substances analysed must have uniform concentrations throughout the arterial system, and must not be produced or consumed within the blood or lymph, so that all production or consumption is confined to organs or well-defined tissues. The substances analysed must not affect blood flow. In order to analyse control in terms of the relative sensitivities of different organs to the arterial concentrations of substances, these concentrations must be the same for the different organs, and this requirement may exclude certain organs, such as the liver with an arterial supply not derived directly from the left heart, from such an analysis. The analysis is simplest and most useful when interactions between organs are mediated by a single substance in the blood plasma, and the analysis may become too complex to be useful when interactions are mediated by several metabolites and/or hormones. Violation of these assumptions and conditions for some blood substances and organs may limit the practical application of this analysis. Nevertheless for many blood substances, the analysis may be of practical and conceptual use.

DEFINITIONS AND THEOREMS

The circulation of the blood to and from the organs of the mammalian body can be represented as in Scheme 1. Blood is pumped from the left heart via the arteries to the organs (including the heart muscle via the coronary artery), and then via veins to the right heart, which then pumps the blood back via the lungs to the left heart. For any substance which attains a steady-state concentration in the blood, and which is not produced or consumed in the blood itself, there must be one or more organs that produce or transport the substance into the blood, and one or more organs that consume or transport the substance out of the blood. We shall call the steady-state net flux of a substance from the blood into any one organ *in vivo* the 'organ flux' of the substance, which will be positive for flux into the organ and negative for flux out of the organ. Thus, if the substance is designated X and the organ is designated i, then the organ flux of that substance (designated J_i^X) will be given by:

$$J_i^X = ([X]_a - [X]_{v,i}) RBF_i \quad (1)$$

Scheme 1 Diagram of blood circulation to and from organs in the body

The arrows indicate the direction of blood flow. The coronary circulation for perfusion of the heart is distinguished from passage of the blood through the ventricles of left and right heart.

where RBF_i is the rate of blood flow through organ i , $[X]_a$ is the steady-state arterial plasma concentration of substance X , and $[X]_v$ is the steady-state venous plasma concentration of substance X for organ i . In any one state of the body, the arterial concentration of a substance is normally the same for all organs, except the lungs and liver (see later), but to remove ambiguity we will take $[X]_a$ to refer to the plasma concentration of X in the aorta. $[X]_a$ will sometimes be abbreviated to $[X]$ or X . In the steady state where $[X]$ is unchanging, the sum of all organ fluxes for all organs in the body is equal to zero, i.e.:

$$\sum_{i=1}^n J_i^X = 0 \quad (2)$$

where n is the total number of organs in the body. This is simply a definition of the steady state.

In what follows we need to distinguish between variables and parameters of the system, parameters being quantities that affect the system behaviour without being influenced by changes in the system (e.g. kinetic constants of enzymes, concentrations of substances that are not variables of the system) and variables being quantities that are influenced by changes in the system (e.g. organ fluxes, concentrations of X or other substances influenced by changes in organ flux).

In vivo in the whole-body $[X]_a$ is a variable determined by the kinetic parameters of the organs, but with the isolated (or effectively isolated) organ $[X]_a$ is determined by the experimenter. In the latter case (where the arterial concentrations of all plasma substances are held constant), the net flux of X into organ i will be called the 'local rate' of organ i , with symbol v_i . Now the local rate of an organ is in general a function of $[X]$ and of both external and internal parameters:

$$v_i = f([X]_a, [Y]_a, \dots, [E_1], [E_2], \dots, k_1, k_2, \dots, RBF_i) \quad (3)$$

The external parameters include the plasma concentrations of effectors or regulators of that flux (e.g. Y), whereas internal parameters include the concentrations ($[E]$) and kinetic parameters (e.g. k_{cat} and K_m values) of the individual enzymes of the organ, and the rate of blood flow through the organ (RBF_i). The venous concentrations of X and Y do not appear in eqn. (3)

because, with a positive blood flow rate, the venous concentrations are determined by the above parameters (i.e. the venous concentrations are dependent variables).

Now the sensitivity of the local rate of X into an organ i to a change in the arterial plasma concentration of X can be defined as follows:

$$\epsilon_X^{v_i} = \frac{\partial v_i}{\partial [X]_a} \times \frac{[X]_a}{v_i} \quad (4)$$

where ϵ is the elasticity coefficient of organ i to substance X , and the concentrations of all other substances in the plasma are constant. This is just the percentage change in the steady-state flux of X into organ i divided by the percentage change in the arterial concentration of X (extrapolated to infinitesimal change), when the concentrations of all other relevant blood substances are held constant. We may also define the sensitivity of the local rate of an organ i to any parameter (e.g. p) as follows:

$$\pi_p^{v_i} = \frac{\partial v_i}{\partial p} \times \frac{p}{v_i} \quad (5)$$

where π is the elasticity coefficient of organ i to parameter p , and where all other parameters and all plasma concentrations are constant. The parameter may be internal (e.g. an enzyme concentration) or external (e.g. an inhibitor).

In what follows we will make the major simplifying assumption that, when $[X]_a$ changes, no other substance (which is capable of changing the organ flux of X) changes plasma concentration significantly (unless otherwise stated). This assumption is not essential for developing a control analysis, but without this assumption the theorems generated are complex and less useful in practice. However, if the organ fluxes of X are regulated by other blood-borne effectors (e.g. hormones or metabolites) or by the autonomic nervous system, and if the arterial concentrations of these effectors (or the level of autonomic stimulation) can be regarded as a unique function of $[X]_a$, then a useful control analysis which differs little from that given below can still be generated (see later).

Eqn. (5) defines the sensitivity of the local rate of an organ i to a change in some parameter p *in vitro* (when all plasma concentrations are held constant). We may also define a 'response coefficient' quantifying the sensitivity of an organ flux to a change in some parameter p (when the plasma concentrations are determined by the system):

$$R_p^{J_i} = \frac{\partial J_i}{\partial p} \times \frac{p}{J_i} \quad (6)$$

This definition of the response coefficient is consistent with that proposed by Schuster and Heinrich (1992). Now when changes in p affect only the local rate of a single organ i , we can define a new coefficient quantifying the relative sensitivities of the organ flux and the local rate to a change in p :

$$C_{v_i}^{J_i} = R_p^{J_i} / \pi_p^{v_i} \quad (7)$$

$$= \left(\frac{\partial J_i}{\partial p} / \frac{\partial v_i}{\partial p} \right) \frac{v_i}{J_i} \quad (8)$$

where the elasticity to p of all organs other than i is zero. Now this 'organ flux control coefficient' (with abbreviated symbol C_i^J) is independent of the nature of p , i.e. the value is the same no matter which internal or external parameter is changed (as long

as p affects only v_i) (see Heinrich et al., 1977). Thus the definition of the control coefficient can be abbreviated as:

$$C_{v_i}^J = \frac{\partial J}{\partial v_i} \times \frac{v_i}{J} \quad (9)$$

The general properties of this type of definition are explored in Schuster and Heinrich (1992). Now if changes in p affect the local rate of only one organ i , then:

$$R_p^J = C_{v_i}^J \pi_p^{v_i} \quad (10)$$

But if changes in p affect the local rate of several organs then:

$$R_p^J = \sum_{i=1}^n (C_{v_i}^J \pi_p^{v_i}) \quad (11)$$

We may also define a 'concentration control coefficient' quantifying the extent to which any particular organ (e.g. organ i) controls $[X]_a$ as:

$$C_{v_i}^{[X]} = \left(\frac{\partial [X]_a}{\partial p} / \frac{\partial v_i}{\partial p} \right) \frac{v_i}{[X]_a} \quad (12)$$

$$= \frac{\partial [X]_a}{\partial v_i} \times \frac{v_i}{[X]_a} \quad (13)$$

where p affects the local rate of only organ i . This coefficient is the percentage change in the steady-state arterial concentration of X divided by the percentage change in the local rate of organ i (altered by changing some internal parameter by an infinitesimal amount), when the local rate of no other organ is changed and there are no changes in inputs to the body or environmental conditions. The coefficient may be abbreviated as C_i^X .

The flux and concentration control coefficients obey summation theorems:

$$\sum_{i=1}^n C_{v_i}^X = 0 \quad (14)$$

$$\sum_{i=1}^n C_{v_i}^J = 1 \quad (15)$$

where n is the total number of organs (and other tissues) in the body. Thus the sum of the concentration control coefficients of all organs (and other tissues) over the arterial concentration of a substance is equal to zero, and the sum of all flux control coefficients for all organs over the flux into any one particular organ is equal to 1. See Kacser and Burns (1973), Giersch (1988a) and Schuster and Heinrich (1992) for proofs of the analogous summation theorem for metabolic pathways. The above theorems for organs can be derived by suitable redefinition of parameters.

We need now a means of determining the control coefficients directly from the elasticities. When there are only two organs involved, one consuming the substance X (organ 1) and one producing the substance X (organ 2), then the organ fluxes must be equal (and of opposite sign); we will call this flux J . The connectivity theorems relating the control coefficients to the elasticities are:

$$C_{v_1}^J e_X^{v_1} + C_{v_2}^J e_X^{v_2} = 0 \quad (16)$$

$$C_{v_1}^X e_X^{v_1} + C_{v_2}^X e_X^{v_2} = -1 \quad (17)$$

Thus, the relative control coefficients of two organs over the common flux of a blood-borne substance is equal to the inverse of their relative elasticities to that substance. Analogous theorems for metabolic pathways are proven in Kacser and Burns (1973) and Giersch (1988b), and the above theorems may be proven by redefinition of parameters.

Combining the connectivity theorems with the summation theorems, we can obtain the absolute control coefficients directly from the elasticities. Thus, for the concentration control coefficients:

$$C_{v_1}^X = -1/(e_X^{v_1} - e_X^{v_2}) \quad (18)$$

$$C_{v_2}^X = 1/(e_X^{v_1} - e_X^{v_2}) \quad (19)$$

Thus, where two organs have equal and opposite fluxes of a substance, they have equal and opposite control coefficients over the concentration of the substance in the blood.

When more than two organs are involved in the metabolism or transport of a substance, then there is more than one flux over which control is exerted. For example, when there are three organs, there are three fluxes, three concentration control coefficients and three flux control coefficients over each flux (i.e. nine flux control coefficients). The connectivity theorems take a more general form derived for metabolic pathways in Kacser and Burns (1973), Westerhoff and Chen (1984) and Giersch (1988b):

$$\sum_{j=1}^n (C_{v_j}^J e_X^{v_j}) = 0 \quad (20)$$

$$\sum_{j=1}^n (C_{v_j}^X e_X^{v_j}) = -1 \quad (21)$$

In order to describe the control of flux into or out of one particular organ (e.g. organ i) we may conceptually group together all other organs as one unit (which we will call the 'rest of the body' or 'rb'). The control coefficient of this 'rest of the body' unit is just the sum of the control coefficients of the individual organs making up the rest of the body. The elasticity of the rest of the body is a complex function of the elasticities and fluxes of the individual organs (Fell and Sauro, 1985). Now the connectivity theorem relating the elasticities and control coefficients of the organ of interest and the rest of the body is the same as before, i.e. for flux control:

$$C_{v_i}^J / C_{v_{rb}}^J = -e_X^{v_{rb}} / e_X^{v_i} \quad (22)$$

In the steady state, $J_{rb} = -J_i$, and thus J_{rb} can be estimated from J_i . Now the relative control coefficients of the different organs that make up the rest of the body are equal to their relative organ fluxes of X , i.e.

$$C_{v_j}^J / C_{v_k}^J = J_j^X / J_k^X \quad (23)$$

$$C_{v_j}^X / C_{v_k}^X = J_j^X / J_k^X \quad (24)$$

where all fluxes are organ fluxes of X . These relations are known as the branching theorems and were derived for branching metabolic pathways in Fell and Sauro (1985) and Sauro et al. (1987).

Now we have shown that (a) the control coefficients of an organ relative to the control exerted by the rest of the body over the organ flux can be determined from the relative elasticities to the substance in question [eqn. (22)], (b) the relative control coefficients of the individual organs making up the rest of the body can be obtained from their relative organ fluxes [eqns. (23) and (24)] and (c) the sum of all organ control coefficients is 1 for fluxes and zero for concentrations [eqns. (14) and (15)]. Thus all the absolute control coefficients may be derived.

Alternatively all control coefficients can be obtained from the elasticities and fluxes of the individual organs, using equations derived by combining the summation, connectivity and branching theorems (Brown et al., 1990; Fell and Sauro, 1985; Sauro et al., 1987). Thus

$$C_{v_i}^X = J_i^X / *e \quad (25)$$

$$C_{v_i}^{J_j} = J_i^X e_{X_j}^{v_i} / *e \quad (26)$$

$$C_{v_i}^{J_i} = (J_i^X e_{X_i}^{v_i} / *e) + 1 \quad (27)$$

where

$$*e = - \sum_{i=1}^n (J_i^X e_{X_i}^{v_i}) \quad (28)$$

These equations (25–28), plus eqn. (11), summarize the control analysis for the whole body. $*e$ [defined by eqn. (28)] is the total sensitivity of the body to changes in the arterial level of X, and is given by the product of the organ flux and elasticity to X summed for all organs. Eqn. (25) gives the concentration control coefficient of any organ in terms of the fluxes and elasticities of all organs. Eqn. (26) gives the control coefficient of an organ over the flux into any other organ in terms of the fluxes and elasticities of all organs. Eqn. (27) gives the control coefficient of an organ over its own flux in terms of the fluxes and elasticities of all organs.

COMPLICATIONS

Non-homogeneous organs, and control within the organ

Metabolic control analysis was originally developed to describe control by individual enzymes (or transporters) over metabolic pathways in cells. How do these flux control coefficients of individual enzymes over cellular pathways relate to the control coefficients of organs over organ fluxes in the whole body (described in this paper)? We can define the flux control coefficient of enzyme E_1 in isolated organ i with local enzyme rate v_{1i} over the steady-state organ flux (V_i) of X into isolated organ i as:

$$C_{v_{1i}}^{v_i} = \left(\frac{\partial V_i}{\partial p} \right) \frac{v_{1i}}{V_i} \quad (29)$$

where the parameter change affects the local rate of no other enzyme. When the parameter changed is the concentration of E_1 , then this definition reduces to that used by Kacser and Burns (1973). We can also define the flux control coefficient of enzyme E_1 in organ i over the organ flux of organ j in the whole body as:

$$C_{v_{1i}}^{J_j} = \left(\frac{\partial J_j}{\partial p} \right) \frac{v_{1i}}{J_j} \quad (30)$$

where p affects the only local rate of E_1 in organ i . It is easily shown that:

$$C_{v_{1i}}^{J_j} = C_{v_i}^{J_j} C_{v_{1i}}^{v_i} \quad (31)$$

The interpretation of these coefficients is straightforward if the organ is homogeneous, in the sense that all components of the organ are equally distributed. However, if the organ is not homogeneous, then the local environment of the enzyme will vary, so that the elasticity of the enzyme is difficult to define because there are a range of substrate, product and effector concentrations of the enzyme, and a range of enzyme rates. Similarly the control coefficients of enzymes measured in isolated cells or organelles will be difficult or impossible to relate to their control coefficients in organs or the whole body if the organs are not homogeneous, as the cells or organelles will be in a range of

environments in the intact system, but presumably not in the isolated system.

Organs may be non-homogeneous because: (a) there are different cell types in the same organ, (b) the same cell type may vary in its enzyme make up in different parts of an organ and (c) there are gradients of substrates, products and effector molecules through organs. The control analysis outlined here for organs does not assume that an organ is a metabolically homogeneous entity, and these non-homogeneities do not invalidate the use of the summation, branching and connectivity theorems as applied to organ fluxes. However, as stated above, these non-homogeneities do complicate the measurement of control by individual enzymes within the organ, and the relation between the control coefficients of an enzyme measured in isolated cells to that in an intact organ *in vivo* becomes problematic in a heterogeneous tissue.

Flux regulators other than X

For some substances, changes in the plasma concentration of the substance of interest [X] cause changes in the plasma concentration of other substances, e.g. [Y], which directly affect the organ fluxes of X. For example, changes in the blood concentration of glucose result in changes in the concentration of insulin and glucagon, which in turn alter the tissue metabolism of glucose. Now if [Y] (or any number of other metabolites and hormones) is a unique function of [X], we can alter the connectivity theorems to take account of the elasticity to these effectors. For example, with two organs and the concentrations of two effectors Y and Z being a unique function of [X], then:

$$C_{v_1}^{v_{X,Y,Z}} e_{X,Y,Z}^{v_1} + C_{v_2}^{v_{X,Y,Z}} e_{X,Y,Z}^{v_2} = 0 \quad (32)$$

$$C_{v_1}^{X} e_{X,Y,Z}^{v_1} + C_{v_2}^{X} e_{X,Y,Z}^{v_2} = -1 \quad (33)$$

where $e_{X,Y,Z}$ is the total response of the organ to changes in X (including the effects of the changing levels of Y and Z resulting from the change in X), and is given by:

$$e_{X,Y,Z}^{v_i} = e_{X_i}^{v_i} + (e_{Y_i}^{v_i} R_{X_i}^Y) + (e_{Z_i}^{v_i} R_{X_i}^Z) \quad (34)$$

where

$$R_{X_i}^Y = \frac{\partial [Y]_a}{\partial [X]_a} \times \frac{[X]_a}{[Y]_a} \quad (35)$$

where $[X]_a$ is altered directly by the experimenter *in vivo*. These relations can be proved in the same way as the simpler connectivity theorems. In order to estimate the relative control coefficients experimentally with isolated perfused organs, the elasticities to [X] and [Y] and [Z] would have to be measured with perfused organs, while the response coefficient (R) of [Y] to [X] would have to be measured *in vivo*. However, if the elasticity measurements are made *in vivo*, then we can simply measure the total response to [X] (i.e. $e_{X,Y,Z}$) directly, because any manipulation of [X] will automatically change [Y] by the appropriate amount. Thus the total response of the organ of interest could be estimated by changing the local rate of the rest of the body and measuring the sensitivity of the organ flux to the induced change in [X], and the total response of the rest of the body could be estimated by changing the local rate of the organ in question and again measuring the dependence of the organ flux on [X]. The summation and branching theorems hold in the usual form for systems where all flux regulators are a unique function of $[X]_a$ (see Brown et al., 1990).

When concentrations of regulators are not a unique function of [X], or when there are two or more interacting fluxes, then, although the analysis can be adapted to describe control in these

systems, in practice it becomes difficult to estimate the control coefficients from the elasticities. Schuster et al. (1993) have discussed some of the difficulties involved, and ways of overcoming them.

The lungs, abdomen and heart

The circulation of the lungs differs from that of other organs in that the arterial blood supply does not derive from the left heart, but rather from the right heart (Scheme 1). Thus the concentration of a substance in the arterial blood supply will differ between the lungs and other organs if the flux of that substance into or out of the lungs is significant. For such a substance, the summation and branching theorems still hold for the lungs, so that the control by the lungs relative to other organs over plasma concentrations and other organ fluxes can be derived. However, a useful form of the connectivity theorem is not derivable, so the control by the lung relative to other organs over lung fluxes cannot be estimated from elasticities.

The arterial supply to the liver is derived in part directly from the left heart (via the hepatic artery) and in part from the veins of stomach, intestine, pancreas and spleen (via the portal vein). The summation theorem still applies to the liver and all abdominal organs, but the branching theorem is not strictly valid when applied to the individual organs of the abdomen, and no useful form of connectivity theorem can be applied to these organs. Thus, if fluxes are significant for both the liver and other abdominal organs, it is necessary to treat the abdominal (or splanchnic) organs as one unit.

The blood may pass through three different parts of the heart during the circulation: the atrium and ventricle of the right heart, the atrium and ventricle of the left heart, and the coronary circulation (Scheme 1). Metabolic fluxes are normally only significant for the coronary circulation. However, for some fluxes such as the maximal metabolic rate, the pumping function of the heart may limit the overall rate. Such control by blood flow cannot be easily incorporated into a control analysis.

Changes in relative blood flow through organs

Changes in the blood concentration of certain substances (particularly CO_2 , O_2 , adenosine and adrenaline) cause changes in the relative blood flow through different organs. Changes in blood flow can affect the organ flux of some substances. Such effects could be described in terms of the total elasticity to the substance of interest, but we would have to be sure that the level of vasoactive substance was a unique function of the concentration of the substance of interest $[X]_a$ in the conditions used. Fortunately, for most substances, the effect on blood flow is unlikely to be significant. When measuring organ elasticities in isolated organs, it is necessary to ensure that the rate of blood flow is identical with that *in vivo*.

Steady states

Concentrations of substances in blood plasma may not reach true steady states *in vivo*. There are two main reasons for this. Firstly, *in vivo* there are many external perturbations occurring at various time intervals, e.g. muscle use, feeding, stress and sleep. In the laboratory, these perturbations can be controlled, but the relaxation time of the steady state, particularly to feeding, may be long. Secondly, changes in the blood concentration of a substance may elicit changes in organ fluxes over various time scales, depending on the mechanism of these changes. Changes in flux due to X acting as a substrate, product or effector may be

relatively fast, changes due to X affecting hormone concentrations may be fast or slow, changes due to secondary effects on gene expression may be relatively slow. Thus the elasticity and control coefficients of an organ may be significantly time dependent. It is essential to be aware of this time dependence, and not compare coefficients of different organs over different time scales.

Production or consumption by the blood or lymph

Some substances are produced or consumed by the blood itself or by the lymphatic system. A control analysis constructed in terms of organs is not suitable for such substances.

EXPERIMENTAL APPLICATION OF THE ANALYSIS

Control coefficients may be measured either (a) directly by estimating the ratio of response and elasticity coefficients to a parameter affecting the local rate of one organ or (b) indirectly by measuring the relative elasticities of different organs to the plasma concentration of the substance involved in the fluxes (X).

The direct method requires a specific effector or treatment of the organ of interest. The relative sensitivities of the steady-state fluxes to this effector when *in vivo* and when the organ is isolated (or effectively isolated *in vivo* by clamping [X]) could then be measured and used to estimate the control coefficients. Alternatively the initial effect of the effector on the organ flux *in vivo* might be used as an estimate of the effector elasticity, and compared with the effect on the eventual steady-state organ flux, to give the ratio of effector response and effector elasticity coefficients. Dividing the response coefficient by the effector elasticity coefficient gives the control coefficient [see eqn. (7)]. However, this requires that the effector is specific to the identified organ, and that the response and elasticity coefficients are measured under the same conditions. If we have an inhibitor or stimulator of organ flux specific to a particular organ, we need to titrate the organ flux with measured concentrations of the effector when $[X]_a$ is held constant, either with the isolated organ or *in situ*. *In vivo* fluxes are then titrated with measured concentration of the effector, when $[X]_a$ is allowed to vary. The organ flux control coefficients are then derived from eqn. (7).

There are several different approaches to estimating control coefficients indirectly from X elasticities. One approach is to use isolated organs perfused in conditions similar to those found *in vivo*, and then alter $[X]_a$ and measure the dependence of the organ flux of X on the arterial concentration of X. The other approach is to measure the elasticity to [X] *in vivo*, [X] being changed by for example intravenous, oral or respiratory administration, or by inhibiting or stimulating flux to other organs with drugs etc. However, it must be ensured that steady-state conditions apply, and perturbations do not cause changes in plasma concentrations of any substance other than X which affect the fluxes of X (except where the concentrations of regulator substances are unique functions of [X]).

If the control coefficients of only one organ are of interest, then the relative control coefficients of all organs except the organ of interest over that organ's flux of X may be determined from the relative fluxes of X. The control coefficient of the organ of interest relative to the rest of the body can be determined from the relative elasticities. The elasticity of the 'rest of the body' could be determined simply by inhibiting or stimulating (or otherwise specifically changing the local rate of) the organ of interest by different amounts and measuring the relation between $[X]_a$ and the flux of that organ. The flux of the 'rest of the body' can be estimated from that of the organ, as they are equal and

opposite in the steady state. $[X]_o$ can be changed without changing the local rate of the 'rest of the body' by inhibiting or stimulating the use of X in the organ of interest.

If local rates are changed *in vivo* by using inhibitors or stimulators, these effectors need to be specific to a particular organ, and such effectors are not always available. However, the local rate of an organ might also be changed by physical damage or removal, local cooling or heating, hormones or afferent nerve stimulation, or if the blood flow is low relative to the total blood flow, by changing the rate of organ blood flow.

Methods for estimating organ fluxes *in vivo* include measuring arteriovenous concentration differences and blood flow, specific radioactivity of continuously infused radiolabels in the blood, uptake of radiolabel into organs and saturation transfer of magnetic resonance. Methods also exist for holding plasma concentrations constant ('clamping') by infusion, e.g. for glucose, insulin and ketone bodies (Best et al., 1981; Radziuk and Lickley, 1985; Keller et al., 1988).

Arterial and venous plasma concentrations of substances can be measured and changed by catheter or microdialysis. Catheters or microdialysis probes can be inserted into arteries or veins to monitor or change plasma concentrations continuously, with usually negligible tissue damage. Thus one possible method for measuring elasticity and control coefficients is as follows. Catheters or microdialysis probes are inserted into the veins draining the organs of interest and into one artery. Organ fluxes of the substance of interest are calculated from arteriovenous concentration differences and organ blood flow rates (which can also be estimated using the probes). After an initial steady state has been established, the state is perturbed by introducing the substance of interest into the blood via one of the probes at a known (relatively low) rate; when a new steady state is established, all the new organ fluxes are measured. From these measurements the elasticities and fluxes, and thus the control coefficients of the organs, can be estimated.

There are considerable data available on the turnover rates of blood substances derived from the plasma dilution of radiolabel method [see, for example, Balasse and Neef (1975) and Sacca et al. (1992)]. In this method a small amount of the isotopically labelled substance of interest is continuously infused into a vein of the whole animal via a catheter, and blood samples are repeatedly taken to measure the blood or plasma level of the substance and its specific radioactivity. From the specific radioactivity and the infusion rate, the rate of transport into and out of the blood can be estimated. If the processes of production and utilization in respective organs are effectively irreversible, then these rates can also be estimated. Continuous infusion of the unlabelled substance allows the plasma concentration to be raised to a new steady-state level, and if rates of production and consumption are continuously measured by a parallel infusion of the labelled substance, then sensitivity of production and consumption to the increased plasma concentration can be measured (Balasse and Neef, 1975). Thus the control coefficients of production or utilization could be estimated from these elasticities. In the next section, this method is illustrated by analysing data obtained by the isotope dilution method on ketone-body turnover in humans.

EXAMPLE OF WHOLE-BODY CONTROL ANALYSIS APPLIED TO KETONE-BODY METABOLISM

In order to illustrate how the concepts set out above can be used to analyse control in the whole body, I will take the example of ketone-body metabolism. Ketone bodies (3-hydroxybutyrate and acetoacetate) are produced almost exclusively in liver in most

conditions from fatty acids supplied from the adipose tissue, and are used in most tissues except the liver [reviewed in Robinson and Williamson (1980), Foster (1984) and Girard et al. (1992)]. Plasma levels and turnover of ketone bodies are considerably increased during prolonged starvation, juvenile-onset diabetes, late pregnancy, lactation and suckling, i.e. conditions where plasma non-esterified fatty acid levels are elevated. The relation between ketone-body utilization rate in the whole body and blood concentration has been shown to be linear and proportional up to fairly high concentrations (2–5 mM) beyond which saturation is approached (Robinson and Williamson, 1980; Dietze et al., 1978; Soling and Seufert, 1978). A similar relationship between blood ketone-body concentration and rate of utilization has been shown by measurements of arteriovenous differences across individual tissues (brain, heart, skeletal muscle and kidney) in various species (human, rat, dog) (Robinson and Williamson, 1980; Dietze et al., 1978; Soling and Seufert, 1978).

The relationship between rates of ketone-body production and blood ketone-body concentrations has not been so well studied, but it has been shown that increasing blood levels by infusion of exogenous ketone bodies inhibits production, and inhibition is 'roughly proportional' to the rate of infusion (Balasse and Neef, 1975; Soling and Seufert, 1978). Balasse and Neef (1975) measured blood levels and turnover of ketone bodies in fasting humans before and during a continuous infusion of exogenous ketone bodies (Figure 1). Production and utilization of ketone bodies was measured by following their specific radioactivities in the blood during constant intravenous infusion of radiolabelled ketone bodies. The effect of infusing high levels of unlabelled ketone bodies was tested, the endogenous rate of production being derived from the total rate of influx into the blood pool minus the rate of infusion of exogenous ketone bodies. In the new steady state, the total rate of influx (or production) is equal to the total rate of efflux from the blood pool (utilization). The infusion increased blood ketone-body levels by 48–93 %, decreased endogenous production by 10–33 % and increased utilization by 19–66 % in six different subjects (Figure 1).

Now the ratio of decrease in production to increase in utilization gives an estimate of the relative elasticities of production and utilization to the blood levels of ketone bodies, and thus gives an estimate of the relative control coefficients. The ratio of the decrease in production to the increase in utilization ranged from 0.19 to 1.33 (mean 0.75) in the six subjects, and thus the estimated ratio of the control coefficients of utilization to production has the same values. The control coefficients of utilization over total turnover estimated from these data range from 0.15 to 0.57 (mean 0.39), and the estimated control coefficient of production over turnover range from 0.43 to 0.85 (mean 0.61). Thus production had on average 61 % of the control over turnover and utilization had 39 %. This means that both production and utilization have control over turnover, thus stimulation of production will also increase utilization, and stimulation of utilization will also increase production. The control coefficients over the blood ketone-body level can be calculated from the overall elasticities [using eqn. (33)], and the overall elasticities can be estimated by dividing the percentage change in production or utilization by the percentage change in ketone-body level. The concentration control coefficient calculated in this way ranged from 0.73 to 1.37 (mean 1.07), being equal and opposite for production and utilization (positive for utilization, negative for production). Thus stimulation or inhibition of production or consumption should cause roughly proportionate changes in blood ketone-body levels.

The measurements of Balasse and Neef (1975) are not ideal for the application of control analysis, particularly because the

Figure 1 Average response of six starved humans to infusion of acetoacetate, measured by Balasse and Neef (1975)

[^{14}C]Acetoacetate was continuously infused into starved adult human volunteers via a catheter, and the levels of plasma glucose, non-esterified fatty acids (NEFA), acetoacetate (ACAC) and 3-hydroxybutyrate (βOHB), and the specific radioactivity of ketone bodies were measured in samples. The total rate of inflow of ketone bodies into the blood was calculated from the specific radioactivity and exogenous infusion rate. The inflow rate is divided into the endogenous rate (i.e. the production rate) and the exogenous rate (i.e. the rate of infusion of exogenous ketone bodies). In a steady state the total inflow rate must equal the total outflow rate (i.e. the rate of ketone-body consumption by utilizing tissues). After 120 min, a high concentration of unlabelled acetoacetate was continuously infused into the volunteers, and the response of plasma concentrations measured. Data and diagram are from Balasse and Neef (1975) with the permission of W. B. Saunders Company.

perturbations to the blood levels were relatively large, but the example shows how relatively easy it can be to estimate control coefficients using the infusion method which has also been used extensively for glucose, fatty acids and amino acids, and could be used for most blood substances.

The control coefficients estimated above are for the overall processes of production and utilization. The control coefficient of utilization can be divided up according to the relative rates of utilization by different organs [eqns. (23) and (24)]. Dietze et al. (1978) have estimated that 85–90% of ketone bodies are used by

brain and muscles in starved adult humans, brain and muscle having roughly equal rates. Thus most of the control exerted by the utilizing processes is shared roughly equally between brain and muscle. The control coefficients of production involve both ketogenesis in and fatty acid supply to the liver. The relative control by these processes could be estimated by analysing the control by different organs over plasma fatty acid levels, and the sensitivity of liver ketogenesis to plasma fatty acid level.

Control coefficients refer to only one state. The control coefficients over ketone-body turnover in the fed state are likely to be very different from those estimated above for the fasted state. In the fed state the blood ketone-body levels are much lower and there is unlikely to be any significant inhibition of ketone-body production by blood ketone-body levels. Thus almost all control over production is likely to be in the production process itself. However, control over the blood level will be equally distributed between production and consumption, and the different tissues consuming ketone bodies will compete for them, having negative control coefficients over each other's fluxes proportionate to the tissue fluxes. At the very high levels of blood ketone bodies reached in untreated diabetes, ketone-body utilization becomes saturated (Soling and Seufert, 1978; Robinson and Williamson, 1980), so that control over production should be mostly in the utilization processes.

DISCUSSION

The control analysis outlined here may have two main uses: (1) as an experimental method for estimating organ control coefficients and thus relating the sensitivity of isolated organs to organs *in vivo*, and (2) as a conceptual tool for thinking about control *in vivo* and the interrelation between organ fluxes.

The definitions of the control coefficients in terms of local rates given in this paper are analogous to the definitions given in Heinrich et al. (1977) and Schuster and Heinrich (1992) for enzymes, and ensure that the summation, connectivity and branching theorems hold also for organs, at least in the simple case where organs interact via a single substance (see Giersch 1988a,b). Because the control coefficients are defined in terms of local rate (rather than total enzyme concentrations), it is not necessary to assume that the organs are homogeneous in terms of metabolite concentrations, cell type or gene expression.

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