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Chapter 15

NUTRIENT–RESPONSE: A "TOP DOWN" APPROACH TO METABOLIC CONTROL

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I. INTRODUCTION

Analysis of nutrient–response curves may appear prosaic to some nutritionists, but these curves contain a wealth of information. Proper interpretation of nutrient–response curves has resulted in astute conclusions. For example, Max Rubner concluded in his monograph, "In spite of the varying chemistry of catabolism which apparently occurs during changes in the form of nourishment, the energy metabolism is the determining factor and focal point around which everything else revolves" (Rubner, 1902). This is a rather accurate word description of the central role played by oxidative phosphorylation in cellular metabolism. This is truly remarkable since the monograph was published approximately 25 years before the isolation and characterization of adenosine triphosphate and nearly 40 years before the identification of the process of oxidative phosphorylation. Clearly, the analysis of nutrient-response curves constitutes a worthwhile facet of mathematical modeling in nutrition.

II. MATHEMATICAL TREATMENT

The first prerequisite to interpretation of nutrient–response in terms of the metabolic fate of a given nutrient is a reasonable understanding of the nutrient-response curve itself. Anyone who has looked at a nutrient–response curve should have been convinced that the curve is nonlinear provided the curve encompasses a reasonable range of nutrient intake. Typical nutrient–response curves are shown in Fig. 1. At higher levels of nutrient intake, inhibition of the response may be observed in any of the foregoing curves. The equation and parameter constraints will be referred to later in the text.

A. INTERPRETATION OF NUTRIENT–RESPONSE CURVES

A variety of techniques have been employed to analyze these nutrient–response curves, the worst of which is the attempt to fit these curves to a single straight line (for example, Keys *et al.*, 1959; Hegsted *et al.*, 1965; Said and Hegsted, 1969). Monod reported that the growth of bacterial cultures in the exponential phase of growth is a hyperbolic function of the limiting nutrient (Monod, 1949). Morgan *et al.* (1975) suggested that the response of higher animals to a nutrient is a rational function of nutrient intake provided that some of the parameters of the rational polynomial are arbitrarily assigned a value of zero. It has been suggested by others that the nutrient–response relationship is described better by a rational polynomial without any arbitrary assumption concerning the magnitude of any of the parameters (Schulz, 1987, 1991).

There has been significant speculation on the reason why nutrient–response curves appear to be described by rational polynomials. It has been

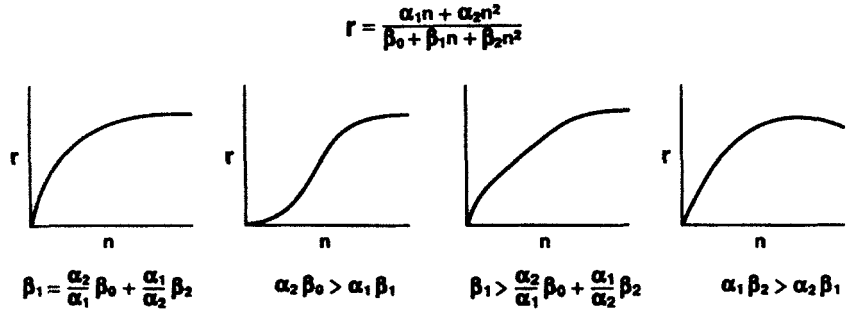

FIG. 1. Possible shapes of nutrient–response curves and the parameter constraints which allow these curves to be described by a 2:2 rational polynomial.

suggested that the nutrient–response curve reflects the kinetic behavior of a rate-limiting enzymic reaction in the pathway by which the nutrient is metabolized (Morgan *et al.*, 1975). On the other hand, Monod (1949) expressed the opinion that bacterial growth is too complex a process to be described by the kinetic behavior of a single enzymic reaction. In regard to this speculation, it is relevant that investigations of metabolic control at the level of individual pathways in which it is possible to obtain quantitative estimates of the control of the flux exerted by individual enzymes in the pathway indicate that, in most cases, the control of flux through a pathway is shared by more than one enzyme (see, for example, Westerhof *et al.*, 1987).

B. DERIVATION OF AN EXPRESSION FOR NUTRIENT–RESPONSE

An alternative to the empirical fitting of nutrient–response curves is the derivation of an explicit equation to describe the nutrient–response relationship (Schulz, 1992). A model for this approach lies in the stochastic approach to enzyme kinetics and biochemical cycle kinetics (Ninio, 1987; Hill, 1989; Mazur, 1991). In this treatment, the nutrient–response relationship is represented as a directed graph (digraph). The intermediate metabolites in the pathway(s) by which the nutrient is metabolized are the vertices of the digraph. It should be realized that some of the vertices may be the same metabolite found in a different pool. The allowable transitions between the metabolites are represented by the edges of the digraph. Each transition is assigned a weight which is the probability of the transition occurring. Thus, a metabolic pathway can be viewed as a series of interconnections of discrete states anyone of which might be visited during a random walk through the pathway represented by the digraph. One can calculate the probability that any specific state might be occupied during a large ensemble of random walks around the digraph. Alternately, if the transition to one or more termination states were to result in an instantaneous transition to the initial state, a similar result could be obtained by calculating the probability that a given state would be occupied at any time during a single continuous walk about the digraph (Hill, 1989). This is given as a conceptual aid and should not be construed as biochemical conversion of the termination state to the starting state.

Figure 2A is an example of a very simple metabolic pathway in which the starting state is vertex 1. This state can be considered to be the dietary nutrient. If states 4 and 5 are termination states, the pathway can be represented as the closed diagram of Fig. 2B. There are two cycles in Fig. 2B one of which can be visualized as giving rise to the observed response. The expression derived for the probability of completion of either cycle is a 1 : 1

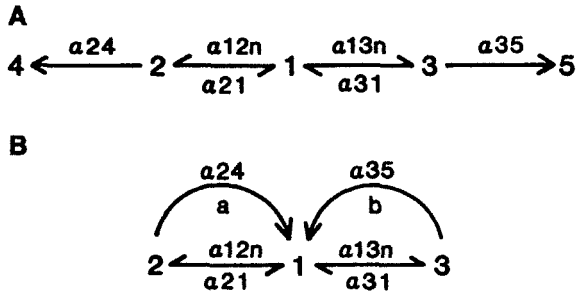

FIG. 2. (A) An open diagram of a metabolic pathway in which state 1 is the starting state and states 4 and 5 are termination states. (B) A closed diagram of the same metabolic pathway.

rational polynomial in nutrient intake. Figure 3 portrays a more complex pathway in which there are convergent paths each of which is dependent on nutrient intake. Figure 3B is the closed digraph if state 5 is the termina-

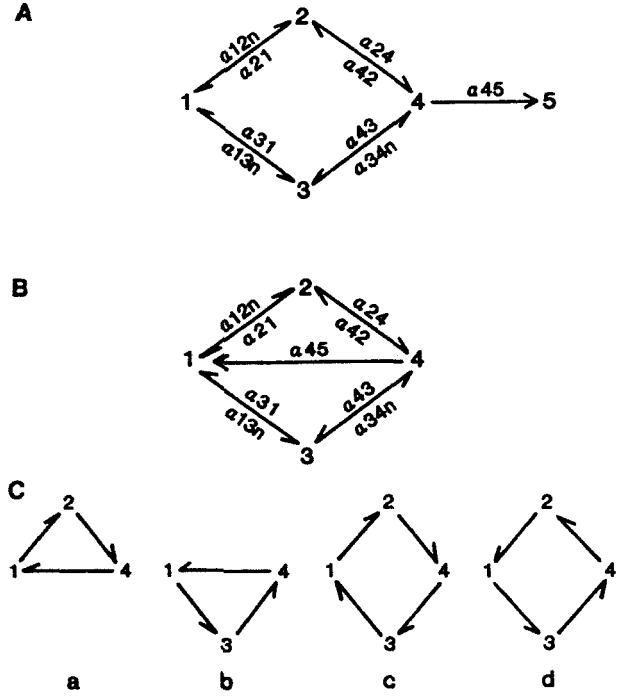

FIG. 3. (A) An open diagram of a metabolic pathway with parallel branches. State 1 is the starting state and state 5 is the termination state. (B) A closed diagram of the same pathway. (C) Cyclic paths of closed diagram.

tion state. The expression for the completion of the cycle is a 2:2 rational function in nutrient intake (Schulz, 1992). A general equation for the nutrient–response relationship as derived from these stochastic principles is

$$r = \frac{\sum_{i=0}^{1} \alpha_i n^i}{\sum_{i=0}^{m} \beta_i n^i}, \; m \geq 1, \; \alpha_i \geq 0, \; \beta_i \geq 0, \text{ where all } \beta_i \neq 0,$$

where r is the response and n is the amount of nutrient. If the measured response is a net response, then $\alpha_0 = 0$. It has been shown that the foregoing general equation can describe all of the observed nutrient–response curves (Schulz, 1987). Reference to Fig. 1 shows that a 2:2 rational function can give rise to the shapes observed in nutrient–response curves if certain constraints are placed on the parameters. This would suggest that the equation for nutrient–response is a rational polynomial, and there is no need to explain the shape of these curves in terms of a rate-limiting enzymic reaction.

It is not surprising that the nutrient–response relationship is described by a rational polynomial for the rate of most biological reactions are described by this type of equation. The dimensions of the parameters in the general equation for nutrient–response are

$$\alpha_i = \text{response} \times (\text{nutrient})^{1-i} \quad i = 0, 1 \ldots l$$
$$\beta_i = (\text{nutrient})^{1-i} \quad i = 0, 1 \ldots m.$$

Thus, the numerator terms are amplitude factors and the denominator polynomial represents the total nutrient processed by the organism. This allows partitioning the nutrient processed into a number of grossly defined pools. The number of pools is determined by the number of the denominator terms. This is entirely analogous to the rational polynomial which describes the steady state rate of an enzyme-catalyzed reaction in which the denominator consists of the enzyme species and the numerator contains amplitude terms.

Estimates of the parameters of the equation for the nutrient–response curve can be obtained by graphical analysis (Schulz, 1987) or by statistical analysis of the rational polynomial (Press *et al.*, 1992). Figure 4 shows the response of rats in terms of accumulation of body nitrogen to three different sources of dietary protein (Phillips, 1981). The sources of dietary protein were casein, peanut protein, and wheat gluten. The parameters were estimated graphically, and these estimates were "fine tuned" by simulation

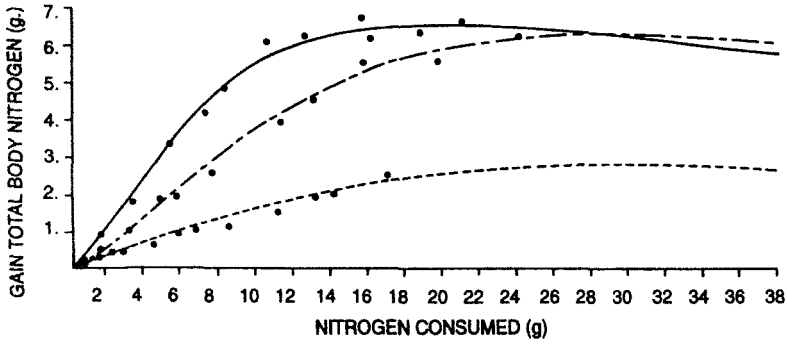

FIG. 4. Nutrient–response curves for three different proteins. The dots represent the data obtained experimentally. The solid line is the line generated for casein, the broken line represents peanut protein, and the dashed line represents wheat gluten.

(Schulz, 1987). The observed points are represented by the dots in Fig. 4. The equation which best fits the data is

$$r = \frac{\alpha_1 n + \alpha_2 n^2}{\beta_0 + \beta_1 n + \beta_2 n^2 + \beta_3 n^3}.$$

The lines in Fig. 4 (solid line for casein, broken line for peanut protein, and dashed line for wheat gluten) were generated from the parameters listed in Table I. Figure 5 shows that f_1, the fraction of nutrient passing through the $\beta_1 n$ pool, increases and then decreases with increasing nitrogen intake while f_2, the fraction of nutrient passing through the $\beta_2 n^2$ pool, increases to a maximum value in an asymptotic manner. However, it should be recognized that the order of the three proteins is reversed in the two parts of Fig. 5.

TABLE I

EQUATION PARAMETERS[a]

	Casein	Peanut protein	Wheat gluten
α_1	10.600	3.700	2.440
α_2	2.600	0.900	0.162
β_0	24.000	15.000	13.000
β_1	1.000	1.000	1.000
β_2	0.270	0.060	0.008
β_3	0.005	0.002	0.001

[a] All parameters are evaluated relative to β_1.

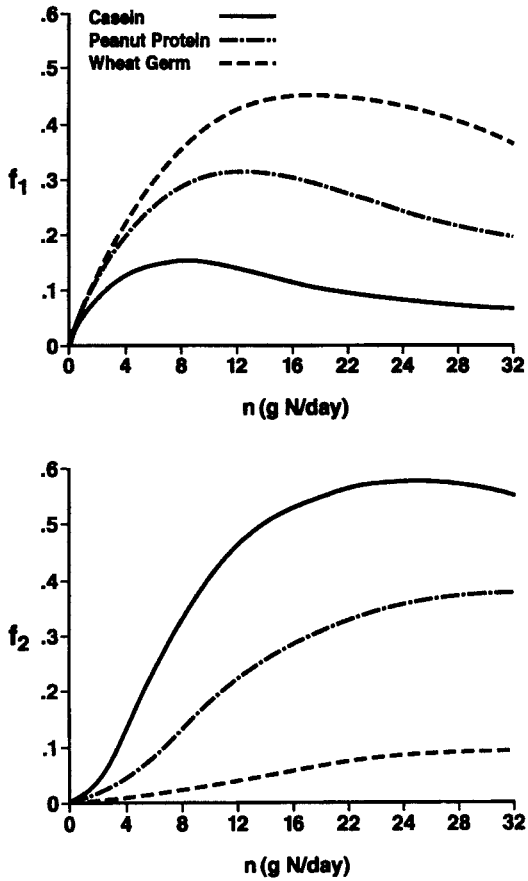

FIG. 5. Plots of f_1 and f_2 vs daily dietary protein nitrogen intake. The solid line represents casein, the broken line represents peanut protein, and the dashed line represents wheat gluten.

C. A BRIEF DESCRIPTION OF METABOLIC CONTROL THEORY

The object of metabolic control theory is to provide a sound mathematical foundation for the quantitative estimation of the role played by individual enzymes on the control of flux through a metabolic pathway and also the control exerted by individual enzymes on the concentration of intermediate metabolites in the pathway. The general principles of metabolic control theory and biochemical systems theory can be visualized by considering the simple metabolic pathway in Fig. 6. The numbers above the arrows

FIG. 6. A simple metabolic pathway.

indicates the enzymes in the pathway, although both enzymes and carriers can be involved. The direction of the arrows indicates the normal flow of flux through the path, but this does not imply irreversibility of any step. The letters M_i both identify and represent the concentrations of the intermediate metabolites. The substance X_0 is an independent variable and lies outside of the pathway. It is important to recognize the distinction between regulation and control of a metabolic pathway. Many enzymes are subject to regulation by modifiers which may be internal and external to the pathway. Thus the activity of these enzymes may vary greatly under different physiological conditions. However, a given regulatory enzyme may or may not exert a significant effect on the control of flux through the pathway. For example, the enzymes phosphorylase and glycogen synthase are both present in liver and muscle in such high amounts that they can exert no significant role in the control of their respective pathways when they are fully active. However, when they are largely in the inactive forms they may exert an important role in the control of their pathways. It is important to recognize that the control of a pathway is a quantitative concept which must be determined by quantitative methods.

A general equation for the rate of any enzymic step in the metabolic pathway is

$$v_j = \frac{V_f \lfloor 1 - \Gamma/K_{eq} \rfloor}{1 + \dfrac{K_{M_i}}{M_i}\left[1 + \dfrac{M_j}{K_{iM_{j_1}}}\right] + \dfrac{M_j}{K_{iM_{j_2}}}}$$

where M_i is the substrate for enzyme j and M_j is the product of the enzymic reaction. The Michaelis constant for the substrate is K_{M_i} and the K_{iM_j} are product inhibition constants. The ratio of substrate to product is the mass action ratio and is represented by the symbol Γ . The sensitivity of the reaction rate to any pathway reactant is termed the elasticity coefficient of the enzyme to the reactant involved, and the elasticity coefficient for enzyme j with respect to substrate M_i is given by the following expression.

$$\varepsilon_i^j = \frac{\partial v_j}{v_j}\Big/\frac{\partial M_i}{M_i} = \frac{M_i}{v_j}\frac{\partial v_j}{\partial M_i} = \frac{\partial \ln v}{\partial \ln M}.$$

The elasticity can be expressed by differentiation of the general equation

for the rate of an enzymic reaction with respect to its substrate and multiplication by M_i/v_j.

$$\varepsilon_i^j = \frac{\Gamma/K_{eq}}{1 - \Gamma/K_{eq}} + \frac{\dfrac{K_{M_i}}{M_i}\left[1 + \dfrac{M_j}{K_{iM_{j_1}}}\right]}{1 + \dfrac{K_{M_i}}{M_i}\left[1 + \dfrac{M_j}{K_{iM_{j_1}}}\right] + \dfrac{M_j}{K_{iM_{j_2}}}}.$$

The elasticity coefficient consists of two terms, the first of which is thermodynamic and the second term is kinetic. The value of the thermodynamic term varies from zero when the reaction is infinitely far from equilibrium to infinity when the reaction is at equilibrium. The kinetic term varies from zero when the enzyme is saturated with substrate to a value of unity when the substrate concentration is very much less than that required to saturate the enzyme. The elasticity coefficient of the enzyme with respect to its product can be obtained in a similar manner.

$$\varepsilon_j^j = -\frac{\Gamma/K_{eq}}{1 - \Gamma/K_{eq}} - \frac{\dfrac{M_j}{K_{iM_{j_1}}}\left[\dfrac{K_{iM_{j_1}}}{K_{iM_{j_2}}} + \dfrac{K_{Mi}}{M_i}\right]}{1 + \dfrac{K_{m_i}}{M_i}\left[1 + \dfrac{M_j}{K_{iM_{j_1}}}\right] + \dfrac{M_j}{K_{iM_{j_2}}}}.$$

The terms on the right-hand side of the foregoing expression are both negative, and this is expected because the product is an inhibitor of the enzymic reaction. It important to note that any enzyme can exhibit sensitivity to the product of the reaction even under the condition that the reaction is very far from equilibrium. The sensitivity of an enzyme to its product is often ignored in the mathematical modeling of whole animals. The reason for this oversight lies in the concept of identifiability (Jacquez and Perry, 1990; Jacquez, Chapter 19, this volume). In studies of the intact animal the effect of the product of an enzymic reaction may be difficult to identify, but that does not justify the assumption that the enzyme is insensitive to the product. Rather it emphasizes that sensitivity and identifiability are two separate concepts both of which must be recognized in mathematical modeling. If the sensitivity of the system to any reactant is not readily identifiable, then it is essential to establish means by which the sensitivity of the system to the reactant can be identified. The elasticity coefficient plays an additional essential role in metabolic control theory for it provides

the link between the domain of the steady-state enzyme kineticist and control of multienzyme systems. The complete development of the principles of metabolic control theory is given elsewhere (see, for example, Westerhof and van Dam, 1987; Cornish-Bowden and Cardenas, 1990) and will not be presented here.

D. BIOCHEMICAL SYSTEMS THEORY AND NUTRIENT–RESPONSE

The general equation for the nutrient–response relationship can be written as

$$r = \frac{\alpha_i n^i + \alpha_{i-1} n^{i-1} + \ldots + \alpha_1 n + \alpha_0}{\beta_i n^i + \beta_{i-1} n^{i-1} + \ldots + \beta_1 n + \beta_0}.$$

This equation can be written in the factored form

$$r = \frac{\Phi (a_i + n)(a_{i-1} + n \ldots (a_i + n)}{\Theta (b_i + n)(b_{i-1} + n) \ldots (b_i + n)}.$$

The foregoing equation in logarithmic form is

$$\ln r = \ln \frac{\Phi}{\Theta} + \ln (a_i + n) + \ln (a_{i-1+} n) + \ldots + \ln (a_1 + n) - \ln (b_i + n) - \ln (b_{i-1} + n) - \ldots - \ln (b_1 + n).$$

Thus, it is not surprising that rational polynomials give rise linear segments when plotted in log–log space. In the case of many biological reactions this linearity extends over a number of order of magnitude (Savageau, 1976). This observation led Savageau to develop a power law formulation of metabolic control based on a Taylor's series when plotted in log–log space (Savageau, 1972).

It is now possible to consider Savageau's power law, first as it applies to Fig. 6, and then as it might apply in a "top-down" approach. For any step in Fig. 6, the truncated Taylor's series logarithmic expression is

$$\ln v_j = \ln v_{j_0} + \left(\frac{d \ln v_j}{d \ln M_i}\right)(\ln M_i - \ln M_{i_0}),$$

where M_i is the substrate for enzyme j and i_0 and j_0 refer to M_i and v_j,

respectively, about some operating point where the differential is evaluated at i_0 and j_0. Let

$$\ln \alpha_j = \ln v_{j_0} - \varepsilon_i^j \ln M_{i_0},$$

then

$$\ln v_j = \ln \alpha_j + \varepsilon_i^j \ln M_i$$
$$v_j = \alpha_j M_i^{\varepsilon_i^j}.$$

If more than one metabolite in the pathway affects v_j

$$v_j = \alpha_j \prod_{k=1}^{m} M_k^{\varepsilon_k^j}.$$

The concentration of an intermediate metabolite is given by

$$\frac{d M_j}{dt} = v_i - v_j = \alpha_i \prod_{k=1}^{m} M_k^{\varepsilon_k^i} - \beta_i \prod_{k=1}^{n} M_k^{\varepsilon_k^j} = 0.$$

The differential equations for the pathway in Fig. 6 are

$$\frac{d M_1}{dt} = \alpha_1 X_0^{\varepsilon_0^1} M_1^{-\varepsilon_1^{-1}} - \beta_1 M_1^{\varepsilon_1^2} M_2^{-\varepsilon_2^{-2}} = 0$$
$$\frac{d M_2}{dt} = \alpha_2 M_1^{\varepsilon_1^2} M_2^{-\varepsilon_2^{-2}} - \beta_2 M_2^{\varepsilon_2^3} = 0.$$

Since ε_i^j is the elasticity coefficient for an enzyme with respect to its product, it is negative because the product is inhibitory, and for convenience $\bar{\varepsilon}_i^j = -\varepsilon_i^j$. The foregoing equations can be rearranged as

$$b_1 = \varepsilon_0^1 y_0 - (\varepsilon_1^2 + \bar{\varepsilon}_1^1) y_1 + \bar{\varepsilon}_2^2 y_2$$
$$b_2 = \varepsilon_1^2 y_1 - (\varepsilon_2^3 + \bar{\varepsilon}_2^2) y_2,$$

where $b_i = \ln \beta_i/\alpha_i$, $y_0 = \ln X_0$, and $y_i = \ln M_i$. The expressions for the logarithmic concentrations of the intermediate metabolites are

$$y_1 = \lfloor \varepsilon_0^1(\varepsilon_2^3 + \bar{\varepsilon}_2^2) y_0 - (\varepsilon_2^3 + \bar{\varepsilon}_0^2) b_1 - \bar{\varepsilon}_2^2 b_2 \rfloor / |D|$$
$$y_2 = \lfloor \varepsilon_0^1 \varepsilon_1^2 y_0 - \varepsilon_1^2 b_1 + (\varepsilon_1^2 + \bar{\varepsilon}_1^1) b_2 \rfloor / |D|,$$

where

$$|D| = \varepsilon_1^2\varepsilon_2^3 + \bar{\varepsilon}_1^1\varepsilon_2^3 + \bar{\varepsilon}_1^1\bar{\varepsilon}_2^2.$$

The sensitivity of the intermediate metabolites to the nutrient is obtained by differentiation of these equations with respect to y_0, and this is the sensitivity of the logarithmic concentration of the intermediate metabolites to the logarithmic concentration of the dietary nutrient.

III. ANALYSIS OF DATA ON THREE DIETARY PROTEINS

The metabolic pools defined in the denominator of the nutrient–response equation can be viewed as intermediate metabolites. Thus, the logarithmic derivative of each metabolic pool with respect to nutrient intake provides an estimate of the sensitivity of the pool to nutrient intake. Figure 7 shows

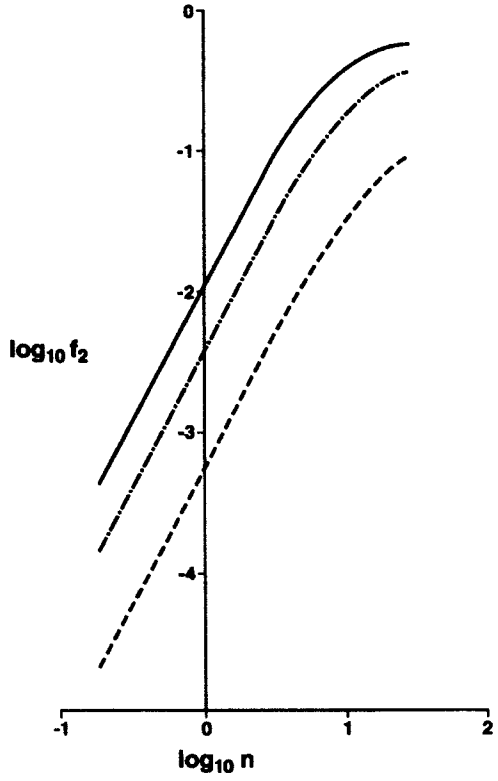

FIG. 7. Log–log plot of f_2 vs daily protein intake.

the log–log plot of f_2, the fraction of the intake flowing through the $\beta_2 n^2$ pool vs intake for each protein. The lines at low to intermediate nutrient intake are parallel, indicating that the sensitivity of the pool to intake were identical and that the proteins differed only in their capacity for providing substrate for the $\beta_2 n^2$ pool. The data presented in Fig. 7 are summarized in Table II together with the sensitivity of pool $\beta^1 n$ to intake and the sensitivity of pool $\beta_2 n^2$ to pool $\beta_1 n$. The sensitivities as indicated by the slopes of the lines are identical for each of the proteins. The sensitivity of f_2 to intake is greater than that of f_1, but there was no significant difference between the dietary proteins. These data suggest that the three dietary

TABLE II

SLOPES AND INTERCEPTS OF LINEAR LOG–LOG RELATIONSHIPS

Nutrient	Slope	Intercept
	Log response vs log intake	
Casein	1.1097 ± 0.0080	−0.2737 ± 0.0043
Peanut	1.1311 ± 0.0063	−0.5264 ± 0.0420
Gluten	0.9702 ± 0.0046	−0.7357 ± 0.0080
	Log f_2 vs log intake	
Casein	1.8066 ± 0.0321	−2.0115 ± 0.0136
Peanut	1.8104 ± 0.0251	−2.4610 ± 0.0163
Gluten	1.8388 ± 0.0185	−3.2708 ± 0.0120
	Log f_1 vs log intake	
Casein	0.8315 ± 0.0291	−1.4392 ± 0.0152
Peanut	0.8264 ± 0.0235	−1.2378 ± 0.0137
Gluten	0.8168 ± 0.0212	−1.1750 ± 0.0145
	Log response vs log f_2	
Casein	0.6128 ± 0.0077	0.9596 ± 0.0128
Peanut	0.6233 ± 0.0098	1.0085 ± 0.0199
Gluten	0.5273 ± 0.0029	0.9892 ± 0.0080
	Log response vs log f_1	
Casein	1.3211 ± 0.0436	1.6300 ± 0.0581
Peanut	1.3790 ± 0.0445	1.1878 ± 0.0462
Gluten	1.1514 ± 0.0200	0.6175 ± 0.0196
	Log f_2 vs log f_1	
Casein	2.2203 ± 0.0486	1.1196 ± 0.0631
Peanut	2.2404 ± 0.0416	1.1878 ± 0.0462
Gluten	2.2996 ± 0.0281	0.6851 ± 0.0266

proteins differ only with respect to their ability to provide the indispensable amino acids. For example, the ratio of the analog of the intercepts of the plots of log of response vs log of intake of peanut protein to casein is 0.56 and the ratio for wheat gluten to casein is 0.34. This is consistent with the ratio of lysine in these proteins, and it is also essentially identical to the relative values of these proteins for maintenance and growth (Finke *et al.*, 1987). The sensitivity of the response to intake, f_1 and f_2, is not identical for all of the dietary proteins. The slopes of the log–log plots for casein and peanut protein do not differ significantly, but the sensitivity of response to wheat gluten is significantly less. This suggests that wheat gluten has an adverse effect on the final stage of protein synthesis.

At the present time there is no apparent explanation for the inhibition of the later stage of protein synthesis by wheat gluten, but this analysis suggests that the differences in response to these three dietary proteins involves more differences in amino acid composition and identifies the portion of the pathway which should be the subject of further research.

IV. CONCLUSIONS

Nutrient–response curves contain a wealth of information concerning the metabolic fate of the nutrient involved. Unfortunately, this fact is often lost in the desire to obtain estimates of parameters to which one can assign high orders of statistical significance even though these parameters may have nebulous biochemical or physiological significance. The alternative described here is the mathematical analysis of the nutrient–response curve in terms of the metabolic fate of the nutrient and the integration of this analysis with the well-established principles developed in metabolic control theory and biochemical systems theory. In recent years there has been considerable progress in extending the concepts of metabolic control from simple metabolic pathways to more complex systems such as cell organelles and tissues (Brown *et al.*, 1990; Kahn and Westerhof, 1991). This paper proposes a further extension of this trend.

Finally, it is important to recognize the essential relationship between the concepts of sensitivity and identifiability in mathematical modeling in nutrition. It is too easy to overlook the fact that flux through a metabolic pathway may be sensitive to the concentration of intermediates in the pathway. If the sensitivity is not readily identifiable, conditions must be sought to render the sensitivity identifiable. However, if the sensitivity is not identifiable readily, it is incumbent on the investigator to recognize the possible effect of such sensitivity.

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