General model for nutritional responses of higher organisms
(bioassay/saturation kinetics/growth responses)

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Communicated by George C. Cotsias, August 19, 1975

ABSTRACT A general saturation equation is derived which is shown to describe a wide variety of nutrient-response relationships in higher organisms. Iterative multiple linear regression analysis is used to obtain least squares estimates of the constants defining theoretical nutrient-response curves. Curves thus generated accurately predict experimentally observed responses. From this treatment, response parameters are developed which are analogous to $V_{\text{max}}$ and $K_m$ of enzyme kinetics. It is proposed that this model be applied in evaluating nutritional requirements and in assessing the relative biological efficiency of nutrient sources.

It is well established that bacterial growth rates obey saturation kinetics with respect to the concentration of limiting nutrient (1, 2). However, the nutritional responses of animals and humans have been less amenable to satisfactory mathematical analysis. Linear, semi-logarithmic, and quadratic equations have been developed to describe the nutrient-response curves of higher organisms (3-5). In general, these models have found utility only within narrow ranges of nutrient intake and possess little, if any, theoretical basis. In this communication, we report the derivation of a general saturation equation and its application to the nutritional responses of higher organisms. A preliminary report of these findings has recently been published (6, 7).

DERIVATION

The rationale for applying saturation kinetics to gross biological responses derives from the following considerations. Organisms absorb and utilize nutrients via sequences of translocations and transformations, and for any particular metabolic state one step of a sequence would be expected to be rate-limiting for the process as a whole. If the identity of the sequence-controlling reaction does not change with time or nutrient intake, then the overall response of the sequence to graded levels of nutrient will reflect the kinetics of the rate-limiting step. Since many translocations and transformations of intermediary metabolism obey saturation kinetics individually, we have explored the possibility that data from feeding experiments might be treated mathematically as manifestations of saturable phenomena.

Visual inspection of a number of nutrient-response curves from literature sources reveals basic similarities to saturation functions. In general, most nutrient-response curves tend to "plateau out," i.e., to approach an asymptotic or limiting response at high nutrient intake. We have observed that the curvature of nutrient-response functions in approaching this asymptote resembles either hyperbolic saturation curves of the Michaelis-Menten type or sigmoidal saturation curves described by the Hill equation (8, 9). However, direct application of either the Michaelis-Menten or Hill equation to nutrient-response curves is ordinarily precluded by the fact that experimental nutrient-response curves rarely pass through the origin of the coordinate axes as required by these equations.

From such considerations, two criteria evolved which led to the development of a general saturation equation having maximum utility in the interpretation of nutritional responses: first, the general equation must be able to treat both hyperbolic and sigmoidal saturation phenomena; secondly, the curve described by the general equation must be free to intersect the ordinate axis at any point required by the experimental data.

The Hill equation [1], which describes sigmoidal saturation curves, fulfills the first requirement since for $n = 1$, it reduces to a hyperbolic saturation function of the Michaelis-Menten type:

$$y = (Y_{\text{lim}}X^n)/(K + X^n) \quad [1]$$

where $y =$ velocity or saturation fraction
$Y_{\text{lim}} =$ asymptotic velocity or saturation fraction
$X =$ concentration of ligand or substrate
$n =$ apparent kinetic order of the velocity or saturation fraction with respect to $X$ as $X$ approaches zero
$K =$ characteristic constant of the system, having the property that for $X = K^{1/n}, y = Y_{\text{lim}}/2$

However, since the Hill equation requires that $y = 0$ when $X = 0$, this equation does not meet the second criterion.

Modification of the Hill equation by translation of the ordinate axis yields a general saturation function satisfying both of the proposed criteria:

$$y = (bK + Y_{\text{lim}}X^n)/(K + X^n) \quad [2]$$

where $b =$ ordinate intercept

and

$$y = (Y_{\text{lim}} + b)/2 \quad \text{when} \quad X = K^{1/n}$$

Equation 2 is quite versatile: reducing to a general equation for the rectangular hyperbola when $n = 1$, to the Hill equation when $b = 0$, and to the Michaelis-Menten equation when $b = 0$ and $n = 1$.

Equation 2 is a general saturation equation applicable both in vivo and in vitro. In practice we find it convenient to adopt a slightly altered terminology when applying this function to nutritional phenomena in higher organisms. Thus, equation 2 may be rewritten:

$$r = (bK_1 + R_{\text{max}}I^n)/(K_1 + I^n) \quad [3]$$

where $r =$ observed response of the organism (i.e., weight gain, plasma concentrations of metabolites, etc.)
$R_{\text{max}} =$ asymptotic or maximum response of the organism

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FIG. 1. Effect of dietary casein on weight gain in rats. Linear transformation of nutrient-response data according to equation 4 is illustrated in the top graph. The regression coefficient for this plot is given on the bottom line of the inset. The lower frame shows the theoretical nutrient-response curve calculated from equation 3 superimposed on experimental points. Values for parameters defining the theoretical curve are shown in the inset. Also shown is \( a \), the intercept of the calculated curve on the intake axis. The data are taken from ref. 12.

\[
I = \text{nutrient intake} \\
\begin{align*}
n & = \text{apparent kinetic order of the response with respect to } I \text{ as } I \text{ approaches zero} \\
b & = \text{calculated ordinate intercept of the nutrient-response curve} \\
K_I & = \text{nutrition constant}
\end{align*}
\]

Additionally we define \( K_{0.5} \) as the nutrient intake required for a “halfway” response, i.e., a response equal to \( (R_{\text{max}} + b)/2 \).

Table 1. Typical nutritional responses obeying the general saturation equation

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Response</th>
<th>Species</th>
<th>Fig.</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Casein</td>
<td>Weight gain</td>
<td>Rat</td>
<td>1</td>
<td>12</td>
</tr>
<tr>
<td>Vitamin K</td>
<td>Blood clotting time</td>
<td>Chick 2</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>Carcass nitrogen</td>
<td>Mouse</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>Vitamin A acetate</td>
<td>Vitamin A in blood</td>
<td>Chick 4</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>Casein</td>
<td>Carcass retention of isoleucine</td>
<td>Chick 5</td>
<td>16</td>
<td></td>
</tr>
<tr>
<td>Vitamin D (as cod liver oil) or irradiated ergosterol</td>
<td>% Ash in femur</td>
<td>Chick 6</td>
<td>17</td>
<td></td>
</tr>
</tbody>
</table>

FIG. 2. Effect of dietary vitamin K on blood clotting time in baby chicks. Linear transformation of nutrient-response data according to equation 4 is illustrated in the top graph. The regression coefficient for this plot is given on the bottom line of the inset. The lower frame shows the theoretical nutrient-response curve calculated from equation 3 superimposed on experimental points. Values for parameters defining the theoretical curve are shown in the inset. Also shown is \( a \), the intercept of the calculated curve on the intake axis. The data are taken from ref. 13.

\[
r = -K_I(r - b)/I^n + R_{\text{max}}
\]

By rearranging equation 3 to the slope-intercept form, it is possible to effect a linear transformation of nutrient-response data:

\[
r = -K_I(r - b)/I^n + R_{\text{max}}
\]

Inspection reveals that equation 4 is a more general version of the familiar Eadie–Hofstee equation (10, 11).

Expansion of equation 4 yields a form of the general saturation equation which is amenable to multiple linear regression analysis for a given \( n \):

\[
r = -K_I(r/I^n) + bK_I(1/I^n) + R_{\text{max}}
\]

METHODS AND RESULTS

Actual nutrient-response data are fitted to equation 5 using iterative multiple linear regression analysis. To accomplish this task we have written a computer program which varies \( n \) independently until the best statistical fit of experimental data is found. The computer then uses the fitted values for \( n, b, K_I, \) and \( R_{\text{max}} \) to construct theoretical response curves according to equations 3 and 4. Actual data points are superimposed on the theoretical curves for direct comparison of model and data in the accompanying figures.

Published data from several laboratories (12–17) conform to the criteria for saturation kinetics imposed by equation 3.
Broad applicability of the model to the nutritional responses of higher organisms is suggested by the conformity of the responses of two mammalian (rat, mouse) and one avian (domestic chick) species to graded intakes of a variety of nutrients, summarized in Table 1 and illustrated in Figs. 1–6.

**DISCUSSION**

The significance of the preceding analysis is that a variety of complex biological responses in both growing and mature animals are shown to obey saturation kinetics with respect to nutrient intake. This model provides specific advantages over previous mathematical treatments of nutritional responses:

1. Data over a wider range of nutrient intakes may be included in the analysis.
2. Definite response parameters are developed analogous to $V_{max}$ and $K_m$ of enzyme kinetics.
3. Theoretical nutrient-response curves generated by the treatment are capable of predicting actual responses with superior accuracy.

The application of saturation kinetics to describe nutritional responses of higher organisms has only rarely been employed. A recent example is the study of Thompson and Leevey in which the relationship between the dose of thiamine administered to human subjects and the amount recovered from urine was found to obey Michaelis–Menten kinetics (18). In our experience most nutritional responses of higher animals are more complicated and follow sigmoidal kinetics.

The application of saturation kinetics to growth phenomena has apparently been limited to the field of microbiology. In a classic study published in 1942, Monod demonstrated that bacterial growth rates obey Michaelis–Menten kinetics with respect to the concentration of limiting nutrient (1). Current models for the kinetics of microbial growth are explicitly based on the saturation model originally proposed by Monod (19). The present results indicate that animal growth responses, as well as microbial growth responses, are saturable functions of nutrient intake.

The adherence of an observed nutritional response to the general saturation equation suggests that nutrient utilization is controlled by a single rate-limiting step within the observed range of intake levels. Outside this range other rate-limiting steps may become response-controlling. For this reason the ordinate-intercept of a calculated nutrient-response curve cannot be equated a priori with the actual response at zero nutrient intake. Neither can it be assumed that ever-increasing intake levels will indefinitely yield responses smoothly approaching the asymptotic or maximum response. Extrapolation of a nutrient-response curve beyond the limits of observation is thus predictive of actual response only if the rate-limiting step remains unchanged.

We stress the generality of the saturation model with respect to the nature of the response-controlling step. Both catalytic and transport processes are known to obey saturation...
kinetics. Sigmoidal responses, so frequently encountered in animal nutrition, imply cooperativity which could derive either from allosteric effects or from more complex metabolic interactions. Thus the analysis of nutrient-response relationships using the general saturation equation is not mechanistically oriented.

Interpretation of nutritional responses as saturable phenomena has important implications for the estimation of nutritional requirements. Sharp breaks in nutrient-response curves have often been sought to define "minimal" or "optimal" intake levels. It is obvious that for responses obeying the general saturation equation, such a search will be fruitless. In determining farm animal requirements, some balance must be struck, taking account of feed costs, time to reach market size, and value of final product. In human nutrition, the practice has generally been to relate requirements or recommended allowances to clinical status, "acceptable" growth rates, and norms of body fluid composition (20). In the future, it may be possible to relate these clinical and laboratory standards to specific portions of nutrient-response curves and thus further to quantitate recommended allowances.

An important objective of quantitative nutrition is the development of bioassay methods providing useful comparisons of the biological efficiency of alternate nutrient sources, e.g., the various forms of vitamin D, the tocopherols, carotenoids and vitamin A derivatives, different forms of iron, and proteins. Hegsted and Chang (12) have commented on the criteria for a satisfactory bioassay, including the necessity for developing some function that yields a linear regression between dose and response. In the Eadie–Hofstee rearrangement of the general saturation equation, such a linear regression between nutrient intake and response is obtained.

The model generates two useful constants permitting comparison between alternate nutrient sources, i.e., $R_{max}$ and $K_{0.5}$. Consider a case in which it is found that different sources of a nutrient yield the same $R_{max}$ at high intake levels, these sources may be considered equal. On the other hand, if dietary levels are limiting, the nutrient source exhibiting the lowest $K_{0.5}$ would be preferred.

The merit of any mathematical treatment of experimental data must be judged in terms of such criteria as statistical and predictive accuracy, range of application, insights provided, and ability to suggest new experimental approaches. We believe that the model derived from saturation kinetics specifically provides such advantages over previous models for the nutritional response.