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the dominance of blue-green algae in a subtidal community.

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SOME THOUGHTS ON NUTRIENT LIMITATION IN ALGAE¹

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SUMMARY

An empirical relation relating specific growth rate in steady state systems to nutrient status with respect to more than one nutrient simultaneously is proposed, based on 3 experimentally verifiable postulates: (1) that uptake depends on the external substrate concentration; (2) that growth depends on the internal substrate concentration; and (3) in a steady state system specific rate of uptake (in the absence of significant excretion) is necessarily the product of the specific growth rate and internal substrate concentration. The implications of this model are discussed in particular in respect to the concept of luxury consumption and Liebig's law of minimum. Some aspects of uptake in transient situations are also discussed.

INTRODUCTION

Any discussion of nutrient limitation should, by rights, start with Liebig (11), that yield (of the soil) is determined by the amount of the nutrient that happens to be in minimal supply. Because yields may only be obtained by growth, it is an obvious, although by no means logically necessary, step to substitute rate of growth for yield, and we have with Lotka (12): "If one essential component is presented in limited amounts any moderate increase or decrease in the ample supply of the other components will have little or no observable influence on the rate of growth." Indeed, this statement is

also implicit in the Monod model for nutrient-limited growth of microorganisms (13):

$$\mu/\mu_m = s/(K_s + s) \quad (1)$$

(see Notation for explanation of symbols); or in Caperon's model (1), which extends the Monod model very elegantly to embrace prey-predator systems.

Also implicit in the classic models is the assumption of constant composition with respect to the limiting nutrient during nutrient-limited growth; thus Monod's other proposition was that the material yield of the growth process was constant.

$$dx/dt = -Y ds/dt \quad (2)$$

Y is the yield coefficient; its reciprocal, Q , which we may term the coefficient of demand, is equivalent to the cell nutrient quota when excretory losses are negligible. Monod's tenet is generally assumed to be broadly true and indeed is stated explicitly in Caperon's model (1). Put another way, to assume a constant yield coefficient is to admit by implication that the demand of cells for limiting nutrients is independent of the parameters of growth and nutrition.

On the other hand, it is now well known that cell composition does vary greatly with conditions and rate of growth (see, for example, 7), so that some limitation in the application of Equation (2) has to be admitted, particularly outside the original context of carbon nutrition. If the variability is associated exclusively with nutrient excess, one is led to the question, when is a nutrient limiting and

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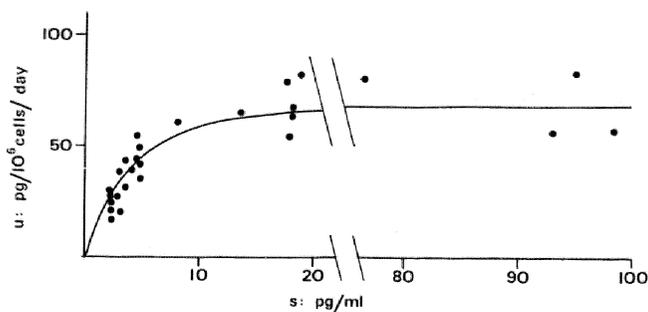


FIG. 1. Specific rate of uptake (u) of vitamin B_{12} by exponentially growing *Monochrysis lutheri* as a function of substrate concentration (s) (4). In the *Skeletonema* and *Monochrysis* experiments (Fig. 1-9 and 12-14), population volume (as determined by Coulter counter) was taken as a measure of biomass. However, the mean cell volume of *Monochrysis* remained so constant ($40\text{--}60\ \mu\text{m}^3$) over the range of conditions used that it was possible to express the *Monochrysis* results in terms of cell numbers rather than population volumes.

when is it not? Where is the sharp line between invariant and variable cell composition?

A related question is concerned with the phenomenon of "luxury consumption." This, as applied to the uptake of phosphate by microalgae, is familiar to planktonologists from the work of Ketchum at Woods Hole (8,9). When phosphorus-depleted algae are placed in fresh culture medium, the medium is very quickly depleted of phosphorus and the initial rates of phosphate uptake are far in excess of the organism's specific growth rate. An accumulation of phosphorus occurs, which is subsequently shared out among the succeeding generations of cells as they multiply. Obviously this statement is not complete. If, for instance, a single cell is put in a large volume of fresh culture medium, does it take up all the phosphorus? If not, what is the limit and what is the maximum rate of uptake and for how long is it continued?

The resolution of this and the previous paradox must involve a model for growth that embraces a number of nutrients, any of which may be limiting to varying degrees. It need, however, involve little more than an empirical statement of the relation between specific growth rate, rates of uptake, cell composition, and substrate concentrations of several nutrients simultaneously.

The attempt that follows to construct such a model draws largely on my experience with vitamin B_{12} limitation in the photosynthetic chryomonad *Monochrysis lutheri*.

METHODOLOGY

Much of the data to be discussed are published (4), and I need not dwell too deeply on practical matters. With nutrients whose essential atom can be labeled (eg, ^{32}P phosphate, ^{59}Fe , or ^{57}Co vitamin B_{12}), it is a simple matter to partition the amounts of the nutrient between the cell and culture medium fractions, and thus obtain the essential information for calculating uptake. Three types of experiment can be performed:

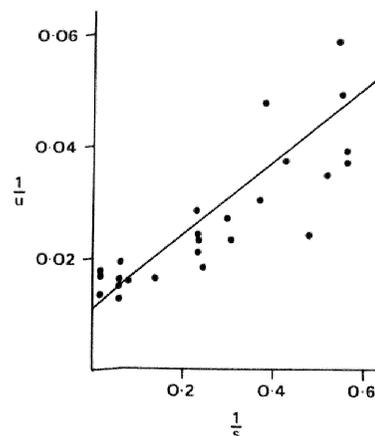


FIG. 2. Reciprocal plot of Fig. 1.

1. Measurement under dynamic conditions in batch cultures growing exponentially in labeled medium. When the inoculum is extremely small a number of generations are possible before the medium begins to show a significant drop in nutrient level, so that conditions are, to all intents, steady state. The specific rate of uptake (u) is then the product of the specific growth rate (μ) and cell nutrient quota (Q).

$$u = \mu Q \quad (3)$$

2. Measurements on continuous cultures, when again we have a steady state, so that

$$u = \mu Q$$

3. Measurement of uptake over the short term by washed suspensions of (usually) log phase cells grown in unlabeled medium and suspended in labeled medium. Here uptake is best given by the rate of increase in cell quota:

$$u = \Delta Q / \Delta t \quad (4)$$

since Δt here is very small compared with the generation time.

BATCH CULTURES

With vitamin B_{12} it is, unfortunately, not possible to obtain a measure of the available substrate in supernatants from even moderately heavy cultures, owing to the presence of excreted protein, which combines with the vitamin to prevent it being taken up by the cells. This meant that one had to rely largely on batch cultures, as described, for information on the effect of substrate concentration on the steady state rate of uptake (4). Presumably other nutrients do not suffer from this defect. The procedure was simply to set up cultures with labeled vitamin B_{12} using very small but known log phase inocula and to harvest after 5 or 6 cell generations but before more than a few percent of the vitamin had been incorporated. Coulter population volume determinations and scintillation counts on the cells and on the medium at the time of harvest are the only measurements required. μ is taken as the difference between the natural logarithms of the harvest and initial populations divided by the time interval. Substrate concentration (s) at the time of harvest is taken as the product of the initial sub-

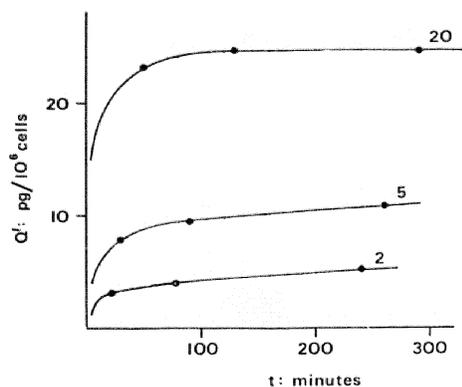


FIG. 3. Time course of uptake of vitamin B₁₂ by washed suspension of *Monochrysis* in media containing initially 2.0, 5.0, and 20 pg vitamin per ml (4). Q' versus t .

strate concentration and the ratio of medium to total scintillation counts. Similarly, cell vitamin is given by the product of the initial substrate concentration and the ratio of cell to total scintillation counts. The latter is divided by the population to obtain the cell quota (Q), which is then multiplied by μ to obtain the specific rate of uptake (u).

The method is highly inaccurate owing to the difficulty of measuring the specific growth rate with so small a number of cells in the cultures. Nevertheless the results so obtained (Fig. 1) could be described fairly satisfactorily by a conventional Michaelis saturation equation of the same form as Equation (1):

$$u/u_m = s/(k_s + s) \quad (5)$$

u_m being the specific rate of uptake when the substrate concentration (s) is very large. k_s is the saturation constant and is numerically equal to the substrate concentration giving half maximal rate of uptake. The reciprocal plot of $1/u$ on $1/s$ (Fig. 2) should give a straight line if Equation (5) fits the data. The constants of the equation however are best derived statistically from the linear regression of u on u/s (4):

$$u = u_m - k_s(u/s) \quad (6)$$

Neither the precise values of these constants nor the statistical niceties need concern us at the moment; I merely want to make the point that the vitamin B₁₂ experiment provides no evidence that the rate of uptake of vitamin B₁₂ during exponential growth depends on substrate concentration in any but a Michaelis fashion.

CELL SUSPENSIONS

I want now to consider the significance of measurements of uptake in short-term experiments on washed cell suspensions. Cell suspensions were set up with the required concentration of labeled vitamin, incubated in the light and aliquots harvested

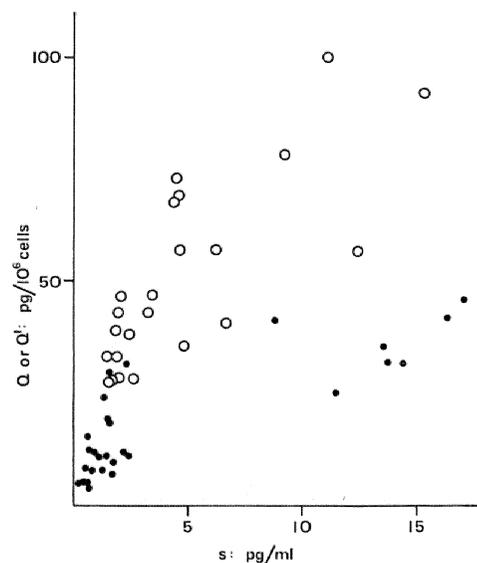


FIG. 4. Comparison of vitamin B₁₂ cell quotas (Q) in exponentially growing cells of *Monochrysis* (open circles) with those (Q') in washed suspensions (filled circles) (4). The quotas are plotted against substrate concentration (s).

at intervals. The substrate concentrations and cell quotas (Q') at the times of harvest were measured and calculated in the same way as was the batch cultures, but the mean rates of uptake between successive harvests were taken as the difference between the cell quotas calculated for these times divided by the time interval. Figure 3 shows a typical time course of uptake by moderately light concentrations of log phase cells suspended in different concentrations of labeled vitamin. Uptake is seen to be initially very great, but saturation is reached quite soon, so that the cell quotas obtained are scarcely a third of those obtained under dynamic conditions with the same substrate concentrations (Fig. 4). Since the substrate does not become exhausted, exhaustion cannot account for the early cessation of uptake. This is made clear in Fig. 5, in which are shown the slopes of the curves of Fig. 3, *ie*, the rates of uptake, plotted against the instantaneous substrate concentrations. It will be observed, especially in the 20 pg/ml curve, that uptake falls to zero with some 11 pg/ml still in the medium. Curve *A* in this figure is the dynamic rate of uptake (the curve in Fig. 1) drawn on the same scale for comparison.

Here, then, is a clear case of what one might call luxury consumption—an initial uptake rate well over 10 times the steady state rate, but which very soon drops to zero. However, to name a thing is not necessarily to understand it, but it is possible to advance a little. In the first place we note that uptake ceases; therefore we do not have steady state conditions in spite of our having used log phase cells. In the second place, we note that the quotas obtained are very low compared to the steady state quotas. Third, there is very high initial uptake.

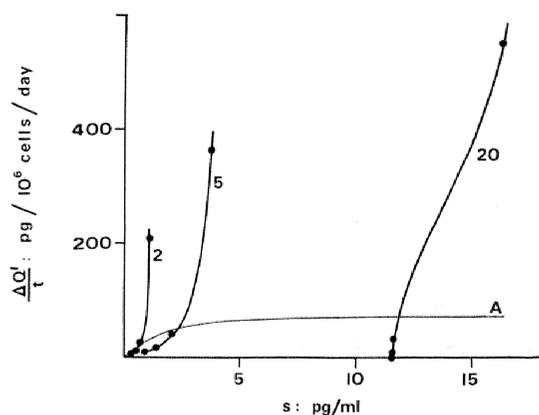


FIG. 5. Rates of uptake (Q'/t) of vitamin B_{12} by washed suspensions of log phase cells of *Monochrysis* in media containing initially 2.0, 5.0, and 20 pg vitamin per ml, as a function of substrate concentration (s); *ie*, the mean slopes of the 3 curves in Fig. 3 plotted against mean substrate concentration (s). Curve *A* is part of the curve of Fig. 1 on the same scale for comparison.

This can be interpreted as absorption to a very limited surface with early saturation. The low cell quotas indicate that transport inward to the functional sites is not taking place, so that uptake had to cease with saturation of the surface. We have, by the act of suspending the cells in new medium, induced a lag that affects the transport but not the adsorption mechanisms.

The reciprocal plot of the plateau values of the cell quota (Q')² and substrate concentration in these short-term experiments always yields a good straight line. This indicates that adsorption here can also be described by a Michaelis equation (or more properly a Langmuir isotherm):

$$Q'/Q'_m = s/(k'_s + s) \quad (7)$$

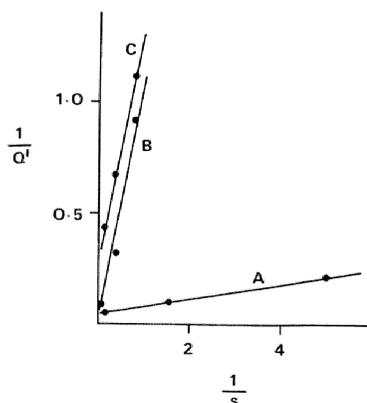


FIG. 6. Reciprocal plateau quotas ($1/Q'$) versus reciprocal substrate concentrations ($1/s$) of vitamin B_{12} in washed suspensions of *Monochrysis* cells (s). *A*: Log phase cells in unused medium. *B*: Log phase cells in used medium. *C*: Stationary phase cells in unused medium.

² Q' , since we are only referring to the cell surface.

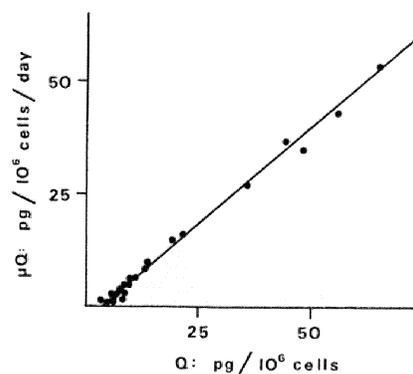


FIG. 7. Uptake (μQ) of vitamin B_{12} by *Monochrysis* as a function of the cell quota (Q) in a vitamin B_{12} -limited chemostat (s).

The reciprocal plot is shown in Fig. 6. The set of data under discussion is the lower of the 3 lines. The next line was given by cells from the same culture suspended in old medium enriched with labeled vitamin. This contained the vitamin B_{12} binding factor; competitive inhibition is clearly shown by the fact that the intercepts are coincident on the ordinate, while the slopes are very different. Competitive inhibition is characterized by the fact that its effects disappear as the abundance of the item competed for becomes very large. The top line was given by vitamin B_{12} -limited stationary phase cells in fresh medium. Here we see that both the intercept and the slope differ. The reciprocal of the intercept is of course Q'_m , *ie*, the Q' plateau that would be obtained with an extremely large substrate concentration; in other words, the surface capacity. The intercepts indicate that the log phase cells have ten times the capacity of cells of the stationary phase.

The hypothesis is, therefore, that the plateau quo-

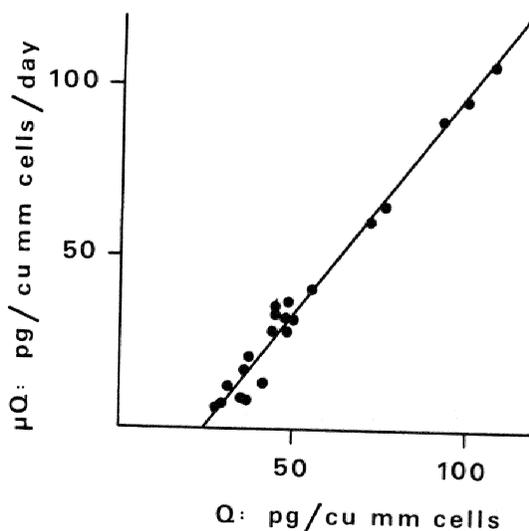


FIG. 8. Uptake (μQ) of vitamin B_{12} by *Skeletonema costatum* as a function of cell quota (Q) in a vitamin B_{12} -limited chemostat (s).

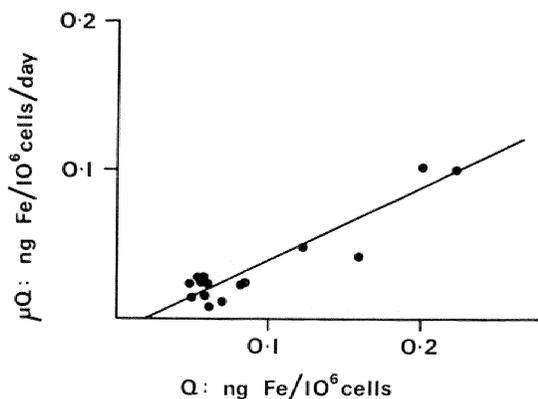


FIG. 9. Uptake (μQ) of iron by *Monochrysis* as a function of cell quota (Q) in an iron-limited chemostat.

tas obtained in short-term experiments on washed cells are a measure of the cell surface capacity and tell very little about the physiological level of requirement. On the other hand, since the 2 saturation constants, k_s and k_s' , in Equations (5) and (7), respectively, are of the same order of magnitude, namely, 1.2 and 2.5 $\mu\text{g/ml}$, it follows that transport inward of vitamin B_{12} is more or less directly proportional to the concentration of vitamin on the cell surface and therefore akin to diffusion. This surface therefore, whether real or virtual, controls the rate of uptake. Now the very high initial rate of adsorption is seen as merely the priming of the pump responsible for uptake.

CONTINUOUS CULTURE

Although the chemostat is not suitable for studying the effect of substrate concentration on uptake rates, it does provide another piece of the jigsaw, possibly the most important piece. This is the relation between cell quota, specific growth rate, and specific rate of uptake.

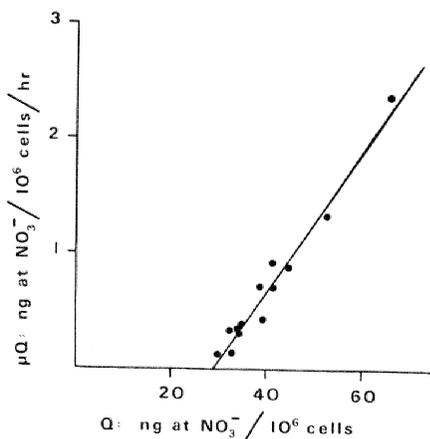


FIG. 10. Uptake (μQ) of nitrate by *Isochrysis galbana* as a function of cell quota (Q) in a nitrate-limited chemostat (data from 2).

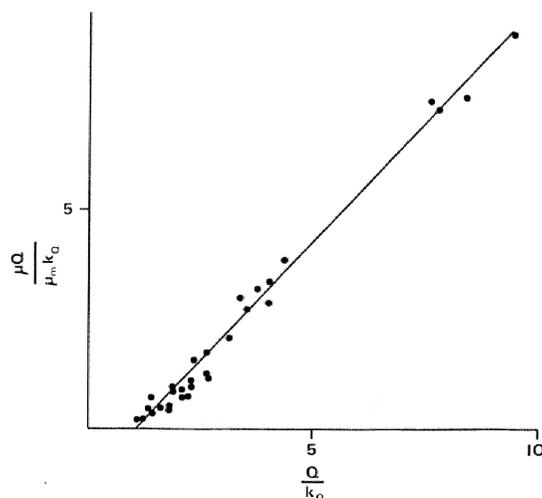


FIG. 11. Uptake ($\mu Q/\mu_m'k_Q$) by *Cyclotella nana* of phosphate as a function of cell quota (Q/k_Q) in a phosphate-limited chemostat, standardized plot (data from 10).

As we have seen, one relation between these parameters, a partial one, is necessarily that uptake is the product of the specific growth rate and the cell quota [Equation (3)]. It is perhaps unnecessary to point out that this relation only applies to steady state situations when Q is constant, the more general case being

$$dQ/dt = u - \mu Q \quad (8)$$

For completeness, we require u in terms of either μ alone or Q alone, which are just what the chemostat can provide.

The essence of the experiment is to let a chemostat equilibrate at different dilution rates, while measuring the cell quota. If a steady state is achieved, D (the dilution rate) and μ are numerically equal when the former is expressed as culture vol-

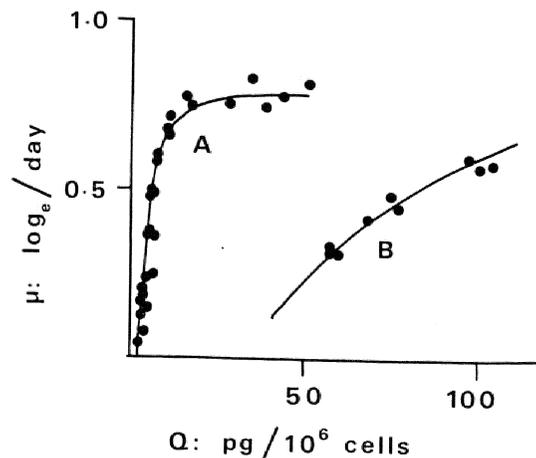


FIG. 12. Specific growth rate (μ) of *Monochrysis* as a function of cell quota (Q) of vitamin B_{12} . A: Chemostat with vitamin B_{12} limiting (data from Fig. 7). B: Chemostat with nitrate limiting.

umes and the latter in natural logarithms. The cell quota (Q) respecting a radioactively labeled nutrient is taken as the product of the chemostat input substrate concentration (s_R) and the ratio of cell-to-total scintillation counts, the whole being divided by the biomass measurement. The product of the cell quota and dilution rate gives us the specific rate of uptake (u).

Invariably μQ (*ie*, u) and Q are found to have a linear relationship when plotted against one another. The measurements are easy to make and the results usually very good indeed, correlation coefficients in excess of 0.98 being not unusual. Some examples follow: Figure 7 shows vitamin B₁₂ for *Monochrysis lutheri* with vitamin B₁₂ limiting (4); Fig. 8 the same for the marine diatom *Skeletonema costatum* (5); Fig. 9 shows iron for *Monochrysis* with iron limiting; while Fig. 10, based on data from Caperon (2), shows nitrogen for *Isochrysis galbana* with nitrate limiting; and Fig. 11 phosphorus for the diatom *Cyclotella nana* with phosphate limiting (10).

In every case the line intercepts the abscissa, so the equation is in the form

$$y = b(x - a) \quad (9)$$

y here being μQ or DQ and x, Q . When Q is very large a becomes unimportant and

$$\mu Q \approx bQ$$

Thus the slope b is the asymptotic value of μ for large values of Q . This we may designate μ_m' [noting that it must differ from μ_m of Equation (1) because it relates to internal (*ie*, Q) and not external substrate concentration³]. When μ approaches zero, Q becomes a . I have previously referred to this intercept as k_Q , the subsistence quota (4,5). Our equation then becomes

$$\mu Q = \mu_m' (Q - k_Q) \quad (10)$$

or rearranging

$$\mu / \mu_m' = 1 - k_Q / Q \quad (11)$$

Plotting μ against Q produces an asymptotic curve with the intercept on the Q axis at k_Q and the asymptote μ_m' . The data for the *Monochrysis* B₁₂ experiment plotted thus are shown in Fig. 12, curve A.

Equation (11) is an empirical statement and is

³ Both μ_m and μ_m' may loosely be termed "maximum growth rates." Both are in a sense mathematical abstractions, being defined by their respective equations and associated assumptions. μ_m is the value to which growth rate tends as the external substrate concentration becomes infinite, and the other likewise when the internal concentration becomes infinite. The two are related in a definite manner [see Equation (15)]. However, μ cannot be greater than μ_m and, indeed if one looks at Equations (1) and (11), infinite s produces a finite Q . Thus μ_m' must be regarded as more of an abstraction than μ_m , but nonetheless useful in its context, as we shall see.

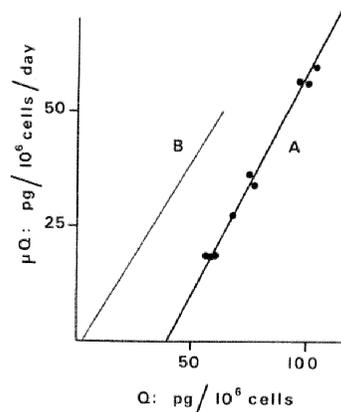


FIG. 13. A: Uptake (μQ) of vitamin B₁₂ by *Monochrysis* as a function of cell quota (Q) in a nitrate-limited chemostat. B: The regression of Fig. 7 placed on the same scale.

therefore useful only as long as it fits the facts and is easy to handle. Fuhs' exponential equation (10) is a possible, though awkward, alternative. Equation (11) can be given the form of a Michaelis equation, thus

$$\mu / \mu_m' = (Q - k_Q) / [(Q - k_Q) + k_Q] \quad (12)$$

We have to assume, however, that k_Q represents a nonlabile component with the function of a saturation constant, while the labile component, $Q - k_Q$, has the function of controlling substrate concentration (2,14). It is not profitable to theorize further on the enzyme analogy, since growth results from a large number of enzyme reactions, any of which could cause the expressions to have the general shape of a Michaelis equation. Suffice to say that our present expression has the edge on that of Monod in that it admits that the coefficient of demand increases with increasing growth rate. Equation (2) therefore requires the proviso of a constant μ . In-

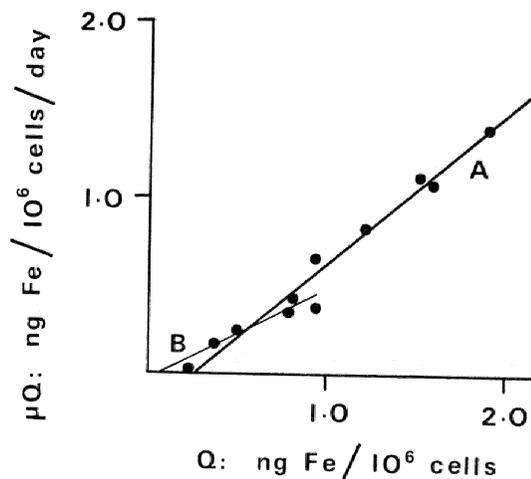


FIG. 14. A: Uptake (μQ) of iron by *Monochrysis* as a function of cell quota (Q) in a nitrate-limited chemostat. B: The regression of Fig. 9 placed on the same scale.

deed, with hindsight it is difficult to conceive of nutrient control of the rate of a cell's growth except through some aspect of the *internal* substrate concentration.

Specific growth rate can be obtained in terms of external substrate concentration by substitution of Equations (3) and (5) into Equation (11):

$$1/\mu = 1/\mu_m' + k_Q/u_m + k_Q k_s/u_m s \quad (13)$$

Monod's equation [Equation (1)], rearranged, gives

$$1/\mu = 1/\mu_m + K_s/\mu_m s \quad (14)$$

so that one can see by analogy that μ_m' is larger than μ_m by a little

$$1/\mu_m' = 1/\mu_m - k_Q/u_m, \quad (15)$$

and that

$$K_s = \mu_m k_Q k_s/u_m \quad (16)$$

[Thus Equations (15) and (16) may be substituted into Equation (13) to produce Equation (14).] This, incidentally, shows that the validity of the Monod equation is not diminished in spite of the severe constraint placed on Equation (2).

NUTRIENTS IN EXCESS

Thus far in our argument we have assumed that all other nutrients were in great excess and without influence. This now prompts the question as to what the relation is between μ and Q , when μ is limited by one of the other nutrients. If the specific rate of uptake depends only on the external substrate concentration according to Equation (5) and is therefore independent of the specific growth rate, Q should increase when μ is limited by exhaustion of some other nutrient.

This indeed does happen in chemostats limited by nutrients other than the one under observation. Figure 12, curve *B*, shows μ versus Q for vitamin B₁₂ for *Monochrysis* in a nitrate-limited chemostat.

The plot of μQ versus Q is again linear and intercepts the abscissa and is therefore also described by Equation (10). Figure 13 shows this, and Fig. 14 shows the same for iron in *Monochrysis* with nitrate limiting. Thus we have a situation in which one equation serves to describe either case: the nutrient limiting (when the dependent variable is μ) and the nutrient not limiting (when the dependent variable is Q), whichever nutrient we choose to consider. The implication of this is that a simple multiplicative modification of Equation (11) could describe an organism's status respecting several nutrients simultaneously, the simplest polynomial being

$$\mu/\mu_m' = (1 - k_{Q_A}/Q_A)(1 - k_{Q_B}/Q_B) \times (1 - k_{Q_C}/Q_C) \dots \quad (17)$$

where the subscripts *A*, *B*, *C*, etc., refer to the various nutrients. Since u/μ can be substituted for Q and

$u_m s/(k_s + s)$ for u appropriately in each bracket, we have a derivative relating growth rate to the various external substrate concentrations. Moreover, neither Equation (17) nor its derivative require any prior postulations as to which nutrients are limiting, nor indeed as to whether any are more severely limiting than the remainder.

The rationale underlying Equation (17) is that if a parameter is independently proportional to 2 or more functions, it is also proportional to their product. The proportionality constant μ_m' is, as was pointed out earlier, a mathematical abstraction; its value is determined by all the factors (nutritional and physical, etc.) excluded from the equation. For instance the value of μ_m' of Equation (11) may differ from that of μ_m' of Equation (17) because the latter equation excludes fewer nutritional factors than does the former. Thus no absolute value of this or the other constants (including μ_m of the Monod equation) can be said to exist and so cannot be found in practice. μ_m' as determined will always be less than its true value because culture media contain finite amounts of all nutrients, and the k_Q values as measured will likewise be too large for the same reason. However, approaches could be made by appropriate choice of conditions, *eg*, for measuring μ_m' all nutrients should be in excess, while for measuring each k_Q the nutrient in question should be as low as possible, with all others in excess.

DISCUSSION

To summarize, the postulates expressed in Equation (17) and its derivative are 2: first that the specific rate of uptake depends on the substrate concentration in a Michaelis fashion irrespective of the internal situation [Equation (5)]; and second that the specific rate of uptake and the cell quota have a linear relationship [Equation (10)]. The apparent anomaly is resolved by a third postulate, namely, that the steady state cell quota is the quotient of the specific rates of uptake and growth [Equation (3)]. For the sake of simplicity, excretory losses have been assumed to be negligible, although they could very well have been taken into account.

It is useful to examine some of the consequences of Equations (11) and (17) and their derivatives. The fact that internal substrate concentration is a variable quantity, even in nutrient-limited conditions, has one important practical implication, namely, that it is inadmissible to use the saturation constant for uptake [k_s in Equation (5)] as a measure of the saturation constant for growth [K_s in Equation (1)] as the 2 parameters are necessarily unequal. The proof of the inequality is simple. If they are equal one has

$$s/(K_s + s) = s/(k_s + s)$$

which, according to Equations (1) and (5), gives

$$\mu/\mu_m = u/u_m$$

$$-ds/dx = Q$$

so

$$u/\mu \ (\equiv Q, \text{ which is a variable}) \\ = u_m/\mu_m \ (\text{which is a constant})$$

With *Monochrysis* one gets a k_s (uptake) for vitamin B₁₂ of 2.59 pg/ml and a K_s (growth) of 0.14 pg/ml (4). Eppley & Thomas (6) found the constants for growth and nitrate uptake to be equal. However they had calculated uptake as increase in cell nitrogen relative to cell nitrogen (rather than to cell mass), *ie*, in the present notation, as $(1/Qx)(dQx/dt)$ [rather than $(1/x)(dQx/dt)$]. Under steady state conditions this would be a specific growth rate measurement. They may have thus hit upon an ingenious way of obtaining growth saturation constants from uptake measurements. This is of great practical importance because uptake is very much easier to measure than growth.

Another consequence of a multiplicative model such as Equation (17) is that the level of nonlimiting nutrients would have to be very high before the aggregate of their effect was negligible. No sharp line is drawn between the state of limitation and that of excess. Simultaneous limitation, although not necessarily of equal degree, is seen as being possible and indeed may well be of common occurrence in nature.

Both the cell quota and the coefficient of demand for each nutrient increase with increased growth rate in this model. Whether the two are synonymous (excretory losses apart) is a philosophical question, unless one specifically defines the coefficient of demand as the cell quota appertaining when all other nutrients are in great excess. There is nothing in the model to contraindicate the demand for nutrient *A* being increased by limitation by nutrients *B*, *C*, *D*, etc., although the model as it stands does not provide for such interactions as limitation by one nutrient depressing the uptake of others, as reported by Ketchum (8) in the case of NO₃ uptake being suppressed by PO₄ limitation; and it does not account, for instance, for the success of the ¹⁴C fixation and acetylene reduction assays for limiting nutrients.

Luxury consumption, however, is predicted by the model: Imagine limitation of growth by nutrient *B* or *C*; neither *B* nor *C* would influence the rate of uptake of *A*. *A* would therefore accumulate inside the cells according to the equation

$$dQ_A/dt = u_A - \mu Q_A$$

until such time as the drop in external concentration of *A* brought its rate of uptake into equilibrium with the specific growth rate, the precise point at which this happens being determined by the amount of *A* in the medium and the amount of cell material. It is clear now that Equation (2) requires the further proviso of a constant external substrate concentration, that is

but only for unique values of both μ and s , and in steady state conditions.

Incidentally, the model makes no prediction of an upper limit (Q_m) to the amount of a nutrient that could be accumulated by a minute population of cells severely rate limited by a different nutrient, since Q_m would be u_m/μ . Common sense demands an upper limit and the point is worth investigating. With large populations, on the other hand, it is easy to see that the external levels of all nutrients will be reduced to near limiting proportions. This accounts for the fact that during a phytoplankton bloom, for instance, the titer of any nutrient one likes to monitor is found to drop drastically. It has occasionally been remarked (*eg*, 15) that nutrient ratios in the sea bore some resemblance to those found in the organisms supported by it. The present model predicts that in the limit

$$Q_A:Q_B:Q_C, \text{ etc.} = s_A u_{m_A}/k_{s_A}:s_B u_{m_B}/k_{s_B}:s_C u_{m_C}/k_{s_C}, \text{ etc.}$$

which is not quite the same thing, although in practice there may not be much difference.

A consequence of this luxury consumption is that it should not usually be possible to gauge the potential of a body of water from the titer of its dissolved nutrients alone. In the absence of grazing, or other perturbations, the potential final crop respecting each nutrient would be given by the quotient of the sum of the cell and dissolved components and the cell subsistence quota:

$$X = (Qx + s)/k_Q$$

where x and s refer to the present cell mass and the dissolved nutrient respectively, Q to the present cell quota, k_Q to the subsistence quota, and X to the final crop. And, of course, the nutrient that yields the smallest X on computation is the "limiting" one. Which brings us back to Liebig, although not to Lotka nor Monod.

Caperon's most recent work with nitrate, published after the present paper was submitted, indicates that with some nutrients and organisms at least there may be a lower limit for external substrate concentration below which no uptake takes place (3). This in itself would only entail relatively minor adjustment to the model developed here. However, they also make an extremely interesting observation that has obvious bearing on my present treatment, which assumes the parameters of uptake to be independent of those of growth. They noted that the maximum uptake rate (u_m) measured in a nonsteady state situation depended on the previous rate of nitrogen supply. This is in agreement with the vitamin B₁₂ work with cell suspensions reported shortly here and discussed in detail previously (4), that the number of sites available for

uptake was lessened when lack of the nutrient in question had previously severely limited growth. The important question for my multinutrient model is whether limitation by one nutrient limits the sites available for uptake of others, for if this is so, one would expect luxury consumption to be suppressed.

NOTATION

Dimensions: ml^{-3}

s = External substrate concentration (mass per unit volume)

x = Biomass (mass per unit volume)

K_s, k_s, k_s' = saturation constants in Equations (1), (5), and (7) (external substrate concentrations giving half maximal rates)

Dimensions: t^{-1}

D = Dilution rate of chemostat (flow volume per culture volume per unit time)

μ = Specific growth rate (increase in biomass per unit biomass per unit time)

μ_m = Maximum specific growth rate at infinite external substrate concentration

μ_m' = Maximum specific growth rate at infinite internal substrate concentration

u = Specific rate of uptake (mass per unit biomass per unit time)

u_m = Maximum specific uptake rate at infinite external substrate concentration

Dimensionless

Q = Demand coefficient, taken in the absence of excretion to be synonymous with the cell quota (internal substrate concentration) (mass per unit biomass)

k_Q = Subsistence quota, *ie.* the Q intercept for zero μ

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