THE NUTRIENT STATUS OF ALGAL CELLS IN CONTINUOUS CULTURE

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(Text-figs. 1–12)

Chemostats were used to study the relation between growth rate, the rate of nutrient uptake and internal and external nutrient concentrations of two nutrients simultaneously. (*Monochrysis lutheri*: phosphorus and vitamin B_{12}.)

Growth rate and internal concentrations of both limiting and excess nutrients are related by simple rectangular hyperbolas.

Control was shown to follow a threshold rather than multiplicative pattern; that is, non-limiting nutrients exert no control at all over the pattern of growth. The limiting nutrient was the one that showed the smallest cell quota:subsistence quota ratio.

Monochrysis populations exhibited two modes of growth. 'Slow adapted cells' differed from 'fast adapted cells' in the values of the constants for the above relation.

Uptake of both limiting and non-limiting nutrients was found to be controlled by internal as well as external substrate concentrations. There was thus a limit to luxury consumption of one nutrient when growth was limited by another.

The mathematical model formulated for growth in a chemostat (equations (23)–(29)) allowed prediction of external and internal substrate concentrations and rates of uptake of two nutrients and of biomass, given only the input concentrations of the two nutrients and the dilution rate. This model should apply equally well to growth in batch cultures; its possible application to natural populations was discussed.

INTRODUCTION

It is my contention that the internal status of an algal cell respecting the various essential nutrients can be as important as or even more important than the concentration of the nutrients in the environment, depending of course on the circumstance (Droop, 1973*a*, *b*). Predictive phytoplankton models (e.g. Dugdale & Macisaac, 1971) commonly make use of the Michaelis Menten enzyme expression for the uptake of a nutrient. This, together with the assumption that there is direct coupling between the uptake of a limiting nutrient and growth, leads to the Monod (1942) equation for nutrient limited growth:

$$\frac{\mu}{\mu_m} = \frac{s}{K_{s(\mu)} + s}. \star$$

(1)

However, since the enzyme reactions that result in growth 'see' internal rather than external substrate concentrations, it can be argued that changes in growth rate must be caused by changes in the concentrations of the various internal nutrient pools, and that the decision for instance as to which nutrient is rate limiting hangs upon the relative concentrations of these pools and not on the external concentrations directly.

★ An explanation of the symbols used throughout the paper is given in Table 9 (p. 854).

It follows from this that a cell's previous history influences its present behaviour, a fact that is well known and that has bedevilled our attempts to describe events in transient systems with the aid of Michaelis kinetics solely in terms of external substrate concentrations. Steady-state systems should not suffer from this difficulty since in them the past is largely eliminated. Such systems have the potential to yield a consistent set of self-contained relationships, which should in principle be applicable to the real world of non-steady-state situations, that is, provided sufficient input information is to hand.

My present objective is to formulate a description of growth of an alga under the steady-state conditions of a chemostat in terms of internal and external concentrations of two essential nutrients, either or both of which may be effecting control.

METHODS

The yellow supra-littoral flagellate *Monochrysis lutheri* (SMBA Strain 60) is a convenient organism for chemostat work. *Skeletonema costatum*, a diatom and another possibility, might have been preferable on the grounds of its ecological importance, but it is less easy to handle and consequently slower to yield results than the flagellate. The two organisms are similar in cell size in their behaviour towards vitamin B_{12} limitation (Droop, 1970). It is however obviously desirable that important conclusions should be confirmed with the diatom.

The details of the apparatus for continuous culture were described previously (Droop, 1966). The capacity of the culture vessels was 295 ml. The culture medium S 88 (Droop, 1968) was prepared as a dry mix without the two nutrients, phosphorus and vitamin B_{12}, which were to be the subject of the experiment. The chemostat reactors were immersed in a water bath at 20 °C and received continuous warm white fluorescent illumination of about a K lux.

The choice of the two experimental nutrients was dictated by the method of measurement. Since the intention was to use radioactive labels the two isotopes had to be mutually distinguishable so that concurrent measurements of both nutrients could be made on a single sample even with the rather unsophisticated well crystal scintillator available to me. ^{57}Co is a γ emitter with energies confined to a narrow band, while ^{32}P is a rather energetic β emitter with an even spread of energies over a very wide band. For accuracy it was advisable that the contribution of the two isotopes to the total count recorded by the apparatus should be of the same order of magnitude. This was achieved when the specific activity of the ^{32}P was 50 times that of the ^{57}Co. Thus the culture medium was charged with ^{57}Co vitamin B_{12} at the rate of some 0·2 μCi/l and with $^{32}PO_4$ at the rate of 10 μCi/l. At this rate it was possible to obtain counts of 1000–50000 cpm on material from 50 ml of culture.

The distinction between the two isotopes was made as follows: Designating the counts (after subtracting background) obtained with four different discriminator bias settings as C_1, C_2, C_3, C_4, then the total count in the sample due to ^{32}P is given by

$$P = \frac{1}{a}\left[C_1 - C_2 - \frac{c}{b}(C_3 - C_4) \right]$$

and that due to ^{57}Co by

$$V = \frac{1}{b}\left[C_3 - C_4 - dP \right],$$

where a, b, c, d are the relative efficiencies respectively of the $C_1 - C_2$ counts for ^{32}P, the $C_3 - C_4$ counts for ^{57}Co, the $C_1 - C_2$ counts for ^{57}Co and the $C_3 - C_4$ counts for ^{32}P; the relative efficiency having been ascertained for each isotope separately and calculated as respectively $(C_1 - C_2)/C_1$ and $(C_3 - C_4)/C_1$. For ^{57}Co and ^{32}P on my apparatus (a $2\frac{1}{2}$ in NaI well crystal and photomultiplier type 6079B with Panax autoscaler Type D697) convenient values for the bias settings were 2, 12, 17 and 25 volts with H.T. setting 1200 volts. With these settings a, b, c, d, were found to be, respectively, 0·386, 0·962, 0·0065 and 0·146.

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We require the following information from the chemostat:
(1) Dilution rate, D ($\equiv \mu$) = flow rate ÷ culture volume.
(2) Cell count, x.
(3) ^{32}P and ^{57}Co radioactivities in cells from an aliquot (*ca.* 50 ml) of culture (designated P_c and V_c respectively).
(4) ^{32}P and ^{57}Co radioactivities in the supernatant from the same aliquot (designated P_s and V_s respectively).
(5) The chemostat phosphorus and vitamin B_{12} input concentrations $s_R(P)$ and $s_R(V)$.
Then the concentrations of phosphorus and vitamin in the supernatant culture medium are given by, respectively:

$$s(P) = \frac{P_s \cdot s_R(P)}{P_c + P_s}$$

and

$$s(V) = \frac{V_s \cdot s_R(V)}{V_c + V_s}$$

and those of the cell quotas for phosphorus and vitamin, respectively:

$$Q(P) = \frac{P_c \cdot s_R(P)}{P_c + P_s} \cdot \frac{1}{x}$$

and

$$Q(V) = \frac{V_c \cdot s_R(V)}{V_c + V_s} \cdot \frac{1}{x}.$$

In harvesting and processing the samples: after withdrawing some 55 ml from the chemostat 1–5 ml was used for a Coulter cell count and the remainder passed through a glass filter pad to retain the cells, in a filter holder so designed as to fit without dismantling into the well of the NaI crystal. The supernatant was then refiltered through a membrane as a precaution and then heated gently with a few mg of ferric chloride to co-precipitate the orthophosphate and then passed through a 2 mm deep 'column' of activated charcoal in another filter holder of the same type. These filter holders were simply standard scintillator beakers with their bases cut off and used upside down with the filter pad (or pad and charcoal) resting on a wire gauze. Accidents apart, GFB glass paper holds back every *Monochrysis* cell, while 2 mm of charcoal holds more than 97% of vitamin B_{12} and 99% of orthophosphate (after precipitation).

Phosphorus was added to the culture medium as orthophosphate (including the label which was 'PBS orthophosphate' from the Radiochemical Centre, Amersham, at a specific activity of *ca.* 100 Ci/mg P). Vitamin B_{12} was obtained from Glaxo Ltd as '100 μg ampoules for injection' and the label, 'CTP Cyanocabalamin for injection', also from the Radiochemical Centre, at a specific activity of usually *ca.* 0·1 Ci/mg vitamin.

Analyses of linear regression were carried out by conventional methods on the data as suggested by the linear derivatives of the equations being tested. 95% fiducial limits were taken as t standard errors from the means, and are shown in parentheses.

INTERNAL SUBSTRATE CONCENTRATION

Introductory

There is increasing evidence from chemostat work that a simple empirical relation exists between the rate of growth of algal cells and the internal concentration (cell quota) of a clearly limiting nutrient. This can be shown to be so with vitamin B_{12} with *Monochrysis lutheri* (Droop, 1968) and with *Skeletonema costatum* (Droop, 1970), with nitrate for *Isochrysis galbana* (Caperon, 1968), with phosphorus for *Cyclotella nana* (Fuhs, 1969), with nitrate for *M. lutheri* and *C. nana* (Caperon & Meyer, 1972 a, b), with phosphorus

for *Nitzschia actinasteroides* (Müller, 1972), with iron for *M. lutheri* (Droop, 1973 b) and with silicon for *Thalassiosira pseudonana* (Paasche, 1973 a, b).

Furthermore, in continuous culture this relation holds whether the nutrient is limiting or not (Droop, 1973 a, b).

Thus, if one considers two nutrients, one of which may or may not be limiting, one has in the chemostat*

$$\frac{D}{D'_m} = 1 - \frac{k'_{Q_A}}{Q_A} = 1 - \frac{k'_{Q_B}}{Q_B} \tag{2}$$

so that

$$\frac{Q_A}{Q_B} = \frac{k'_{Q_A}}{k'_{Q_B}} \tag{3}$$

which is to say that the ratio of internal concentrations of the various nutrients is that of their apparent subsistence quotas and is independent of the specific growth rate.

k'_Q is a variable quantity, which should be distinguished from the 'true' subsistence quota k_Q. The precise relationship between k_Q and k'_Q depends on the model adopted for multinutrient limitation, but in any case k_Q is the value k'_Q takes when the nutrient in question is clearly limiting.

Thus

$$k'_{Q_L} = k_{Q_L} \tag{4}$$

and

$$k'_{Q_N} = k_{Q_L}\frac{Q_N}{Q_L} \tag{5}$$

so that we have

$$R = \frac{k'_{Q_N}}{k_{Q_N}} = \frac{k_{Q_L}}{k_{Q_N}} \cdot \frac{Q_N}{Q_L} = \frac{k_{Q_L}}{k_{Q_N}} \cdot \frac{s_{R_N} - s_N}{s_{R_L} - s_L} \tag{6}$$

since necessarily

$$Q = \frac{s_R - s}{x}. \tag{7}$$

R we may term the 'luxury coefficient' because it is a measure of the relative excess of a non-limiting nutrient over a limiting one. Equation (2) can now be written

$$\frac{D}{D'_m} = 1 - \frac{Rk_Q}{Q}. \tag{8}$$

I have suggested and discussed (Droop, 1973 a, b) a multiplicative model for multi-nutrient limitation in the polynomial

$$\frac{D}{D'_m} = \left(1 - \frac{k_{Q_A}}{Q_B}\right)\left(1 - \frac{k_{Q_B}}{Q_B}\right)\left(...\right), \text{ etc.,} \tag{9}$$

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* This paper is concerned principally with chemostat steady-states when specific growth rate and dilution rate are theoretically equal. The symbol for dilution rate, D, has been used, since that is the parameter actually measured.

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but there is a very obvious alternative, more in keeping with Leibig's 'law of minimum', in what we may term a 'threshold model', namely

$$\frac{D}{D'_m} = 1 - \frac{k_{Q_A}}{Q_A}, \quad \text{or} \quad \frac{D}{D'_m} = 1 - \frac{k_{Q_B}}{Q_B}, \text{ etc.} \tag{10}$$

Essentially the multiplicative model states that all nutrients exert at least some control all the time, while the threshold model states that control is always exerted by a single nutrient, the one that happens to be in shortest supply relative to the needs of the cell.

It will be clear that if equation (9) is true then equation (2) cannot be strictly so under all conditions, since when for example two nutrients are equally and distinctly limiting so that both $k'_{Q_A} = k_{Q_A}$ and $k'_{Q_B} = k_{Q_B}$, we get from equation (9)

$$\sqrt{\frac{D}{D'_m}} = 1 - \frac{k_{Q_A}}{Q_A} = 1 - \frac{k_{Q_B}}{Q_B} \tag{11}$$

which is not compatible with equation (2). Therefore, one would expect either non-linearity in equation (2) or a contradiction of equation (9). Equation (10) on the other hand predicts linearity in equation (2) under all conditions.

At the other extreme, when one of the nutrients is very much more limiting than the remainder, the difference between the two models disappears.

One can go further and state that, unlike the multiplicative model, the threshold model predicts that the observed relation, equation (2) applied to a limiting nutrient is un-affected by the cell status respecting any nutrient, providing the same nutrient remains limiting, however marginally.

Thus to distinguish between the two hypotheses it is necessary to have the two nutrients as nearly equally limiting as we can, that is we need to have

$$\frac{Q_A}{k_{Q_A}} = \frac{Q_B}{k_{Q_B}},$$

whereas to establish value for k_{Q_A} and k_{Q_B} in the first place we require first the one and then the other nutrient to be clearly limiting.

In summary, the objectives are: first to confirm that equation (2) holds where two nutrients are examined simultaneously in a single culture under a variety of conditions of relative input concentrations. Secondly, to decide between the two models for multiple limitation expressed in equations (9) and (10), and also, incidentally, to decide whether the distinction is at all important from a practical point of view.

Results

Two chemostats were operated continuously from early January to late November 1973. To begin with one received 1·44 nM/ml phosphorus and 68 fM/ml vitamin B_{12} (Experiment I) and the other 14·4 nM/ml phosphorus and 6·8 fM/ml vitamin B_{12} (Experiment II). In May the input concentrations were changed to 1·44 nM/ml phosphorus and 21·4 fM/ml vitamin B_{12} (Experiment III) and 1·44 nM/ml phosphorus and 6·8 fM/ml vitamin B_{12} (Experiment IV). Experiment I had phosphorus clearly limiting and the vitamin in great excess while in Experiment II the vitamin was clearly limiting
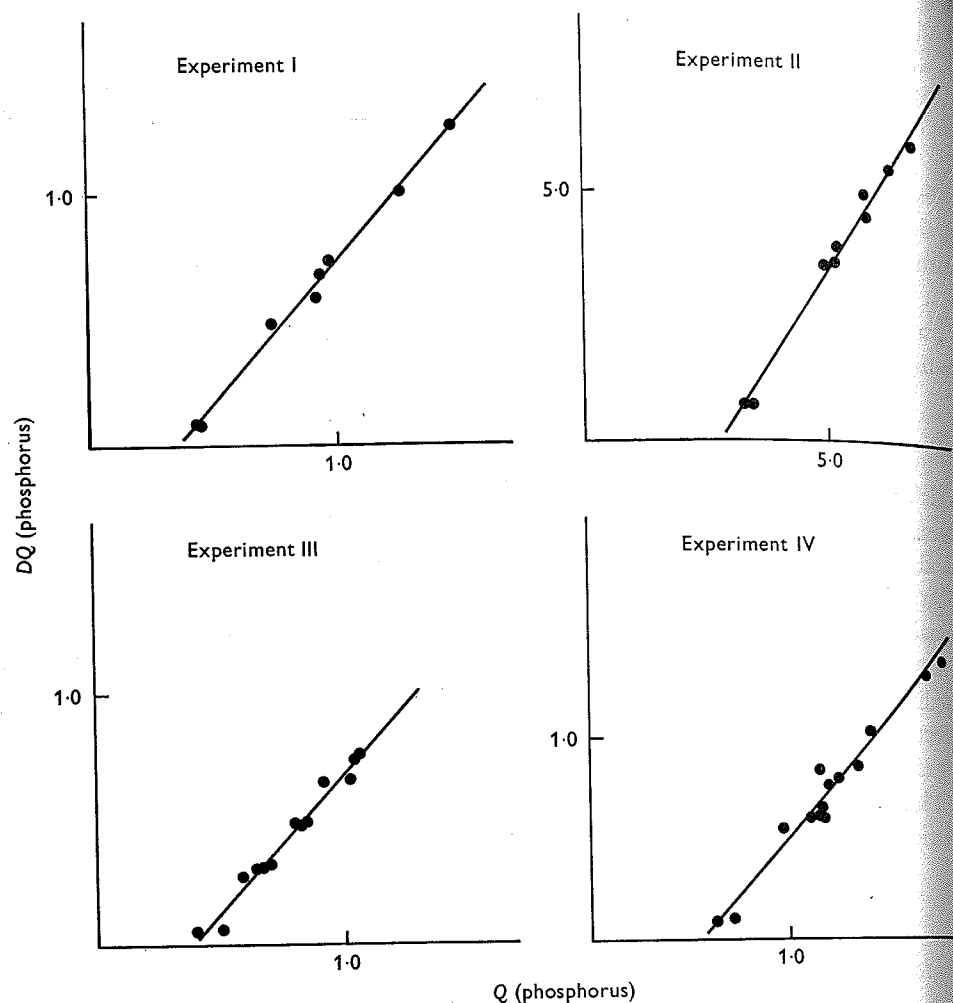
Fig. 1. Regressions of DQ on Q for phosphorus from Experiments I–IV. Q in nM/million cells. DQ in nM/million cells/day.

and phosphorus was in excess. Experiment III proved to have a slight excess of the vitamin and Experiment IV a slight excess of phosphorus.

It gradually became apparent during the experiments that *Monochrysis* exhibited two modes of growth, which we may term respectively 'fast adapted' and 'slow adapted'. This phenomenon will be discussed in a later part of this paper. For the moment, suffice to say that the two modes could readily be distinguished and the switch from one to the other was fairly predictable. The detailed discussion will be confined to results, since they were more precise than the other set, obtained with the 'fast adapted' cells – i.e. to those obtained from the end of February to the end of April and from the end of July to mid-November. The 44 steady-states recorded during these two periods are set out in Table 1. (The 48 steady-states for slow adapted cells recorded mainly in January and February and again between June and September are shown in Table 6, p. 849.)
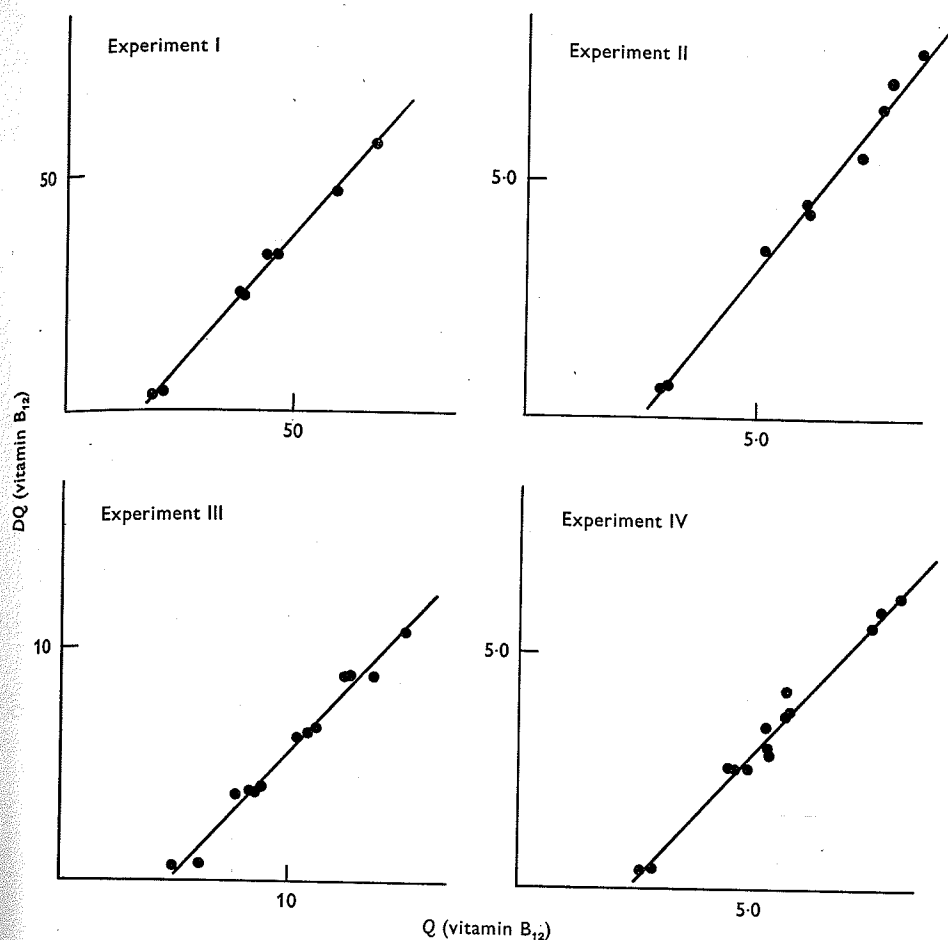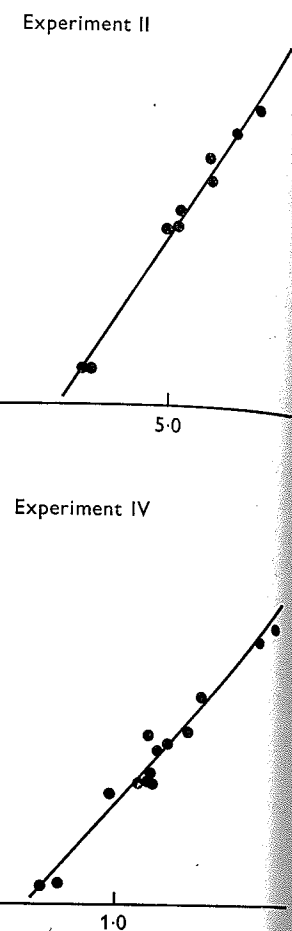
Experiment II

Experiment I

Experiment II

Experiment IV

Experiment III

Experiment IV

Q (vitamin B$_{12}$)

Fig. 2. Regressions of DQ on Q for vitamin B$_{12}$ from Experiments I–IV. Q in fM/million cells. DQ in fM/million cells/day.

I–IV. Q in nM/million cells.

Equation (2)

The first task is to show that equation (2) adequately describes these steady-states both in respect of phosphorus and vitamin B$_{12}$ regardless of which of the two nutrients was the more limiting. We may examine the eight regressions of DQ on Q and the eight of D on $1/Q$ according to the two linear derivatives of the equation,

and

$$DQ = D'_m Q - D'_m k'_Q \tag{12}$$

$$D = D'_m - D'_m k'_Q \frac{1}{Q}. \tag{13}$$

Eight regressions, since we have two nutrients and four experiments. The regression of DQ and Q are shown in Figs. 1–2 and the coefficients derived from them in Table 2. The regression of D on $1/Q$ are likewise shown in Figs. 3–4 and the coefficients in Table 3. The regression of DQ and Q is statistically questionable in that the ordinate and abscissa

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Monochrysis exhibited two apted' and 'slow adapted'. s paper. For the moment, ed and the switch from one will be confined to results, h the 'fast adapted' cells – April and from the end of g these two periods are set recorded mainly in January own in Table 6, p. 849.)

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are not independent since they both contain Q, but is none the less useful in being rather more graphic than the other and, moreover, DQ is the rate of uptake. The correlation coefficients of this regression are naturally very high indeed. On the other hand, high negative correlation coefficients of the regression of D on $1/Q$ are good
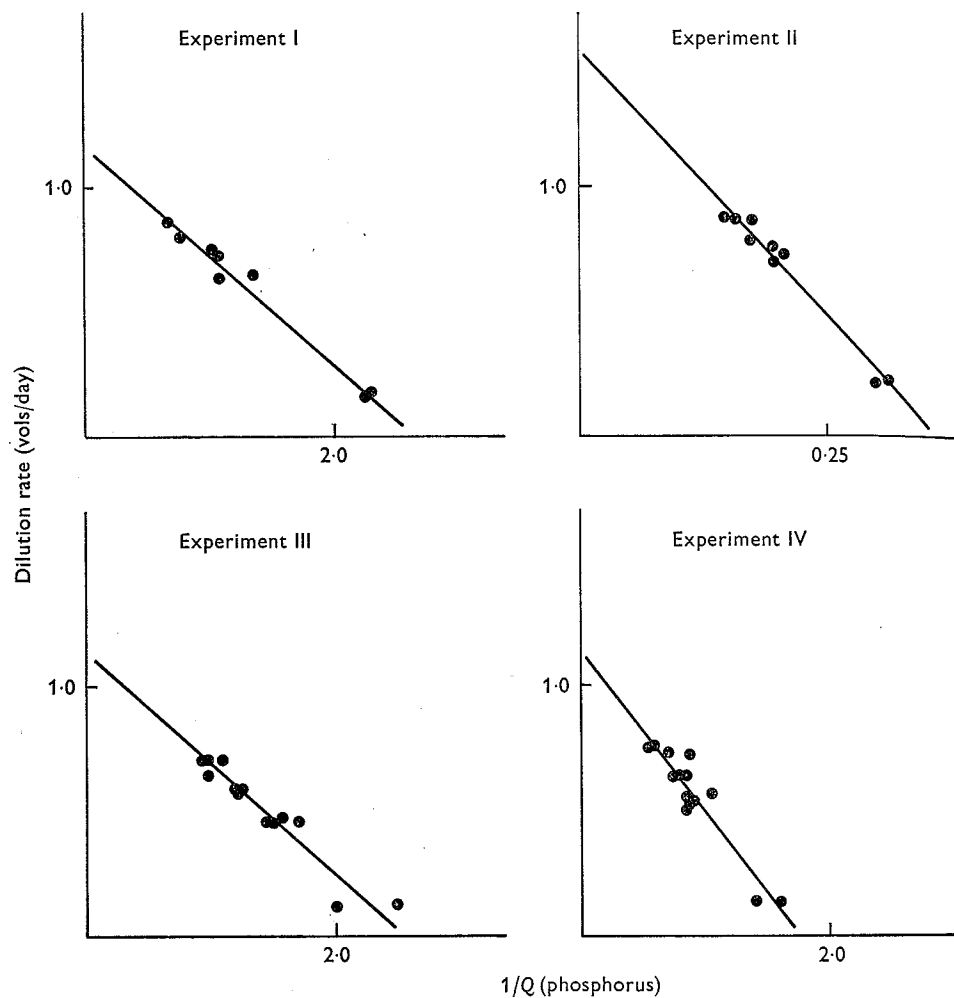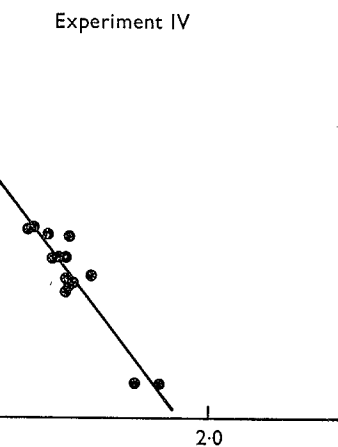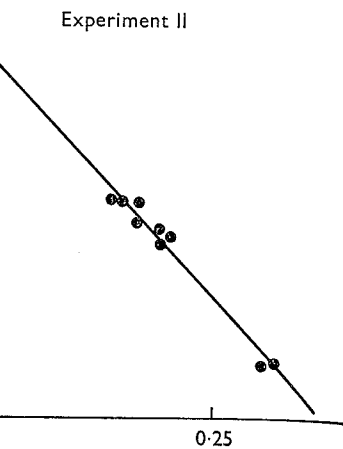

Fig. 3. Regressions of D on $1/Q$ for phosphorus from Experiments I–IV. D in vols/day. $1/Q$ in million cells/nM.

evidence of the appropriateness of the equation for the data. Reference to the tables shows the lowest coefficient to be 0·982 for the DQ on Q regressions and −0·944 for the D on $1/Q$ regressions. These are indeed impressive and we may conclude that equation (2) accurately describes the relation between D and Q for the chemostat steady-states over a very wide range of nutrient input concentrations.

One would expect D'_m to be the same for all the regressions and especially from the phosphorus and vitamin pair in each experiment. This was generally the case, but

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Experiment II showed a higher D'_m than the other three. I cannot explain this anomaly, especially as conditions other than those varied intentionally were uniform throughout the series. We note that k_Q for vitamin B_{12} in the two vitamin-limited cultures was respectively 2·37 and 2·54 fM/million cells. This compares quite well with the figure of
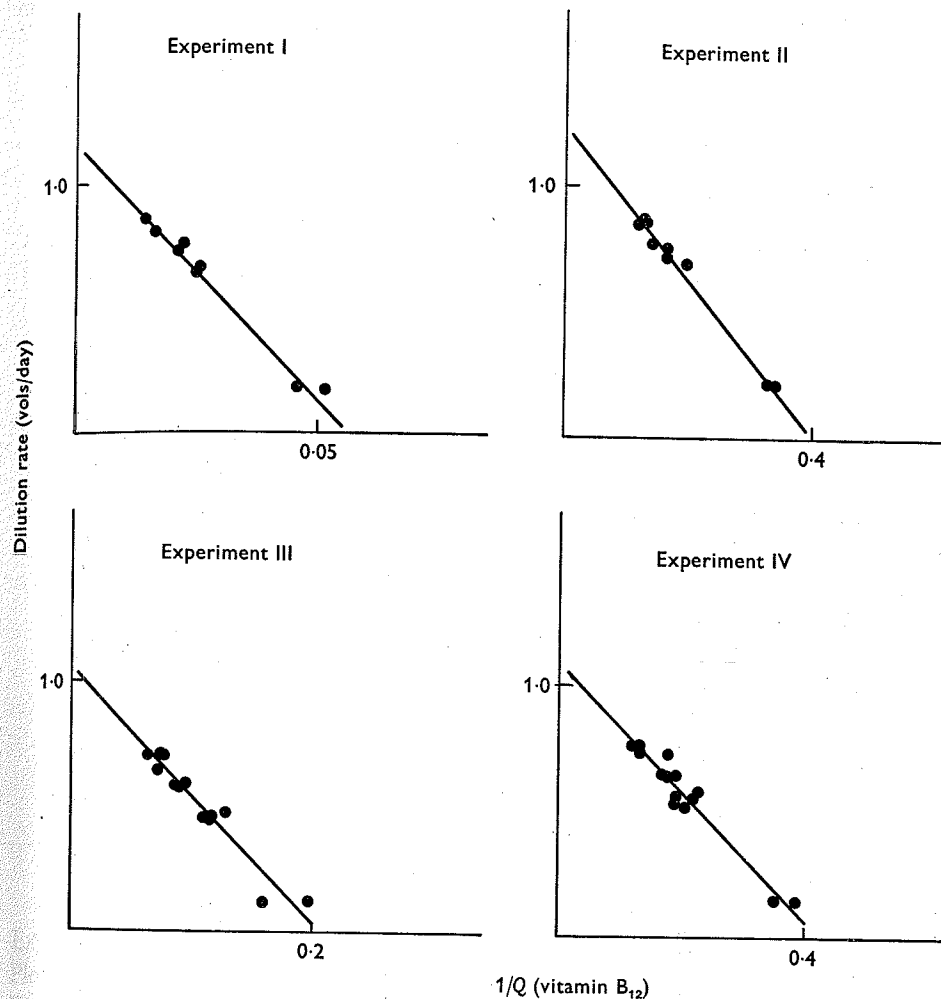
Experiment II

Experiment IV

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Experiments I–IV. D in vols/day.
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Fig. 4. Regressions of D on $1/Q$ for vitamin B_{12} from Experiments I–IV. D in vols/day. $1/Q$ in million cells/fM.

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2·29 obtained with the same organism in 1967 (Droop, 1968). We may take it that the true k_Q of vitamin B_{12} for *Monochrysis* is of this order. Similarly the k_Q for phosphorus probably lies between 0·36 and 0·38 nM/million cells.

TABLE 1. CHEMOSTAT STEADY-STATES, 'FAST ADAPTED' CELLS (s HERE REFERS TO TOTAL DISSOLVED SUBSTRATE)

Date	D (vols/day)	x (millions/ml)	s (P) (nM/ml)	s (V) (fM/ml)	Q (P) (nM/million cells)	Q (V) (fM/million cells)
I. s_R (P) = 1·44 nM/ml, s_R (V) = 68 fM/ml (i.e. vitamin in great excess)						
1. iii.	0·633	1·48	−0·0138	6·28	0·916	39·0
6. iii.	0·650	1·62	0·247	5·50	0·733	38·5
12. iii.	0·724	1·35	0·179	6·15	0·930	45·7
16. iii.	0·752	1·40	0·0812	6·57	0·967	43·8
19. iii.	0·800	1·03	0·144	7·77	1·26	58·5
23. iii.	0·855	0·939	0·0590	5·13	1·47	67·0
6. iv.	0·162	3·01	0·114	9·60	0·440	19·4
19. iv.	0·175	2·65	0·290	10·2	0·433	21·8
II. s_R (P) = 14·4 nM/ml, s_R (V) = 6·8 fM/ml (i.e. phosphorus in great excess)						
1. iii.	0·694	1·18	8·27	0·728	5·16	5·14
6. iii.	0·719	1·05	9·21	0·415	4·93	6·10
12. iii.	0·774	0·804	9·65	1·00	5·87	7·21
16. iii.	0·754	0·970	9·31	0·949	5·21	6·03
19. iii.	0·862	0·812	9·18	0·603	6·39	7·63
23. iii.	0·871	0·719	9·40	1·14	6·92	7·88
28. iii.	0·858	0·728	10·1	0·633	5·83	8·48
6. iv.	0·211	1·94	7·78	1·06	3·39	2·95
19. iv.	0·219	1·87	8·29	1·09	3·25	3·05
III. s_R (P) = 1·44 nM/ml, s_R (V) = 20·4 fM/ml (i.e. vitamin in slight excess)						
1. viii.	0·458	1·97	0·127	3·01	0·664	8·81
8. viii.	0·452	1·97	0·0656	3·45	0·695	8·58
11. ix.	0·459	2·24	0·130	1·63	0·584	8·38
19. ix.	0·475	2·16	0·0574	3·65	0·637	7·74
25. ix.	0·591	1·67	0·0954	3·00	0·803	10·4
28. ix.	0·587	1·57	0·155	2·79	0·815	11·2
1. x.	0·586	1·66	0·0781	2·42	0·818	12·6
3. x.	0·704	1·43	0·124	2·38	0·917	15·0
8. x.	0·709	1·23	0·128	1·91	1·06	15·0
10. x.	0·705	1·31	0·0678	3·96	1·04	12·5
15. x.	0·644	1·38	0·0256	1·61	1·02	13·6
2. xi.	0·116	2·77	0·0663	3·23	0·496	6·21
12. xi.	0·123	3·39	0·0885	3·19	0·398	5·08
IV. s_R (P) = 1·44 nM/ml, s_R (V) = 6·8 fM/ml (i.e. phosphorus in slight excess)						
1. viii.	0·559	1·12	0·122	0·804	1·17	5·35
8. viii.	0·546	1·28	0·0195	0·870	1·11	4·65
13. viii.	0·572	1·37	0·110	0·619	0·965	4·50
11. ix.	0·531	1·11	0·141	0·856	1·17	5·38
19. ix.	0·518	1·20	0·0277	0·909	1·18	4·92
25. ix.	0·644	1·14	0·0539	0·682	1·21	5·35
28. ix.	0·643	1·06	0·101	0·619	1·26	5·84
1. x.	0·642	1·04	0·0276	0·848	1·36	5·74
3. x.	0·729	1·03	0·238	0·841	1·16	5·76
8. x.	0·760	0·749	0·0449	0·674	1·86	8·18
10. x.	0·763	0·798	0·0360	0·612	1·76	7·76
15. x.	0·736	0·829	0·263	0·563	1·43	7·58
2. xi.	0·140	1·86	0·113	1·45	0·713	2·88
12. xi.	0·134	2·02	0·169	1·47	0·626	2·63

D' CELLS (s HERE REFERS
TE)

Q (P) (nM/million cells)	Q (V) (fM/million cells)
n in great excess)	
0·916	39·0
0·733	38·5
0·930	45·7
0·967	43·8
1·26	58·5
1·47	67·0
0·440	19·4
0·433	21·8
orus in great excess)	
5·16	5·14
4·93	6·10
5·87	7·21
5·21	6·03
6·39	7·63
6·92	7·88
5·83	8·48
3·39	2·95
3·25	3·05
min in slight excess)	
0·664	8·81
0·695	8·58
0·584	8·38
0·637	7·74
0·803	10·4
0·815	11·2
0·818	10·8
0·917	12·6
1·06	15·0
1·04	12·5
1·02	13·6
0·496	6·21
0·398	5·08
horus in slight excess)	
1·17	5·35
1·11	4·65
0·965	4·50
1·17	5·38
1·18	4·92
1·21	5·35
1·26	5·84
1·36	5·74
1·16	5·76
1·86	8·18
1·76	7·76
1·43	7·58
0·713	2·88
0·626	2·63

Equation (3)

A corollary of equation (2) is that the quotas of the two nutrients are always in the same ratio as the respective apparent subsistence quotas (equation (3)). In the event, although individual steady-states showed some variation in the Q ratios in each experiment the means of the ratios agreed very well with the calculated k'_Q ratios (Table 4).

TABLE 2. COEFFICIENTS ASSOCIATED WITH THE REGRESSIONS,

(A), $DQ = D'_m Q - D'_m k'_Q$ AND (B), $Q = \frac{1}{D'_m} DQ + k'_Q$

(D'_m derived from (A) and k'_Q from (B). 95 % fiducial limits shown in parenthesis. Data from Table 1.)

Experiment	Nutrient	D'_m (Vols per day)	k'_Q (Moles per 10^6 cells)	r
I		1·13 ($\pm$0·03)	0·361 ($\pm$0·05) 10^{-9}	0·996
III	Phosphorus	1·10 ($\pm$0·03)	0·390 ($\pm$0·05) 10^{-9}	0·989
IV		1·11 ($\pm$0·04)	0·539 ($\pm$0·09) 10^{-9}	0·982
II		1·52 ($\pm$0·05)	2·76 ($\pm$0·27) 10^{-9}	0·993
I		1·16 ($\pm$0·02)	17·3 ($\pm$1·7) 10^{-15}	0·998
III	Vitamin	1·07 ($\pm$0·03)	4·91 ($\pm$0·54) 10^{-15}	0·990
IV		1·09 ($\pm$0·02)	2·42 ($\pm$0·28) 10^{-15}	0·991
II		1·27 ($\pm$0·03)	2·56 ($\pm$0·23) 10^{-15}	0·998

TABLE 3. COEFFICIENTS ASSOCIATED WITH THE REGRESSIONS,

(A), $D = D'_m - D'_m k'_Q \frac{1}{Q}$ AND (B), $\frac{1}{Q} = \frac{1}{k'_Q} - \frac{1}{D'_m k'_Q} D$

(D'_m derived from (A) and k'_Q from (B). 95 % fiducial limits shown in parenthesis.)

Experiment	Nutrient	D'_m (Vols per day)	k'_Q (Moles per 10^6 cells)	r
I		1·16 ($\pm$0·09)	0·373 ($\pm$0·032) 10^{-9}	−0·989
III	Phosphorus	1·09 ($\pm$0·12)	0·393 ($\pm$0·040) 10^{-9}	−0·957
IV		1·15 ($\pm$0·13)	0·599 ($\pm$0·069) 10^{-9}	−0·994
II		1·56 ($\pm$0·13)	2·86 ($\pm$0·19) 10^{-9}	−0·987
I		1·17 ($\pm$0·08)	17·6 ($\pm$2·0) 10^{-15}	−0·992
III	Vitamin	1·06 ($\pm$0·11)	4·97 ($\pm$0·48) 10^{-15}	−0·964
IV		1·09 ($\pm$0·08)	2·45 ($\pm$0·20) 10^{-15}	−0·974
II		1·24 ($\pm$0·06)	2·48 ($\pm$0·14) 10^{-15}	−0·994

Experiment III provided the worst fit, but even here the difference was not significant. Plots of Q (phosphorus) against Q (vitamin) for Experiments III and IV can be seen in Figs. 8 and 9 (pp. 839, 840). It would appear to be a legitimate conclusion that the Q ratios do reflect the k'_Q ratios and that they are constant for any given input nutrient ratio regardless of the dilution rate.

Equations (9) and (10)

To distinguish between the threshold and multiplicative models for simultaneous limitation it is necessary to rely mainly on data from the two experiments with the two nutrients more or less evenly balanced, namely Experiments III and IV.

The point was made earlier that when two nutrients are equally or nearly equally limiting equation (9) and equation (2) could not both be strictly true: the multiplicative
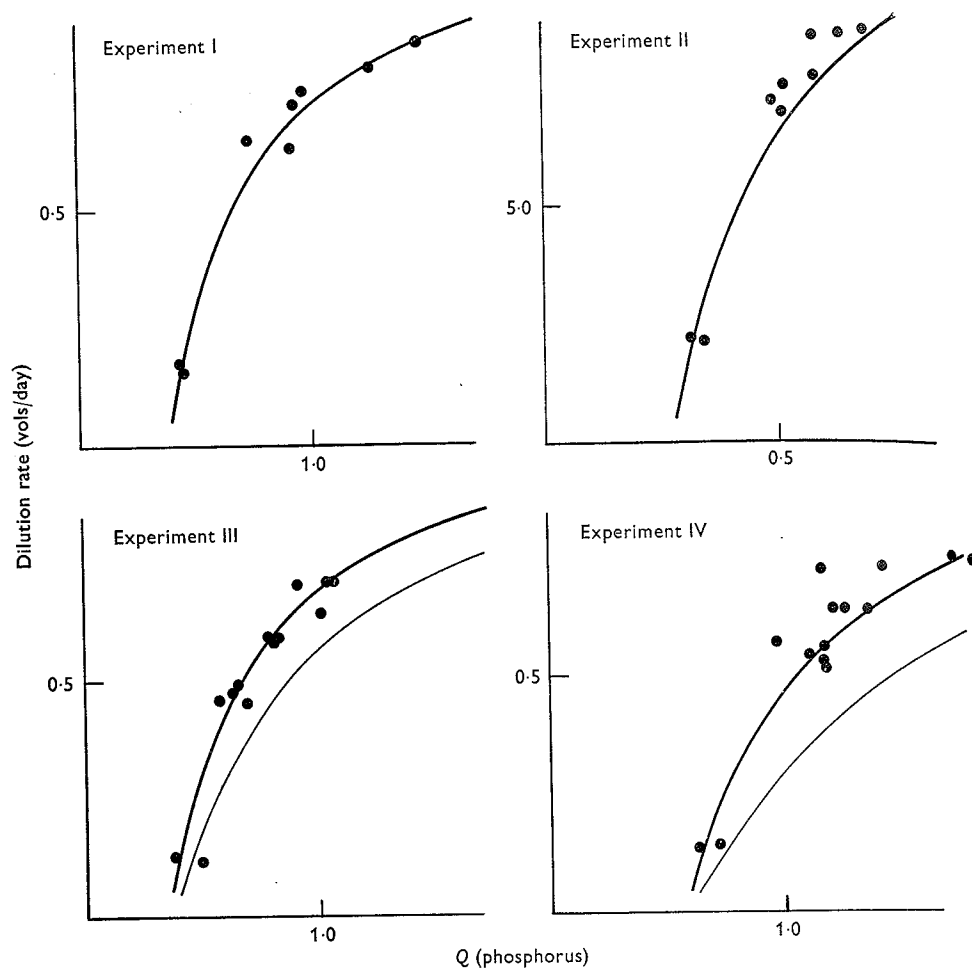
Fig. 5. Plot of D on Q for phosphorus from Experiments I–IV. D in vols/day. Q in nM/million cells. Lighter curves: prediction according to multiplicative hypothesis (equation (9)). Heavier curves: prediction according to threshold hypothesis (equation (10)).

TABLE 4. MEAN RATIOS OF CELL QUOTA OF PHOSPHORUS (Q(P)) TO THAT OF VITAMIN B_{12} (Q(V)) FROM TABLE 1 COMPARED WITH THE MEAN RATIOS OF k'_Q FOR PHOSPHORUS (k'_Q(P)) TO THAT OF VITAMIN B_{12} (k'_Q(V)) TAKEN FROM TABLES 2 AND 3

(95 % fiducial limits in parentheses (those of the k'_Q ratios were calculated by the 'Monte Carlo method').)

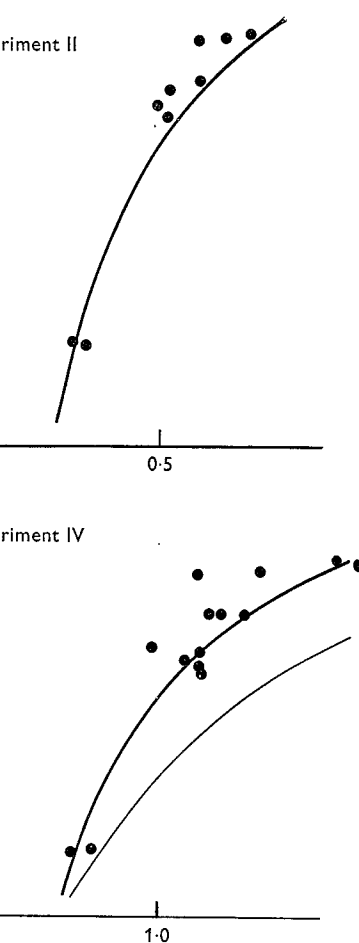| Experiment	Mean ratio Q(P):Q(V)	Mean ratio k'_Q(P):k'_Q(V)
I	0·0213 ($\pm$0·0013) 10^6	0·0210 ($\pm$0·0042) 10^6
III	0·0764 ($\pm$0·0026) 10^6	0·0792 ($\pm$0·0162) 10^6
IV	0·224 ($\pm$0·009) 10^6	0·234 ($\pm$0·055) 10^6
II	0·923 ($\pm$0·12) 10^6	1·11 ($\pm$0·19) 10^6

Fig. 6. Plot of D on Q for vitamin B_{12} from Experiments I–IV. D in vols/day. Q in fM/million cells. Lighter curves: prediction according to the multiplicative hypothesis (equation (9)). Heavier curves: prediction according to the threshold hypothesis (equation (10)).

model requires non-linearity in equation (2). However, as we have seen equation (2) appears to fit the data even when the two nutrients are nearly equally limiting, so we might conclude that the threshold model is the better model. We have not, however, shown that the two nutrients were sufficiently neatly balanced in Experiments III and IV for differences between the two hypotheses to manifest. This is done by calculating D by the two methods (equations (9) and (10)) from the measured steady-state Q values.

The eight plots of D on Q are shown in Figs. 5–6. The curves calculated from equation (9) are shown as light and those calculated from equation (10) as heavy lines. k_Q for each nutrient was taken as the mean of the two lower values of k'_Q of Table 2 and D'_m as the mean value for the appropriate experiment, also from Table 2. In the case of Experiments I and II the pairs of calculated curves were so nearly superimposed that only one

could be shown at the scale of the diagrams. This confirms that any difference between the models disappears with extreme imbalance of the two nutrients. All the other curves clearly indicate that it is the threshold hypothesis that gives the better fit of the facts. The multiplicative model underestimates the growth rates.
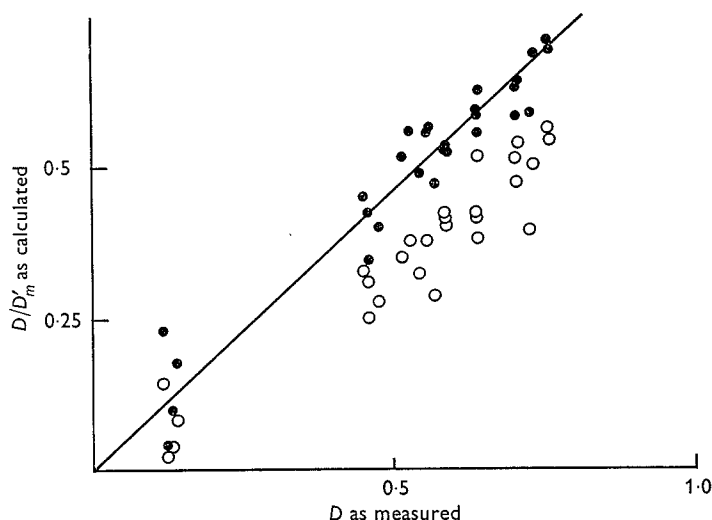

Fig. 7. Computed values of D/D'_m, against D as measured (Experiments III and IV). Open circles D/D'_m computed by equation (9) (multiplicative hypothesis). Close circles D/D'_m computed by equation (10) (threshold hypothesis). The line is the graph of D/D'_m on D, D'_m being given the mean of the values obtained in Experiments III and IV as shown in Table 2.
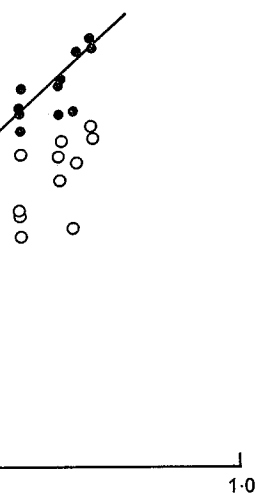
A further check was made by calculating D/D'_m first by equation (9) and then by equation (10) from the steady-state values of Q (phosphorus) and Q (vitamin) in Experiments III and IV and those of k_Q as above and plotting D/D'_m so obtained against D as measured. This should give a straight line with slope $1/D'_m$ passing through the origin. The open circles in Fig. 7 are calculated by equation (9) and the closed circles by equation (10). The slope of the line drawn is that of the reciprocal of the mean D'_m of Experiments III and IV taken from Table 2. Again it is clear that the good fit is provided by equation (10) and not equation (9). And again one observes the multiplicative model underestimating the growth rate.

Figs. 8 and 9 show the steady-states of experiments III and IV plotted on Q (phosphate) and Q (vitamin) coordinates. The actual position of the points is indicated by the decimal point. The numbers are the steady-state dilution rates. The contours are those of D calculated from equation (9) in Figure 8 and from equation (10) in Figure 9. Once again the better fit is seen to be given by the contours calculated from equation (10).

Equation (4)

It is now necessary to check that, as predicted by the threshold model, the relation between specific growth rate and cell quota of a limiting nutrient is unaffected by status respecting any nutrient provided the same nutrient remains (even marginally) limiting.

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sis that gives the better fit of the fac
owth rates.

That this is true when one alters the input concentration of the non-limiting nutrient while keeping that of the limiting one constant is shown by the fact that the parameters of the regressions for phosphorus in Experiments I and II, between which there was a threefold difference in vitamin B_{12} input, do not differ significantly; as also do not those for vitamin B_{12} in Experiments II and IV, between which there was a tenfold difference in the phosphorus input.

Fig. 8. Contours of D on Q (phosphorus) and Q (vitamin) coordinates computed according to equation (9) (multiplicative hypothesis). Experimental steady-states of Experiments III and IV are indicated by the position of the decimal points.

red (Experiments III and IV). Open circles
pothesis). Close circles D/D'_m computed by
e graph of D/D'_m on D, D'_m being given the
IV as shown in Table 2.

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It was not possible from the four experiments already carried out to observe the effect of altering the concentration of the limiting nutrient while holding the non-limiting one constant. In a fifth experiment therefore, both chemostats were given input concentrations of 14·4 nM/ml phosphorus and 13·6 fM/ml vitamin B_{12}, that is with the same phosphorus as Experiment II but with double the amount of vitamin. The latter was still clearly limiting, however, so that a direct comparison could be made with the vitamin data of Experiment II.

In this experiment D'_m was 0·955 ($\pm$ 0·06) and k_Q 1·83 ($\pm$0·28), based on the regression of D on $1/Q$. These values are somewhat smaller than those obtained from Experiment II, but it will be recalled that that experiment yielded abnormally high

values. The only conceivable effect of raising the input concentration of the limiting nutrient would be to increase k_Q. That it was marginally decreased merely increased the margin of error and does not disprove the hypothesis.
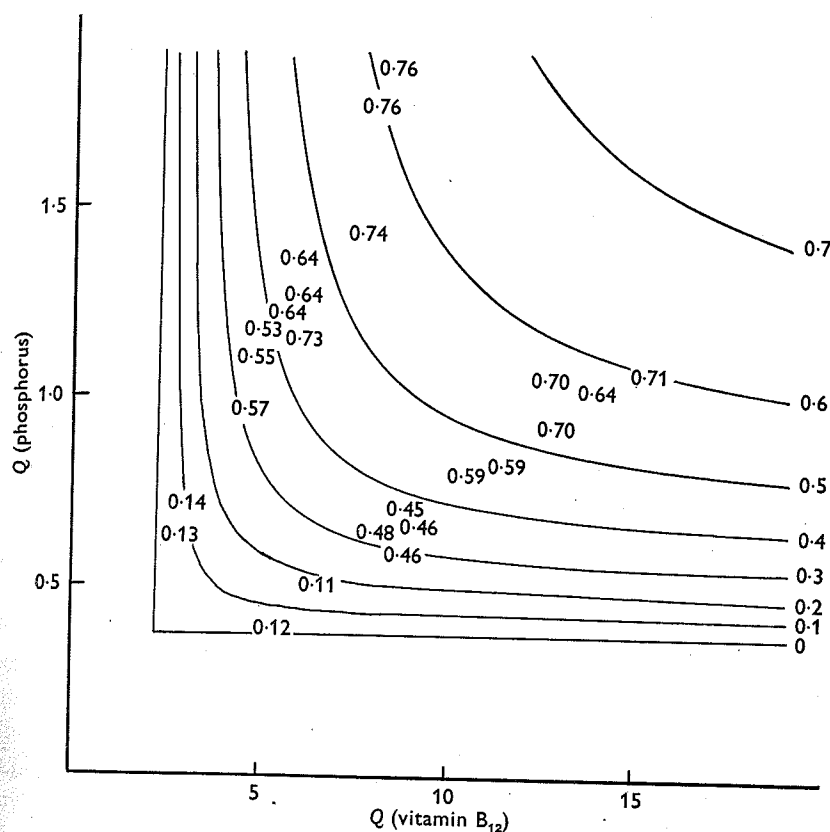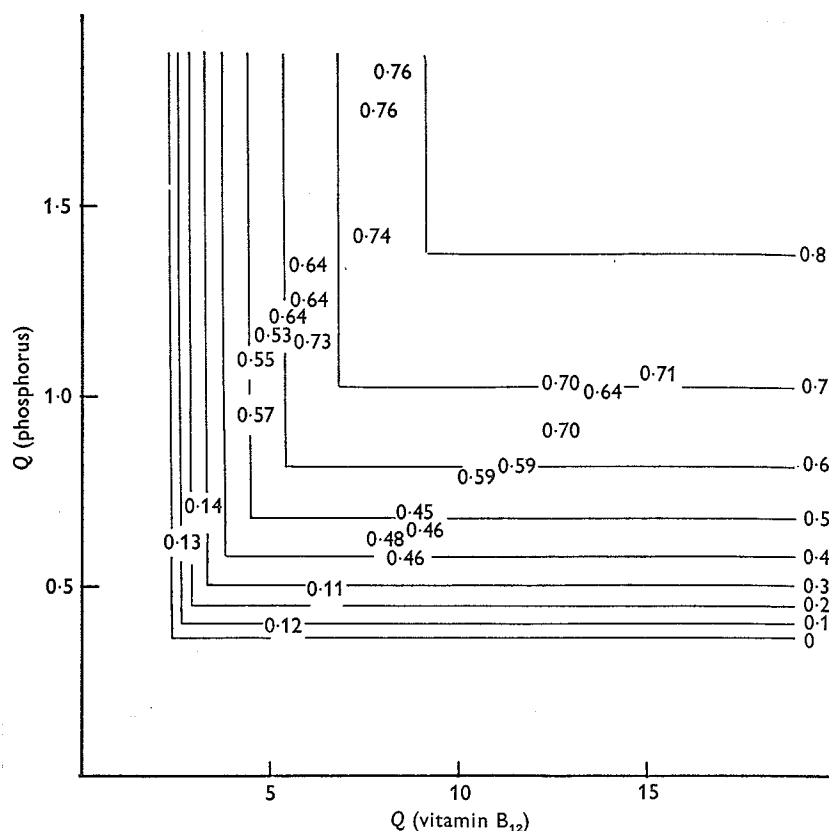

Fig. 9. Contours of D on Q (phosphorus) and Q (vitamin) coordinates computed according to equation (10) (threshold hypothesis). Experimental steady-states of Experiments III and IV are indicated by the position of the decimal points.

Comment and conclusions

Equations (2) and (3) have been convincingly confirmed, namely that under the steady-state conditions of a chemostat it is possible to express growth rate in terms of the internal concentration of both a limiting and a non-limiting nutrient, and that the ratio of these concentrations is independent of the growth rate.

Secondly, the subsistence quota of a limiting nutrient is invariant and independent of the cell's status respecting other nutrients and it is considered a useful measure of the level of requirement. The apparent subsistence quota of a non-limiting nutrient, on the other hand, is larger and variable and its ratio to the true subsistence quota is a measure of the degree of excess of that nutrient. The term 'luxury coefficient' was coined for this ratio.

Thirdly, limitation, at any rate as far as phosphorus and vitamin B_{12} are concerned,

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follows a threshold pattern. That is either one or the other effects control. The controlling nutrient shows the smaller quota to true subsistence quota ratio ($Q:k_Q$) and for it the true and apparent subsistence quotas are identical. The alternative suggestion, that the two nutrients might be exerting simultaneous, though not necessarily equal, control the so-called multiplicative model (Droop 1973 a, b)) has definitely been shown not to be applicable.

Although I believe these conclusions to be well founded, they do only refer to the steady-state conditions of a chemostat, and we shall require to consider how generally relevant they are. A key question in this context is how it comes about that the rates of uptake of two nutrients are in constant proportion regardless of the dilution rate, given constant inputs; the proportion being

$$R(k_{QN}/k_{QL}).$$

The parameter R is apparently determined solely by the input nutrient ratio and should, therefore, be expressible in these terms. The rectangular hyperbola defining R_m

$$\frac{1}{R} = \left(1 - \frac{1}{R_m}\right)\frac{s_{RL}}{s_{RN}} \cdot \frac{k_{QN}}{k_{QL}} + \frac{1}{R_m} \tag{14}$$

provides a moderately good fit. It has the property that when the two nutrients are equally limiting $R = 1$, while at the other extreme $R = R_m$. R_m is thus seen as a reflexion of the limit to luxury consumption of one nutrien in relation to limitation by another. R_m for vitamin B_{12} with phosphorus limiting is very large ($1/R_m = -0.012$, 95 % limits ± 0.026); indeed R appears to be directly proportional to the ratio of input concentrations. When vitamin B_{12} is limiting, on the other hand, we get R_m for phosphorus ca. 11·0 ($1/R_m = 0.091 \pm 0.034$). It would appear that the luxury uptake of phosphorus is more dependent on the uptake of vitamin B_{12} than is that of the vitamin on the uptake of phosphorus.

Since $u = DQ$ in the steady-state, for any given values of s_{RN} and s_{RL} from equation (6):

$$\frac{u_N}{u_L} = \frac{s_{RN} - s_N}{s_{RL} - s_L}, \tag{15}$$

so

$$u_N = \frac{s_{RN} - s_N}{s_{RL} - s_L} \cdot F(s_L), \tag{16}$$

where $F(s_L)$ is to be determined.

If now one considers a batch culture of infinite capacity in which a few cells have achieved a steady-state of growth the same relations apply. Interpreting s_R as the initial substrate concentration, the steady-state can be said to persist until such a time as s differs significantly from s_R. Thus even in a batch culture, although the uptake ratio of two nutrients is $F(s_L, s_N)$, namely $(s_{RN} - s_N)/(s_{RN} - s_L)$, it is not necessarily (s_N/s_L).

It is also possible to extend the reasoning to the non-steady-state condition of incipient nutrient exhaustion. However now

$$\frac{dQ}{dt} = u - \mu Q \tag{17}$$

so that

$$Q = Q^0 + \int_t^0 (u - \mu Q)\, dt \qquad (18)$$

and it is not immediately plain during the transitional phase that Q_N/Q_L and u_N/u_L are equal. That this is the case can be demonstrated by computer simulation. I hope to discuss the practical application of the model to batch cultures at a later date.

EXTERNAL SUBSTRATE CONCENTRATION

Introductory

Since nutrients are provided by the external environment their availability for growth depends, *inter alia*, on the kinetics of uptake. The Michaelis Menten equation, as I mentioned earlier, has usually been employed to describe the dependence of uptake upon substrate concentration (e.g. Wright & Hobbie, 1966; Eppley & Thomas 1969; Eppley, Rogers & McCarthy, 1968; Caperon & Meyer, 1972b) and indeed was an essential part of the model for nutrient limitation discussed by myself (Droop, 1968, 1973a, b).

The Michaelis equation is based on the following reactions.

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X \overset{k_3}{\to} P + E.$$
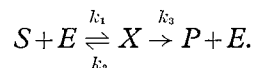
The first reaction is reversible whereas the second is not. The overall reaction is thus unidirectional with no equilibrium state and its rate is proportional to the concentration of the intermediate (X). One would therefore expect the rate of uptake to relate to substrate concentration independently of the specific growth rate and the status of the cell respecting other nutrients, and limitation of growth by one nutrient should lead to continuing luxury consumption of the remainder and hence to there being no limit to the nutrient content of cells, which is an absurdity. This model leaves the Monod equation (equation (1)) for substrate limited growth intact (Droop 1973a, b) and we should therefore expect to find the growth rate/substrate curve passing through the origin.

Alternative hypotheses including some sort of 'product control' could be formulated by making the second reaction above reversible:

$$S + E \underset{k_2}{\overset{k_1}{\rightleftharpoons}} X \underset{k_4}{\overset{k_3}{\rightleftharpoons}} P + E.$$
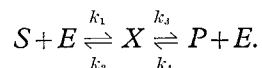
Now the velocity of the reaction is no longer simply proportional to the concentration of the intermediate but is also influenced by the concentration of product. The overall reaction now has an equilibrium state in which net uptake is zero from a finite substrate concentration. Consequently the growth rate/substrate curve would no longer pass through the origin and the simple form of Monod's equation would have to be abandoned.

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The chemostat data

At first sight the substrate data from present experiments are not easy to interpret. Apart from anything else, by comparison with the internal substrate data they are extremely erratic.

The plots of D, u and Q on the measured substrate concentrations are shown in Fig. 11. One may observe first that in no case is there any suggestion that the curves for D and u pass through the origin, as would be predicted by the Monod equation. Secondly, all the sets of vitamin B_{12} data show negative slopes in part so that the curves seem to have the form of a C. Furthermore, accepting the threshold hypothesis for nutrient limitation, it follows from equation (6) that a negative slope on the curve for a non-limiting nutrient could only be produced by a negative slope on the curve for a limiting one. Accordingly, we may infer that the slopes of the phosphorus curves for Experiments I and III are also negative in part. Incidentally, it can also be inferred from equation (6) that any scatter in the substrate concentration of a limiting nutrient that is not due to errors of measurement is necessarily reflected magnified R (k_{Q_N}/k_{Q_L}) times as scatter in the observed concentration of a non-limiting nutrient. Thus, the poor showing of the vitamin substrate data in Experiments I and III results from the poor phosphorus sub-strate data in these experiments.

Previously I was able to show that the negative slope in the curves of D and u on s in vitamin B_{12}-limited chemostat cultures was due to a combination of two factors, *neither of which alone could produce it* (Droop, 1968). These were, first, sequestration of the vitamin by a protein excreted by the cells, which rendered it unavailable to them, and second, a small amount of excretion of the vitamin. The two factors could be quantified and the ensuing modification to the Monod equation was

$$\frac{D}{D_m} = \frac{\beta[s-s_o]}{\beta[K_{s(D)}+(s-s_o)]},\qquad(19)$$

where βs represents the total bound plus free vitamin as measured and βs_0 the residual (free plus bound) vitamin, being the concentration at which excretion and uptake are equal and in equilibrium, i.e. at zero growth rate. β is the ratio of total to free vitamin.

$$\beta = \left(\frac{\tau}{\kappa}\right)\cdot\frac{x}{D}+1.\qquad(20)$$

(τ/κ), the binding constant for vitamin B_{12} and *Monochrysis*, was calculated as 3·27 (when x was expressed as millions/ml and D as volumes/day). This figure may be used in the interpretation of the present vitamin data.

The analogous equation for uptake is simply obtained from equation (19) since both D and Q, and u and Q are uniquely related (equations (2) and (17)):

$$\frac{u}{u_m} = \frac{\beta(s-s_0)}{\beta[k_{s(u)}+(s-s_0)]},\qquad(21)$$

u_m being
$$\frac{D_m D'_m k_Q}{D'_m - D_m}\quad\text{and}\quad k_{s(u)},\ K_{s(D)}\left[\frac{u_m}{k_Q D'_m}+1\right].$$

The plot of D on s (i.e. calculated free vitamin, $\beta s \div \beta$) for the two vitamin-limited experiments (Experiments II and IV) is shown in Fig. 10. Both $K_{s(D)}$ and s_0 had a value of *ca.* 0·03 fM/ml. The 1968 figure for $K_{s(D)}$ was 0·095 fM/ml, but this must also have included s_0, a fact which escaped me at the time.
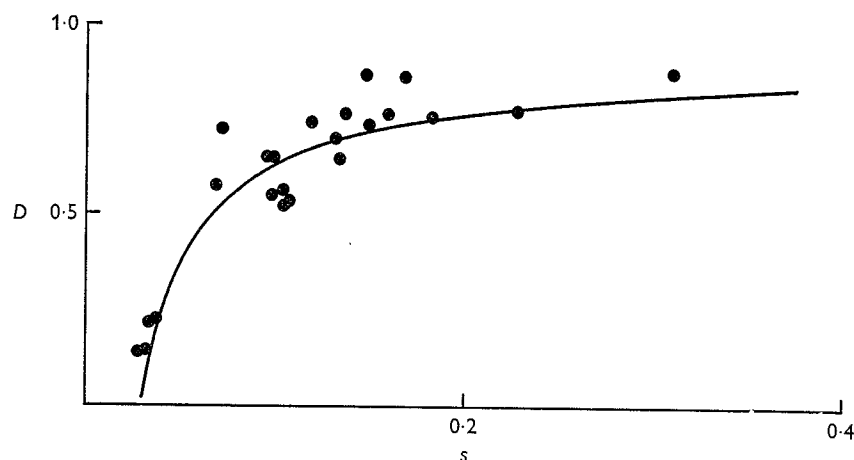

Fig. 10. Plot of D against s corrected for binding. Data from Experiments II and IV (vitamin B_{12} limiting). The curve computed according to equations (19) and (20) with $K_{s(D)} = s_0 = 0·03$ fM/ml and $D_m = 0·9$ vols/day.

Turning now to the phosphorus substrate data, it was pointed out that qualitatively these must have some of the properties of the vitamin data, in particular a negative slope, so that they might with profit be treated mathematically in the same way (without, however, necessarily inferring that phosphorus excretion and phosphorus binding were involved). Equation (20) would be used for the calculation of τ/κ, substituting for β,

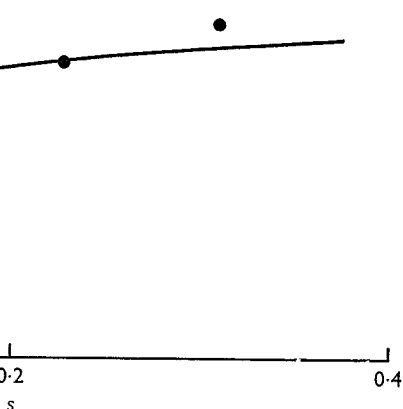$$\frac{K'_{s(D)}}{K_{s(D)} + s_0}.$$

$K'_{s(D)}$ is the apparent saturation constant obtained by applying the Monod equation (equation (1)) to the actual data:

$$K'_{s(D)} = \left(\frac{D_m}{D} - 1\right)\beta s. \tag{22}$$

Unfortunately the phosphate data in Experiments I and II were too scattered to allow any useful calculation by this method, so values τ/κ, s_0 and $K_{s(D)}$ were chosen by trial and error. These are entered in Table 5 under phosphorus. They are emphatically not presented as estimates of the individual parameters; all one can say is that the product $(K_{s(D)} + s_0)(\tau/\kappa)$ is of the correct order.

We are now in a position to simulate the complete operation of the chemostat from a single set of constants and to express this simulation in terms of any pair of parameters associated with a limiting or a non-limiting nutrient. The seven equations providing a complete empirical description of the steady-state in terms of two nutrients (one of which

e vitamin, $\beta s \div \beta$) for the two vitamin-limi
s shown in Fig. 10. Both $K_{s(D)}$ and s_0 ha
re for $K_{s(D)}$ was 0·095 fM/ml, but this
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TABLE 5. CONSTANTS FOR EQUATIONS 23–39 USED IN
COMPUTING THE CURVES IN FIG. 11

	Phosphorus		Vitamin B$_{12}$	
D'_m vols/day ...		1·18		
u_m moles/10^6 cells/day*	1·4 10^{-9}		9·3 10^{-15}	
$k_{s(u)}$ moles/ml*	0·017 10^{-9}		0·12 10^{-15}	
s_0 moles/ml	0·004 10^{-9}		0·03 10^{-15}	
k_Q moles/10^6 cells	0·37 10^{-9}		2·45 10^{-15}	
τ/κ ml/10^6 cells/day	2·13		3·27	
R_m	11·0 (vitamin limiting)		∞ (phosphorus limiting)	
	Expt I	Expt III	Expt II	Expt IV
s_R (phosphorus) moles/ml	1·44 10^{-9}	1·44 10^{-9}	14·4 10^{-9}	1·44 10^{-9}
s_R (vitamin) moles/ml	68·0 10^{-15}	20·4 10^{-15}	6·8 10^{-15}	6·8 10^{-15}

* See equation (21).

s limiting) are re-stated for convenience below. The constants used in the computations
are listed in Table 5.

$$u_L = \frac{u_{mL}(s_L - s_{0L})}{k_{s(u)L} + (s_L - s_{0L})}, \tag{23}$$

$$D = D'_m\left(1 - \frac{k_{QL}}{Q_L}\right), \tag{24}$$

$$u = DQ \tag{25}$$

$$Qx = s_R - \beta s, \tag{26}$$

$$\frac{u_N}{u_L} = \frac{s_{RN} - \beta_N s_N}{s_{RL} - \beta_L s_L} = R\frac{k_{QN}}{k_{QL}}, \tag{27}$$

$$\frac{1}{R} = \left(1 - \frac{1}{R_m}\right)\frac{k_{QN}}{k_{QL}} \cdot \frac{s_{RL}}{s_{RN}} + \frac{1}{R_m}, \tag{28}$$

$$\beta = \frac{\tau}{\kappa} \cdot \frac{x}{D} + 1. \tag{29}$$

rate data, it was pointed out that qualitati
of the vitamin data, in particular a neg
eated mathematically in the same way (with
horus excretion and phosphorus binding
for the calculation of τ/κ, substituting for

$$\frac{K'_{s(D)}}{s(D) + s_0}.$$

t obtained by applying the Monod equ

$$\left(\frac{D_m}{D} - 1\right)\beta s.$$

xperiments I and II were too scattered to
values τ/κ, s_0 and $K_{s(D)}$ were chosen by tri
r phosphorus. They are emphatically not
rameters; all one can say is that the pr

ne complete operation of the chemostat
simulation in terms of any pair of param
ng nutrient. The seven equations provi
dy-state in terms of two nutrients (one of

ng. Data from Experiments II and IV (vitamin B
quations (19) and (20) with $K_{s(D)} = s_0 = 0·03$ fM/ml

he computations of D, Q and u vs βs, i.e. dilution rate, cell quota and uptake against
al external substrate concentrations have been superimposed on the data in Fig. 11.
It cannot be claimed that the prediction of the experimental results is perfect. Never-
less, having regard for the scatter shown by the external substrate data, the large
mber of experimentally determined constants necessarily involved in the computation
the large range of input ratios covered by the four experiments, the correspondence
encouraging. Experiment III appears to be the least satisfactory of the four. Certainly
ission of either β or s_0 from the model drastically reduces the correspondence.
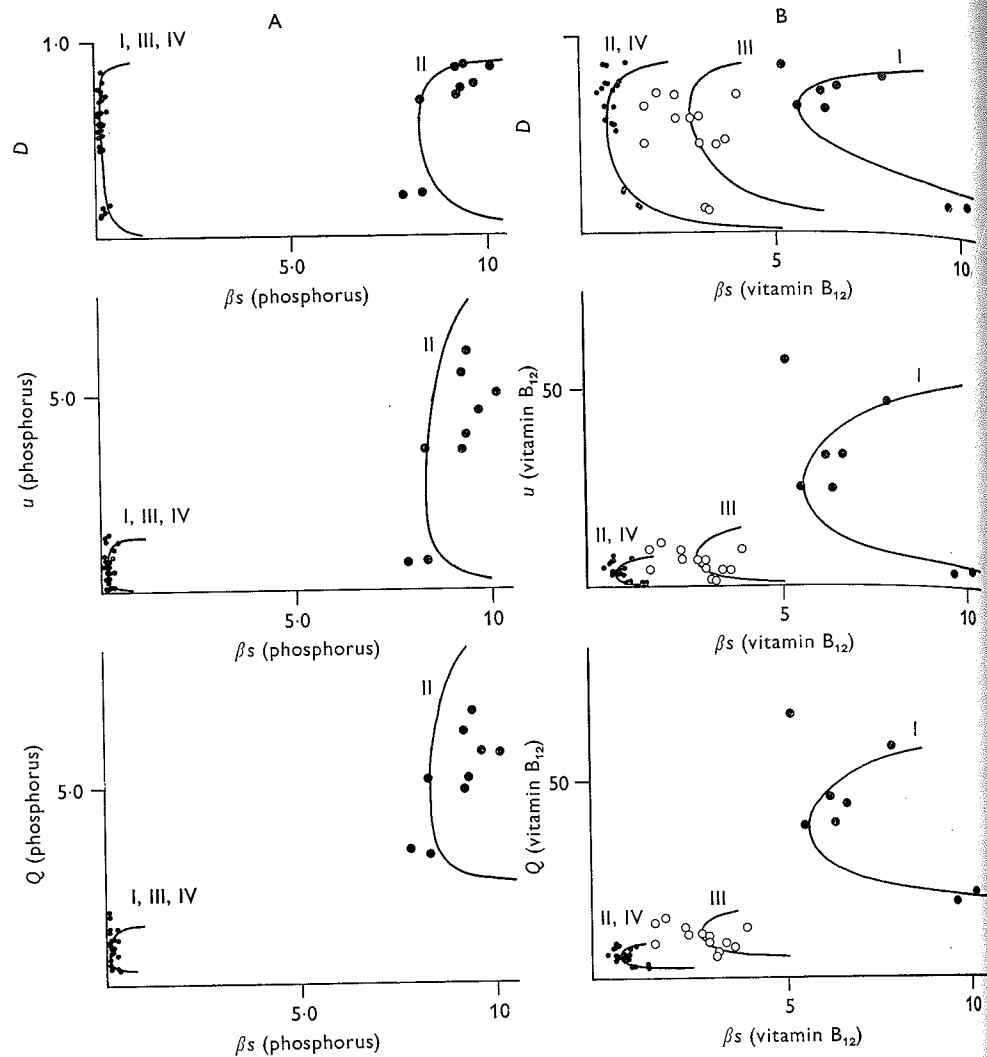
Fig. 11. Chemostat steady-states (Experiments I–IV): plots of D, u and Q on external substrate concentrations (βs) as actually recorded. The curves are computed according to equations (23)–(29), with the constants as set out in Table 5. A, phosphorus. D as vols/day, βs as nM/ml, Q as nM/million cells, u as nM/million cells/day. B, vitamin B_{12}. D as vols/day, βs as fM/ml, Q as fM/million cells, u as fM/million cells/day.

Comment and conclusions

According to our model, therefore, while uptake of a limiting nutrient is a function of available substrate as defined by equation (23), uptake of a non-limiting nutrient is proportional to that of the limiting nutrient regardless of the growth rate. This implies that control of u_N is exerted by Q_N. On the other hand, control is exerted also by the substrate available since R is a function of s_{RN}/s_{RL}. Thus we have uptake of a non-limiting nutrient controlled by both substrate and product.

R defines the limit of luxury consumption of a non-limiting nutrient and, in as far as

βs (vitamin B$_{12}$)

βs (vitamin B$_{12}$)

βs (vitamin B$_{12}$)
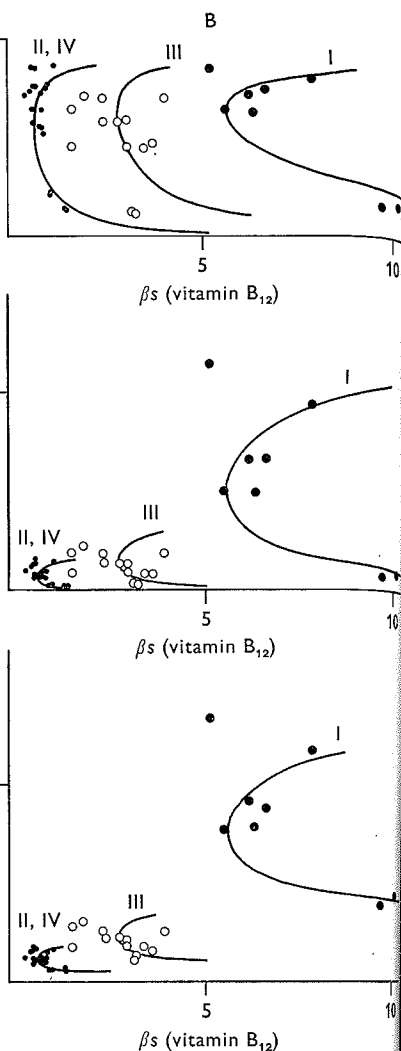
plots of D, u and Q on external substrate
computed according to equations (23)–(29),
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D as vols/day, βs as fM/ml, Q as fM/million

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er hand, control is exerted also by
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non-limiting nutrient and, in as fa

R is circumscribed by R_m, we have both a relative and absolute limit to luxury consumption.

The fact that the growth and uptake curves of limiting nutrients intercept the s axis at s_0 implies an equilibrium at zero growth rate,

$$\text{uptake} = \text{excretion},$$

and of necessity implies product control in some form. To put $(s - s_0)$ for s in the Michaelis equation, though from a practical point of view the obvious thing to do, knocks away any theoretical foundations the equation may have had. All one can say is that the equation in its new form (i.e. equation (23)) may be regarded as an empirical approximation to some very complex equation in terms of rate constants that would describe the kinetics of a fully reversible chain of chemical reactions such as

$$S + E \underset{k_3}{\overset{k_1}{\rightleftharpoons}} X \underset{k_4}{\overset{k_2}{\rightleftharpoons}} P + E.$$
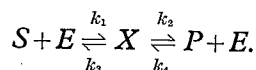
These conclusions refer to vitamin B$_{12}$ and probably to phosphorus but they may be of more general application. Several workers in this field have found it necessary to introduce a term s_0 in their equations describing substrate limited uptake and growth; for example Müller (1972) (phosphate-limited *Nitzschia actinasteroides*), Caperon & Meyer (1972b) (nitrate-limited *Cyclotella nana*) and Paasche (1973b) (silicon-limited *Thalassiosira pseudonana*).

My data suggest that phosphorus behaves as if it were bound and made unavailable in much the same way as vitamin B$_{12}$, only to a lesser degree. This may be so, but the evidence hardly warrants more than a tentative suggestion. Müller (1972), while working with phosphate limitation in *N. actinasteroides*, obtained C-shaped growth rate/substrate curves, and was of the opinion that the explanation was increased mortality at very low growth rates. It is difficult to see how this in itself could prevent the curve passing through the origin.

There is an interesting consequence of the chance (if indeed chance it is) that s_0 and $K_{s(D)}$ appeared to be equal in value. Equation (19) can then be written, assuming $K_{s(D)} = s_0$,

$$\frac{D}{D_m} = 1 - \frac{s_0}{s} \tag{30}$$

which, in combination with equation (2) gives

$$\frac{Q}{Q_m} = \frac{s}{k_{s(Q)} + s}, \tag{31}$$

where

$$Q_m = \frac{k_Q D_m'}{D_m' - D_m} \tag{32}$$

and

$$k_{s(Q)} = \frac{s_0 D_m}{D_m' - D_m}. \tag{33}$$

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Thus in a steady-state system

$$u = \frac{DQ_m s}{k_{s(Q)} + s}.$$

(34)

Therefore, if one were to attempt to express uptake by an equation of Michaelis form, one should find 'u_{max}' varying with the growth rate. Caperon & Meyer (1972b) did just this when they followed the time course of nitrate uptake by *Cyclotella* cells in the growth chamber of a chemostat in the minutes following the stopping of the flow. Their parameter b is the equivalent of Q_m above. It is significant that $K_{s(D)}$ and s_0 in their experiment also appeared to be equal in value. It follows that while u_m of Caperon & Meyer's equation 4 (p. 630) is equivalent to DQ_m, while u_m of their equation 2 on the same page is the equivalent of $D_m Q_m$ and it is therefore not admissible to insert b in their equation 2. By the same token k_s in the two equations stand for different things, namely $k_{s(Q)}$ and $k_{s(u)}$. The confusion arises from the fact that when s_0 is introduced into the Michaelis equation for uptake and, in so far as it can be equated to $K_{s(D)}$, then the equation for Q vs s, and not u vs s has the form of a Michaelis equation (or Langmuir isotherm).

This implies a very simple 'envelope' relationship between internal and external substrate concentration, namely that of reversible adsorption, for equation (31) describes the equilibrium

$$\text{free substrate} \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{adsorbed substrate.}$$
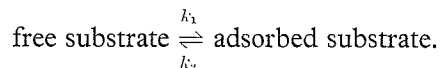
One notes that the curve for Q vs available substrate would pass through the origin were it not for the binding factor and the fact that Q cannot drop below k_Q.

SLOW AND FAST ADAPTED GROWTH

One hesitates to suggest that algal populations may show adaptation to different growth conditions, since it considerably complicates any ecological interpretation that might be put on laboratory results. However, since I based my thesis on only half of the steady-states recorded – it could have been based on the other half with, however, rather less precision – it is incumbent upon me to substantiate my reasons for suggesting that population adaptation is taking place.

The steady-states not used are set out in Table 6, and the coefficients associated with the eight regression of DQ on Q in Table 7. It will be noted that the latter are very different from and invariably lower than their respective counterparts in Table 2. Fig. 12 shows the DQ on Q regression for phosphorus in Experiment II. The 'slow adapted' cells are shown as open circles and the fast adapted as filled circles. There is no doubt that we have here two different populations (in the statistical sense) and the significant thing is, as will be seen in Experiment II in Tables 1 and 6, that, with the exception of the very last reading of all, the steady-state judged to be slow adapted occurred before 1 March. Some time between 23 February and that date the character of the population changed. A similar change occurred at about the same time in Experiment I.

A summary of the history of the four cultures will help to make clear what I believe to have been happening. Experiments I and II were set up in January with inocula

ROOP

$$\frac{Q_m s}{(_Q)+s}.$$

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DAPTED GROWTH.

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TABLE 6. CHEMOSTAT STEADY-STATES, 'SLOW ADAPTED' CELLS.
(s HERE REFERS TO TOTAL DISSOLVED SUBSTRATE)

Date	D (vols/day)	x (millions/ml)	s(P) (nM/ml)	s(V) (fM/ml)	Q(P) (nM/million cells)	Q(V) (fM/million cells)
	I'. s_R(P) = 1·44 nM/ml, s_R(V) = 68 fM/ml (i.e. vitamin in great excess)					
22.i.	0·364	5·51	0·0353	3·83	0·254	11·6
23.i.	0·364	4·96	0·165	4·23	0·257	12·9
25.i.	0·388	3·51	0·104	11·2	0·380	16·2
29.i.	0·487	2·37	0·167	3·40	0·536	27·2
31.i.	0·411	2·23	0·0840	8·03	0·608	26·9
5.ii.	0·423	1·96	0·0927	9·04	0·685	30·1
9.ii.	0·423	1·89	0·106	8·37	0·736	33·0
12.ii.	0·538	1·65	0·105	5·58	0·809	37·9
16.ii.	0·488	1·33	0·0419	9·91	1·05	43·7
20.ii.	0·542	1·67	0·0570	7·09	0·825	36·4
23.ii.	0·513	1·79	0·115	6·68	0·740	34·3
18.v.	0·410	1·54	0·0976	12·4	0·868	36·0
	II'. s_R(P) = 14·4 nM/ml, s_R(V) = 6·8 fM/ml (i.e. phosphorus in great excess)					
22.i.	0·372	2·34	—	0·570	6·55	2·67
23.i.	0·372	2·45	5·03	0·372	3·82	2·63
25.i.	0·427	1·47	8·76	0·369	3·81	4·37
29.i.	0·524	1·24	4·48	0·687	7·60	4·94
31.i.	0·445	1·16	11·8	0·323	2·27	5·60
5.ii.	0·454	1·06	6·65	0·814	7·28	5·64
9.ii.	0·431	1·40	5·08	0·771	6·63	4·30
12.ii.	0·584	1·69	4·80	0·571	5·67	3·69
16.ii.	0·539	1·33	7·23	0·869	6·68	5·55
20.ii.	0·551	1·67	8·15	0·771	5·38	5·22
23.ii.	0·327	1·79	6·32	0·231	5·19	4·24
18.v.	0·487	1·07	8·42	1·15	5·53	5·01
	III'. s_R(P) = 1·44 nM/ml, s_R(V) = 20·4 fM/ml (i.e. vitamin in slight excess)					
20.vi.	0·194	3·87	0·0955	2·82	0·346	4·53
28.vi.	0·191	3·66	0·0938	3·91	0·367	4·51
3.vii.	0·203	7·44	0·0685	2·72	0·184	2·38
11.vii.	0·334	5·15	0·145	1·62	0·251	3·64
18.vii.	0·341	3·28	0·121	3·63	0·401	5·11
25.vii.	0·334	2·59	0·132	3·98	0·504	6·34
23.viii.	0·309	2·03	0·126	3·50	0·647	8·34
30.viii.	0·311	1·95	0·0915	3·60	0·689	8·60
4.ix.	0·311	2·05	0·120	5·12	0·640	7·47
21.xi.	0·322	2·09	0·0863	4·25	0·648	7·74
28.xi.	0·314	2·11	0·0766	5·19	0·644	7·20
	IV'. s_R(P) = 1·44 nM/ml, s_R(V) = 6·8 fM/ml (i.e. phosphorus in slight excess)					
5.vi.	0·199	2·19	0·186	1·62	0·570	2·36
15.vi.	0·203	2·44	0·0884	1·69	0·552	2·09
20.vi.	0·191	2·00	0·0472	1·74	0·697	2·54
28.vi	0·262	1·51	0·0457	1·32	0·925	3·64
3.vii.	0·311	1·41	0·115	1·86	0·935	3·49
11.vii.	0·323	1·57	0·0813	0·881	0·866	3·78
18.vii.	0·443	1·10	0·0834	1·22	1·23	5·06
25.vii.	0·426	1·24	0·117	0·385	1·06	5·18
23.viii.	0·396	1·23	0·121	0·901	1·07	4·80
30.viii.	0·393	1·20	0·145	1·02	1·08	4·83
4.ix.	0·393	1·11	0·110	1·13	1·20	5·13
21.xi.	0·331	1·15	0·107	1·75	1·15	4·38
28.xi.	0·328	1·36	0·109	0·765	0·976	4·43

54-2

TABLE 7. COEFFICIENTS ASSOCIATED WITH THE REGRESSIONS,
(A), $DQ = D'_m Q - D'_m k_Q$ AND (B), $Q = (1/D'_m) DQ + k'_Q$

(D'_m derived from (A) and k'_Q from (B). 95 % fiducial limits shown in parentheses.)

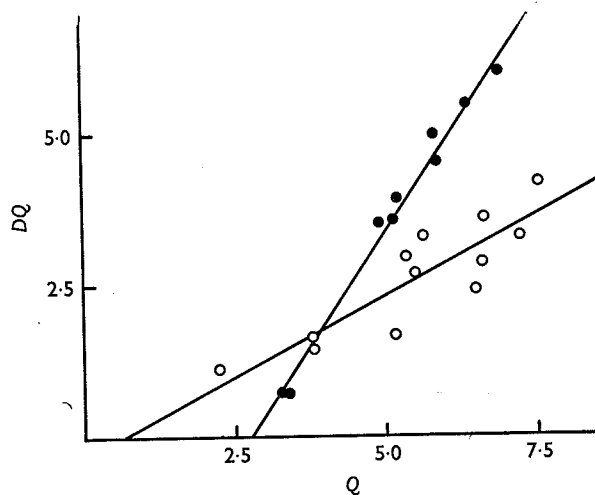| Experiment	Nutrient	D'_m (Vols per day)	k'_Q (Moles per 10^6 cells)	r
I'		0·536 ($\pm$0·05)	0·090 ($\pm$0·13) 10^{-9}	0·967
III'	Phosphorus	0·359 ($\pm$0·02)	0·082 ($\pm$0·09) 10^{-9}	0·965
IV'		0·613 ($\pm$0·03)	0·421 ($\pm$0·19) 10^{-9}	0·958
II'		0·545 ($\pm$0·06)	0·777 ($\pm$2·2) 10^{-9}	0·884
I'		0·554 ($\pm$0·03)	4·82 ($\pm$5·7) 10^{-15}	0·968
III'	Vitamin	0·365 ($\pm$0·02)	1·11 ($\pm$1·4) 10^{-15}	0·959
IV'		0·592 ($\pm$0·02)	1·67 ($\pm$0·54) 10^{-15}	0·980
II'		0·587 ($\pm$0·06)	0·913 ($\pm$1·6) 10^{-15}	0·897

Fig. 12. Phosphorus data from Experiment II; plot of DQ on Q. Open circles, 'slow adapted' cells; filled circles, 'fast adapted' cells. Q as nM/million cells; DQ as nM/million cells/day.

presumably 'slow adapted', from the culture collection. The chemostats were run at low dilution rate at first and the rate was gradually increased over the next six weeks, during which time some eleven steady-states were recorded in each experiment and, although there was a great deal of scatter, a clear pattern did emerge. We have now reached the end of February and dilution rates in excess of 0·5/day were being recorded. The next readings showed a dramatic change in both chemostats, which was particularly pronounced in the phosphorus Q values in Experiment II (the one shown in Fig. 12) where there was a big drop.

In the weeks that followed, during which time the dilution rates were increased still further, a new pattern, characterized by a doubling of the slope of the DQ on Q regression, emerged with some clarity in both experiments. At the beginning of April the dilution rates were reduced suddenly to below 0·2/day, but in spite of this the new pattern was apparently maintained for more than a fortnight, as indicated by the four steady-states recorded during this period. At the end of April my absence from the

TED WITH THE REGRESSIONS,

D (B), $Q = (1/D'_m) DQ + k'_Q$

% fiducial limits shown in parentheses.)

	k'_Q	
ay)	(Moles per 10^6 cells)	r
05)	$0.090 (\pm 0.13) 10^{-9}$	0.967
02)	$0.082 (\pm 0.09) 10^{-9}$	0.965
03)	$0.421 (\pm 0.19) 10^{-9}$	0.958
06)	$0.777 (\pm 2.2) 10^{-9}$	0.884
03)	$4.82 (\pm 5.7) 10^{-15}$	0.968
02)	$1.11 (\pm 1.4) 10^{-15}$	0.959
02)	$1.67 (\pm 0.54) 10^{-15}$	0.980
06)	$0.913 (\pm 1.6) 10^{-15}$	0.897
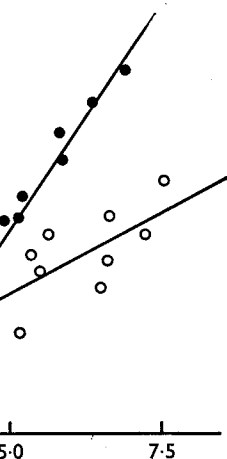
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M/million cells; DQ as nM/million cells/day.

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t the end of April my absence from

country forced me to let both chemostats idle at zero dilution rate for a full month. When they were set going again and allowed to equilibrate at between 0.4 and 0.5/day all the readings from both chemostats fell in with the earlier pattern; evidently the prolonged forced idleness had caused the population once again to become slow adapted.

The input nutrient concentrations were now changed and Experiments III and IV started. Once again the dilution rate was made slow at first and increased gradually and once again a change in the pattern occurred when the rate topped 0.45/day at the beginning of August. Lowering the dilution rates to between 0.4 and 0.3/day on 13 August now caused an immediate reversion to the earlier pattern, and increasing the rate once again (on 4 September) immediately caused the later pattern to return. This was then maintained for the next six weeks, during which time the dilution rates were kept at a high level. As in the earlier two experiments when the dilution rates were suddenly drastically lowered (on 15 October) and kept low the later pattern was maintained for a good month but finally reverted after a period of forced idleness during my absence from the laboratory.

If one accepts that there are two patterns of growth, the one associated with dilution rates below 0.45 and the other with rates above this, what can one infer about the conditions effecting the switch from one pattern to the other? In the first place, if the population is 'slow adapted' it will remain so as long as the dilution rate is kept low, while if it is 'fast adapted' that mode will be maintained so long as the rate is kept high. Secondly, if the population has been maintained without change of mode for a long time sudden and drastic change in dilution rate effects no immediate change in mode, whereas if a change in mode has recently taken place a change in dilution rate across the threshold value (0.45/day?) is followed more or less immediately by an adjustment to the appropriate mode. It is possibly significant that the threshold dilution rate is not too different from the D'_m associated with the slow adapted mode.

It is not possible on the available evidence to decide whether the change in mode is induced or whether we are dealing with a genetic inhomogeneity in the clone of *Monochrysis*, which is now 20 years old. The effect observed in both cases would be population adaptation.

Since the change in mode occurred simultaneously in phosphorus limited and vitamin limited cultures one cannot identify the immediate cause as either phosphorus or vitamin B_{12} limitation. The immediate cause would appear to be a change in the growth rate and the machinery affected directly to be 'down stream' of, or in parallel to, both the phosphorus and vitamin B_{12} uptake mechanisms.

DISCUSSION

The description of the steady-state chemostat in terms of two nutrients embodied in equations (23)–(29) (p. 845) is satisfactory in so far as it enables one to predict any variable associated with a particular steady-state solely from the three controllable variables, namely the dilution rate and the input concentrations of the two nutrients. Could it also be used to simulate a batch culture correctly its potential as a predictive phytoplankton model would be greatly enhanced. This possibility is being investigated.

What an empirical model cannot do of course is to provide any precise picture of the biochemical control mechanisms, though in this case it seems to be fairly clear that the model for uptake based on the Michaelis–Menten enzyme equation should be replaced by one which contains an element of product control, an observable feature of which is that it should not generally be possible for an organism completely to deplete its environment of a limiting nutrient.

The residual concentration of available nutrient, however, is likely to be small and possibly at the limit of detection and therefore not of great practical consequence. On the other hand, if there is sequestration of the nutrient by cell excretions, although the residual available may be small the total might be large and easily detectable, as indeed it is when the nutrient is vitamin B_{12}. Evidence for sequestration of vitamin B_{12} by excreted proteins is secure and moreover not derived merely from steady-state kinetic data (Kristensen, 1956; Daisley, 1961; Droop, 1968), but the latter can only be satisfactorily explained in terms of a phenomenon, which if it is not sequestration is analogous. Furthermore, they make it quite plain that no model involving algal cells and vitamin B_{12}, whether in the laboratory or in the field, can claim any veracity unless it takes full account of the binding phenomenon. Thus, the ratio of total to available vitamin is a function of the time integral of the algal biomass, which in a steady-state system simplifies to equation (20) (p. 843).

I suggested that phosphorus was behaving kinetically in a similar manner in the chemostats and drew attention to the work of Müller (1972) though I do not believe his explanation, i.e. cell death at a low growth rate, could adequately account for the typical C-shaped curves. On the other hand, although one would hesitate to postulate protein binding of orthophosphate, there are other forms of sequestration, such as precipitation of ferric phosphate by the algal secretion of hydroxyl ions.

Without doubt the most far-reaching feature of our model for algal growth is the inderdependence of specific growth rate and internal nutrient concentration; and the most important implication of this is that the potential of a body of water for supporting further growth may depend as much on the nutrient already inside the cells, as that yet to be taken up (Droop, 1973 a, b).

$$x_{\max} = \frac{Qx+s}{k_Q}. \tag{35}$$

Consequently, except in the early stages of a spring increase, particulate nutrient would have the greater bearing on the subsequent course of events, though it is clearly desirable that both parameters should be measured.

The late John Strickland (see Eppley & Strickland, 1968) was probably the first to express the idea of internal substrate dependent growth and a subsistence level below which no growth was possible. He was also aware that the latter could vary according to external nutrient conditions, a fact which has led Eppley & Renger (1974) to question the utility of growth models based on the cell quota parameter. However the present observations of the cell status respecting non-limiting nutrients lead one to distinguish between a 'true' (and constant) and an 'apparent' (and variable) subsistence quota, the former associated with the controlling nutrient, the latter with the remainder. Furthermore, we can use the discrepancy between the apparent and true subsistence quotas to gauge the

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of a spring increase, particulate nutrient w
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degree to which a nutrient is in excess of requirement. I coined the term 'luxury coefficient' (R) for this. The constancy of the true subsistence quota is of course hypothesis but is essential to the model developed here. In point of fact the subsistence quota for vitamin B_{12} and *Monochrysis* is as constant as any parameter I have attempted to measure. I now have four independent estimates of the vitamin B_{12} k_Q for *Monochrysis* from four chemostat runs in which the vitamin was the limiting nutrient, but between which there was a wide range in the ratio of phosphorus to vitamin input concentrations. These are set out in Table 8. With phosphorus also there is considerable agreement between subsistence quota values. I am indebted to my colleague Paul Tett for the following computations from the literature: *Chaetoceros gracilis* (Thomas & Dodson, 1968), 0·001 at P/at. C; *Cylotella nana* (Fuhs, 1969), 0·009 at P/at. C (this is the lower of several values); and *Monochrysis lutheri* (the present paper), 0·0007 at P/at. C.

TABLE 8. SUBSISTENCE QUOTAS FOR VITAMIN B_{12} ESTIMATED FROM VITAMIN LIMITED CULTURES WITH WIDELY VARYING PHOSPHORUS INPUTS

Input concentrations (s_R)			
P (nM/ml)	V (fM/ml)	k_Q (V) (fM/10^6 cells)	
57·5	6·8	2·3	Droop, 1968
14·4	6·8	2·56	Experiment II
14·4	13·6	1·86	Experiment V
1·44	6·8	2·42	Experiment IV

Another factor which persuades me that ecological models should pay more attention to the cell quota parameter is that it has become abundantly clear from chemostat work that the relation between cell quota and growth rate is on a much more sure experimental footing than any relation proposed here or elsewhere between external substrate concentration and specific growth rate. Not only do interesting external substrate concentrations tend to be at the limit of measurement but too frequently they are not what they seem.

Tett *et al.* (1974) have recently used my model with some success to describe events in a Scottish sea loch (Loch Creran) during a spring increase. Dr Tett informs me that the model also permits the construction of an annual phytoplankton pattern that includes spring and autumn maxima and a summer low without the necessity of introducing variable zooplankton grazing, a feature that is virtually absent from Loch Creran.

I have remarked on the similarity of models developed by myself and those developed by Caperon (Caperon & Meyer, 1972b). Each owes something to the other. It is a curious fact, however, that the picture presented by vitamin B_{12} should so resemble those presented by nitrate, phosphate and silicon. One is driven to the conclusion that the biochemical details of uptake and utilization of the various nutrients have very little bearing on the appearance of the kinetic relationship between substrate concentration and growth. It is unlikely that for example the whole phosphorus, or as Paasche (1973a) remarked, silicon content (cell quota) of cells has control over growth rate, for nearly all silicon in diatom cells occurs as inert wall material and phosphorus may be stored as polyphosphate. All one can suppose is that within the confines of our experimental

conditions the active pool is proportional to the total. The burden of this argument holds some comfort for ecologists, for it suggests that they may be spared the necessity of becoming biochemists in addition to being mathematicians.

Mathematical models of phytoplankton growth have great relevance in the laboratory but their predictive value in the field is severely limited, apart from anything else, by their being concerned with single species or even single clones. Their value would be greatly enhanced if, and perhaps it is not too fantastic an idea, they could be applied to mixed populations and even to adapting species and species successions. The phytoplankton of a region might be regarded as an envelope, the sum of its component parts, and it would be interesting to know whether the kinetic properties (μ, μ'_m, k_Q, etc.) of this envelope show any coherence.

TABLE 9. NOTATION EMPLOYED IN EQUATIONS (1)–(35)

Rates (dimensions t^{-1})

Variables:

μ Specific growth rate $\left(\dfrac{1}{x}\cdot\dfrac{dx}{dt}\right)$

D Dilution rate in chemostat: rate of flow per unit volume of vessel (in steady-state $D = \mu$)

u Rate of uptake per unit biomass $\left(-\dfrac{1}{x}\cdot\dfrac{ds}{dt}\right)$

Constants:

μ_m Value of μ if s were infinite
D_m Value of D if s were infinite
D'_m Value of D if Q were infinite
u_m Value of u if s were infinite

Concentrations (dimensions $m\,l^{-3}$)

Variables:

x Biomass
s External available substrate concentration
s_R Concentration of substrate in inflow of chemostat (also initial substrate concentration of a batch culture)

Constants:

s_0 Value of s when μ, D or u are zero
$K_{s(\mu)}$ $\Big\}$ Saturation constant in equation relating μ or
$K_{s(D)}$ D to s

K'_s Apparent value of K_s ($= \beta K_s$)
$k_{s(Q)}$ Saturation constant in equation relating Q to s
$k_{s(u)}$ Saturation constant in equation relating u to s

Coefficients (dimensionless)

Variables:

Q Cell quota or demand coefficient: mass of internal nutrient per unit biomass
R Luxury coefficient (Ratio of $k'_Q : k_Q$)
β Ratio of total to available nutrient in external medium

Constants:

k_Q Subsistence quota of a limiting nutrient: Q at zero growth rate
k'_Q Apparent subsistence quota
Q_m Value of Q if s were infinite
R_m Value of R if the ratio $s_{RN} : s_R$ were infinite

Other

τ/κ (Dimensions $l^3\,m^{-1}\,t^{-1}$) Binding constant

Additional suffixes

A, B Referring respectively to nutrients A and B
L Referring to the limiting nutrient
N Referring to a non-limiting nutrient

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