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ENZYME ACTIVITY IN TERRESTRIAL SOIL IN RELATION TO EXPLORATION OF THE MARTIAN SURFACE

Professor A. D. McLaren

1 January 1972

Department of Soils and Plant Nutrition

Space Sciences Laboratory Series 13, Issue 1

UNIVERSITY OF CALIFORNIA, BERKELEY

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PREFACE

Our objectives are to explore enzyme activities in soil, including abundance, persistence and localization of these activities, and to develop procedures for detection and assay of enzymes in soils suitable for presumptive tests for life in planetary soils.

Thus far we have developed a sensitive test for soil urease, based on hydrolysis of heat-stable ^{14}C-urea and have described the urease activity of ancient and buried soils.

We have also explored in a general way the behavior of enzymes in non-classical systems, e.g. on surfaces, in gels and coacervates, and at low humidity, as an aid to understanding enzyme action in heterogeneous systems such as in soils.

Mathematical models have been developed, based on enzyme action and microbial growth in soil, for rates of oxidation of nitrogen as nitrogen compounds are moved downward in soil by water flow. These bio-geo-chemical models should be applicable to any percolating system, with suitable modification for special features, such as oxygen concentrations, types of hydrodynamic flow, etc.

We have developed a suitable extraction procedure for soil enzymes and have been measuring activities in extracts in order to study how urease is complexed in soil organic matter. Nearly 30 percent of soil enzymes can be isolated as colloidal, clay-free suspensions.

Concerning the Location, Persistence, and Origin of Soil Urease

ABSTRACT

Urease activity in soil is persistent for long periods under low water, low temperature, and sterile regimes, and it has been suggested that some form of enzyme-protective mechanism exists in soil.

Dublin soil was extracted by sonication in water followed by adding a mixture of salts. Urease activity is associated with the organo-mineral complex thus obtained and is resistant to the activities of proteolytic enzymes. Clay-free soil organic matter prepared subsequently by filtration also exhibits urease activity which is resistant to proteolysis. Models consisting of enzymes with bentonite and lignin were found to mimic this resistance to proteolysis.

A model system is presented which suggests both the origin and location of soil ureases and a reason for their persistence in nature.

Additional Key Words for Indexing: soil enzymes, soil organic matter.

INTRODUCTION

Many of the fundamental questions concerning the origin, location, and persistence of soil enzymes remain unanswered. Soil enzymes are doubtless in part extracellular, being liberated during microbial and plant root metabolism and death. They are also intracellular as part of the soil biomass. Although most organic materials are metabolised rapidly by microorganisms, both in vitro and in vivo, many enzyme-proteins persist as active moieties in the soil for very long periods of time (Skujins and McLaren 1968). The addition of urease to soil increases urea hydrolysis only temporarily (Conrad 1940, Moe 1967, Stojanovic 1959, Roberge 1970) suggesting that added urease is either inactivated or destroyed by proteolysis. The presence of a constant background level of enzyme activity, independent of microbial proliferation, again suggests the existence of a protective mechanism (Paulson and Kurtz 1969). Urease activity has been detected in soils stored for decades and correlated better with organic matter content than microorganisms (Skujins and McLaren 1968, 1969). It is therefore apparent that some form of enzyme protection system exists in soil.

In the study of extracellular enzyme activity it is desirable to inhibit any activity of soil microorganisms without either destroying the organisms (with the subsequent release of intercellular enzymes)

or changing the physical and chemical properties of the soil. The use of bacteriostatic agents, such as toluene, and high energy radiation (Skujins and McLaren 1969) have been used to approximate this condition.

The stability of enzymes within the soil matrix has invoked notions of two types of enzyme protection systems: first as an adsorption reaction involving enzymes and clays (Ensminger and Gieseking 1942; Pinck, Dyal and Allison 1954), and secondly as an enzyme-organic matter interaction involving physical or chemical binding mechanisms (Conrad 1940, McLaren 1963). As much of the amorphous soil organic matter is intimately associated with the clay colloids, forming the organo-mineral complex (Kononova 1966), it is difficult to relate enzyme persistence to either (or both) mechanism(s) without prior separation. Ideally, this separation must be achieved without destruction of enzyme activity. Recently we have succeeded in isolating a clay-free urease-active organic matter fraction from soil (Burns, El Sayed and McLaren 1971) and its properties have now been more fully explored.

MATERIALS AND METHODS

Soil

A Dublin clay-loam soil of the following characteristics was used throughout these investigations: sand 24%, silt 35%, clay 42%, organic matter 2.9%, pH 7.2.

Measurement of Enzyme Activity

Soil urease, commercial urease (3X N.F., from Nutritional Biochemical Corporation, Cleveland, Ohio), and pronase (B grade, from Calbiochem, Los Angeles, California) activities were determined quantitatively by ammonia evolution from either urea (urease) or benzoylargenine amide (pronase). Measurements were performed with a modified Conway diffusion dish, as described by McLaren, Reshetko and Huber (1957), or by $^{14}CO_2$ production from ^{14}C-urea (Skujins and McLaren 1969).

Separation of Soil Inorganic Fractions

Dublin soil was separated into its "sand," "silt," and "clay" fractions by a sedimentation procedure assuming the applicability of Stoke's Law. A total of 60 minutes sonication (see below) of the soil suspension (15 minutes between each of four successive sedimentations) served to disintegrate aggregates and allowed for a reasonably accurate separation of inorganic components. Previously, sedimentation without prior sonication was used but aggregated silt and clay particles tended to settle out with the sand fraction. This explains the comparatively low enzyme activities in "silt and clay" fractions as reported recently (Burns et al. 1971), and in certain experiments described herein.

Extraction of Enzyme-active Organic Matter

The extraction technique, as described previously (Burns et al. 1971), has been somewhat modified. Twenty-five g of Dublin soil is suspended in 250 ml of distilled water and sonicated in a water bath for 20 minutes using a Circo 60 watt ultrasonic generator. A mixture of salts, to yield final concentrations of sodium citrate 0.95 M, sodium dihydrogen phosphate 0.05 M, glycine 0.05 M, and sodium chloride 2.0 M, is slowly added to the soil suspension with constant stirring. After dissolution of the salts has occurred, the pH is adjusted to 6.3 and 1 ml of toluene is added as a biostatic agent. The mixture is next agitated for 2 hours at 10°C and then centrifuged at 18,000 g for 30 minutes. The supernatant sol is passed through a bacteriological filter (Mandler Diatomaceous Filter Cylinder No. 3, 5-in. x 1-in., from Allen Filter Co., Toledo, Ohio) in order to remove clay material. The sedimented soil is next extracted four more times, at pH 6.5, once buffered at 0.25 M in Na phosphate (0.01 M in glycine), and three times buffered at 0.05 M in Na phosphate (0.01 M in glycine). A shaking time of 30 minutes was used on each occasion. Each additional extract is also filtered, but the five filtrates are dialyzed separately (Cellulose Dialysis Tubing, average pore radius permeability of 24 A, from Van Waters and Rodgers, San Francisco, California), initially against running tap water for three days, and then against distilled water for one day. During dialysis floculation occurred.

The resulting precipitates were concentrated by centrifugation at 18,000 g for 60 minutes and the supernatants, now with no activity, were discarded. The enzyme-active residues are suspended in 0.001 M Na phosphate (pH 7.0) to a final volume of between 5 and 20 ml depending on their viscosity. The urease activity of the combined extracts varies between 100 and 120% of that found in the original soil. This apparent increase in activity may be related to the release of enzymes and enzyme sites in a similar manner as described below for the soil inorganic components. It is probable that the activity in the organic matter extract is only a small fraction of the total theoretical activity due to soil urease.

Preparation of Bentonite-Urease Complex (BUC)

One ml of Jackbean urease solution at pH 7.0 was added to 0.1 g of bentonite clay (either 0.005 g or 0.01 g enzyme per g clay) in the outer diffusion chamber of a Conway dish. After six hours, maximum expansion of the clay lattices was considered to be complete (Estermann, Peterson and McLaren 1959) and the urease activity of the BUC was measured with the addition of urea. The stability of BUC towards proteolysis was tested by the addition of 1 ml of 500 ppm pronase and allowing 12 hours for reaction before urea was added for assay.

Preparation of Bentonite-Urease-Lignin Complex (BULC)

Urease-bentonite complexes were prepared as above and then lignin (0.01 g per 1 g clay) (Estermann et al. 1959) was added, followed by thorough mixing of the complex into a paste. This paste was allowed to dry, at room temperature for 72 hours, and then was ground into a fine powder. Urease activities of both BUC and BULC were measured by NH_3 evolution from urea (McLaren et al. 1957).

RESULTS

Urease Activity Associated with Soil Inorganic Fractions

As seen in Table 1, a high proportion of soil urease activity is associated with the clay fraction. In addition, the total activity of the clay + silt + sand is in excess of that attributable to the original soil in the ratio of 41/19. It is probable that the extraction procedure, in its disintegration of colloidal aggregates, both increases surface area and releases previously unavailable enzyme sites, thereby enhancing the possibility of enzyme-substrate interaction.

Effect of Pronase on Urease Activity

Persistence of Pronase in Soil

In order to measure the persistence of the proteolytic enzyme mixture "pronase" (Nomoto, Narahasi and Murakami 1960), 5 ml of soil

suspension ("silt-clay" fraction) and 1 ml of pronase (0.001 g enzyme) were incubated together in a water bath at 37°C. One ml of benzoyl-argenine amide (BAA) 0.3 M was added to 1-ml samples of the mixture, and the subsequent release of ammonia was used as a measure of pronase activity. The results are presented in Table 2. The total ammonia evolved consists of three components: that from the effect of pronase on nitrogen-containing substrates already present in the soil, that from the breakdown of pronase itself by soil enzymes, and the ammonia produced as the pronase reacts with the BAA substrate. The data reveal that pronase activity is nearly constant between 1 and 4 days and could act on soil urease if the urease were not protected.

Effect of Pronase on Commercial Urease in Vitro

Pronase and commercial urease in like concentrations (500 ppm) were allowed to react for 20 hours at pH 7.0 in a water bath held at 37°C. The activity of urease, when compared to the controls, dropped from 54.5 μmoles of NH_3/hour to 0.64 μmoles NH_3/hour. It is evident that the proteolytic enzyme pronase is capable of the rapid destruction of urease.

Effect of Pronase on Soil Urease Activity in Vivo

In an attempt to discover the effect of high levels of proteolytic enzymes on soil urease, pronase and soil suspensions ("silt-clay") were incubated at 37°C for 24 hours. Pronase-soil organic matter ratios of 3.3:1 and 0.7:1 were used (compare Ladd and Brisbane 1967). The results in Table 3 show that soil urease activity is unimpeded regardless of the pronase concentration.

Activity of Urease Amended Soil and its Resistance to Pronase

Soil suspensions ("silt-clay") and commercial urease (500 ppm, pH 7.0) were allowed to associate for times ranging from 0 to 120 hours. Following this they were subjected to 6 hours pronase treatment (500 ppm). Figure 1 shows a large reduction in urease activity due to proteolysis. The endogenous soil urease activity was unaffected whilst the soil plus urease, with no pronase treatment, shows an initial high activity but indigenous proteolytic enzymes soon reduced it to that of the untreated soil.

Activity of Soil Organic Matter Extract and its Resistance to Pronase

Organic matter extracted from soil was subjected to pronase attack (500 ppm) for 6 hours at room temperature in an attempt to discover if clay-free organic matter afforded protection to endogenous urease. The results in Table 4 show that there is no depression of soil urease activity in pronase treated extracts.

Stability of Urease in BUC and BULC to Pronase Attack

As reported previously (Burns et al. 1971), bentonite-urease complexes were not resistant to proteolysis whereas bentonite-urease-lignin complexes were. It is worthwhile to note that initially the adsorption of urease onto bentonite increased its activity above that shown by the urease alone.

DISCUSSION

The proteolytic enzyme mixture pronase (from Streptomyces griseus) is very active in the breakdown of Jackbean urease and other proteins. We would therefore expect pronase to hydrolyse soil proteins, including urease unless some mechanisms for "protection" of soil proteins are extant. Clearly, pronase does not deactivate autochthonous urease in soil, regardless of concentration or time of contact. It is therefore evident that urease is shielded from the normal proteolytic effects of pronase. It is also obvious that this protective mechanism does not prevent soil urease-urea interaction.

Pronase added to soil is unstable, but its activity is persistent enough to attack soil urease if the enzymes could combine. On the other hand, plant urease added to soil is not resistant to proteolytic attack by either indigenous soil enzymes or added pronase and therefore increases in urea turnover are ephemeral.

Organic matter extracted from soil by our procedure was free of clays, yet it has a urease activity which is resistant to pronase. This indicates that urease, at least in this instance, is primarily associated with the soil organic matter and not with the clay colloids. Any apparent correlation of enzyme activity with soil clays may be due to the occurrence of organic matter in organo-mineral complexes.

In fact, bentonite clay alone does not protect Jackbean urease from pronase degradation. The observation that urease activity was increased upon adsorption by bentonite is contrary to many reports indicating a reduction in enzyme activity upon adsorption (Durand

1964, 1965; Paulson and Kurtz 1970) although increases have been discussed (Greenland 1965). At this stage it is difficult to explain this observation and, as it is outside the main line of the present investigation, it is suffice to mention that urease may dissociate upon adsorption and in consequence expose many more active sites than present in polymeric form in solution (Reithe and Robbins 1967). Addition of a lignin to the bentonite-urease complex affords protection to urease from pronase attack as expected (Estermann et al. 1959).

Our results with soils and models suggest that urease exists in soil as an enzyme-organic matter complex. This association protects the enzyme from attack by proteolytic enzymes and yet allows diffusion of substrate molecules to and product molecules from active enzyme sites. The comparative molecular size of the components lends itself to this hypothesis.

In soil the organic matter is associated with minerals and it has been suggested that enzymes are situated within the organic matter per se (McLaren 1963). The nature of this association is represented schematically in Fig. 2. The non-persistence of urease added to soil and of bentonite-urease mixtures, and the persistence of urease activity native to soil and in bentonite-urease-lignin complexes leads one to believe that for an enzyme to be persistent in soil it needs to be incorporated into the organo-mineral complex. Presumably, as the enzymes are liberated during digestion of plant roots, micro-organisms, etc., they may be internally complexed with organic matter during humic acid synthesis (Kononova 1966). Synthetic high polymer-

enzyme systems are well known and have similar properties (McLaren and Packer 1970).

This hypothesis goes some way towards explaining the site and persistence of enzyme activity in soil whilst the authors realise that a considerable amount of ephemeral enzyme activity may be due to free, unassociated enzymes in soil (Briggs and Spedding 1963).

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Table 1 - Urease activity of soil components

Soil fraction	Activity in μmoles NH_3 evolved/g/hour
Soil	19
Clay	35
Silt	5
Sand	0.8

The activities of clay, silt, and sand are adjusted so as to represent their contribution to 1 g of undivided soil.

Table 2 - Persistence of pronase in soil

Time, hours	μmoles NH_3 evolved / g / hour		
	BAA → NH_3	Pronase → NH_3 soil N → NH_3	Total NH_3
1	1.0	0.8	1.8
2	1.1	0.8	1.9
3	0.6	1.0	1.6
6	1.2	1.0	2.2
12	1.5	2.2	3.7
24	1.7	2.3	4.0
48	1.8	2.6	4.4
72	1.9	2.7	4.6
96	1.9	3.0	4.9
120	1.3	3.5	4.8
144	1.0	3.4	4.4
192	0.8	1.8	2.6

Table 3 - Effect of pronase on soil urease activity in vivo

Pronase: organic matter	µmoles NH_3 / g / hour				Expected* (D)
	No pronase		Pronase		
	Soil (A)	Soil & urea (B)	Soil (C)	Soil & urea (D)	
3.3 : 1	0.12	0.25	0.35	0.44	0.47
0.7 : 1	0.12	0.25	0.16	0.27	0.29

* A + (B - A) + (C - A) = D = D Expected - if there is no reduction in urease activity due to addition of pronase.

Table 4 - Activity of extracted soil organic matter and its resistance to pronase

Treatment	µmoles of NH_3 evolved/ml/hour	
Organic matter + urea	12	13.9
Organic matter + pronase	1.9	
Organic matter + pronase + urea	15	

Fig 1

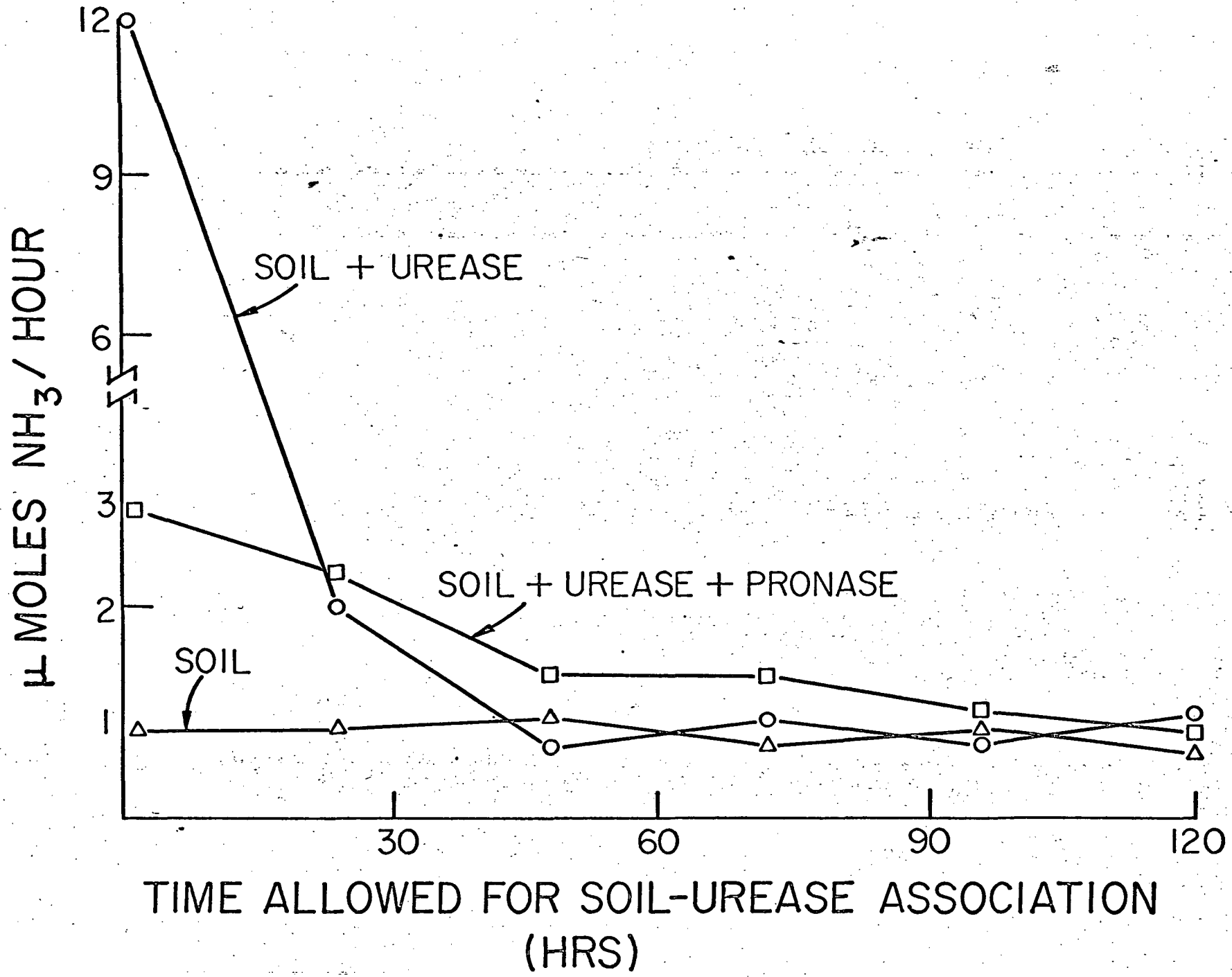
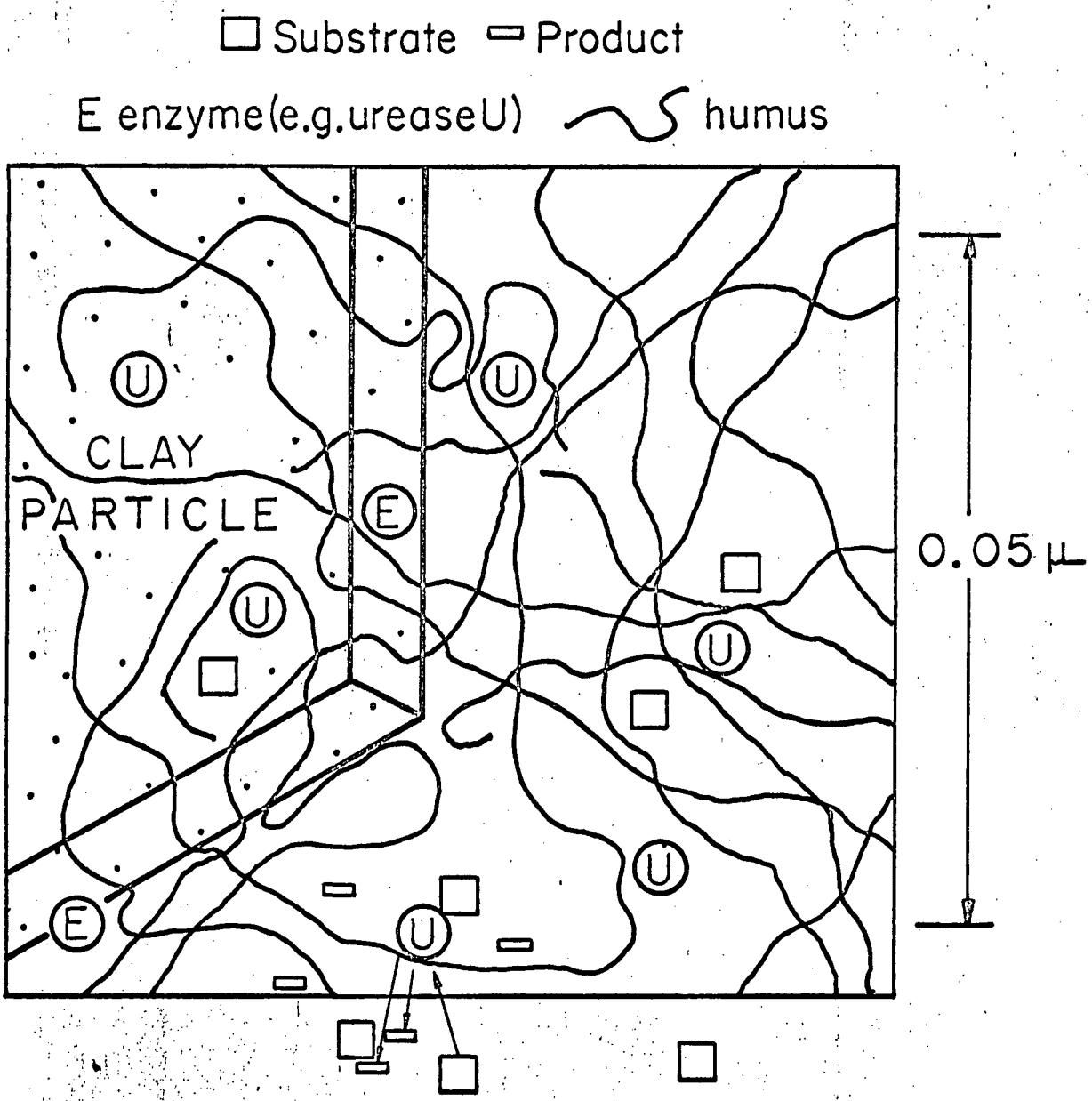

12
9
6
3
2
1
μ MOLES NH3 / HOUR
SOIL + UREASE
SOIL + UREASE + PRONASE
SOIL
30
60
90
120
TIME ALLOWED FOR SOIL-UREASE ASSOCIATION
(HRS)

Fig 2


Substrate
Product
E enzyme(e.g.ureaseU)
humus
CLAY
PARTICLE
U
E
0.05 μ

CAPTIONS TO TABLES

Table 1. Urease activity of soil components.

Table 2. Persistence of pronase in soil.

Table 3. Effect of pronase on soil urease activity in vivo.

Table 4. Activity of extracted soil organic matter and its resistance to pronase.

LEGENDS TO FIGURES

Fig. 1. Action of endogenous proteolytic enzymes and of these plus added pronase on the survival of urease added to soil.

Fig. 2. A model for soil enzyme location and activity.

Competition between Species during Nitrification in Soil

ABSTRACT

Previously we have derived equations which describe populations of nitrifying organisms growing in an idealized soil column perfused with nutrient solutions. Population growth in the soil column at any particular depth is here discussed in terms of a kinetic model that allows for exhaustion of nutrient or space, i.e. surface area limitation, whether or not death occurs, and competition between two species in the same niche. The model in its various ramifications is subject to laboratory tests and points up inadequacies in current understanding of the microscopic ecology of nitrification.

Additional Key Words for Indexing: microbial ecology, kinetics of microbial growth.

The rate of nitrification in soil during re-perfusion with ammonium salts has often been found to first increase and then decrease to nearly zero as the supply of ammonium substrate is exhausted. Lees and Quastel (1946) suggested that during the re-perfusion the population of nitrifiers reached a maximum possible number for the soil because, on providing a fresh supply of ammonium, the rate of nitrification was nearly constant. During oxidation of the first batch of ammonium the rate of growth of nitrifiers, dN/dt, seems to obey an equation of the form (Quastel and Scholefield, 1951; Chase, Corke and Robinson, 1968)

$$dN/dt = \gamma N (1 - \delta N/N_{max}) \qquad [1]$$

where N is the population of ammonium oxidizers at time t after the addition of ammonium, γ is the maximum specific growth constant in the absence of any kind of inhibition of growth (any dependence of γ on substrate concentration is without significance in this discussion (McLaren 1971)), and N_{max} is the maximum population realizable in a cc of soil. The quantity $(1 - \delta N/N_{max})$ is the influence of a population on the growth constant γ, and δ weights the influence of N on this quantity; is usually taken as unity. N_{max} corresponds to the carrying capacity of the soil , be it space, surface area, or the total initial amount of ammonium available, whichever is population-limiting. Lees and Questel suggested that N_{max} was to be identified with a population supported by the total soil surface area available to the nitrifiers and on which nitrification takes place. Indeed, microbial counts of nitrifiers accompanying nitrification of the ammonium initially supplied seems to support

this; the population does increase to a maximum as suggested by Equation [1] (Chase et al. 1968, Morrill and Dawson 1967); following the maximum a decline in numbers may also be observed prior to the addition of more substrates (NH_4^+ or NO_2^-). It has since been found, however, that if a fresh supply of substrate is added and the soil again re-perfused, the population continues to increase, even though the rate of oxidation is constant, and so on for further additions and re-perfusions with added batches of substrate and with successive exhaustions of oxidizable nutrient between periods of re-perfusion (Nishio and Furusaka, in press). The rate-limiting step with zero order kinetics may be the constant rate of solution of oxygen in the perfusing solutions, but in any case the N_{max} observed under these conditions cannot be associated with any limitation of surface area for growth of the nitrifiers.

Clearly, if nitrifiers multiply in numbers beyond the carrying capacity of the soil surface, excess cells could be carried out of the soil sample by the perfusing liquid and be partially entrapped by a soil filtering action during re-perfusion. In a perfused column, however, (McLaren 1971) the excess population of organisms could be permanently removed from the soil system during continuous perfusion and the soil might eventually exhibit a maximum population.

The re-perfusion method of Lees and Quastel has been useful for studying the role of inhibitors on nitrification and for enrichment purposes, but work with soil columns should give results of greater applicability to field work.

Unless the soil has been first sterilized and then inoculated with nitrifiers, other organisms will also occupy some of the soil internal surface area available for growth of microorganisms. Alternately artificial soils, glass beads, and the like can be used to support growth of one or more organisms (Marshall 1971). We now wish to examine the question of what may be expected in the way of population increases of two organisms competing for the same surface area and depending on the same nutrient for energy, i.e. having the same niche.

THE MODEL

If two chemoautotrophic species a and b oxidize ammonium nitrite in a column of soil continuously infiltrated with this substrate, with sufficient concentrations of other metabolites such that $\gamma = \gamma_\infty$ at all times, Equation [1] can be modified to read, for each species,

$$dN_a/dt = \gamma_a N_a \left(1 - \frac{N_a}{N_{max_a}} - \frac{\phi N_b}{N_{max_a}}\right) \qquad [2]$$

and

$$dN_b/dt = \gamma_b N_b \left(1 - \frac{N_b}{N_{max_b}} - \frac{\theta N_a}{N_{max_b}}\right) \qquad [3]$$

This seems to be consistent with the most simple assumptions, since so little is known about growth of microbes on surfaces (Alexander 1971). The term $(1 - N/N_{max})$ represents the influence of N on γ as before; in other words, the rate of growth of a population declines as the usable space between cells is reduced. The cells need not be close-packed in anything approaching a "crystalline" array in the limit, but only spread in such a way as to tend to inhibit mutual growth of other cells at the surface. In Equations [2] and [3], N_{max_a} and N_{max_b} are the maximum populations to be found if species a and b are grown separately, as in Equation [1], and ϕ measures the inhibitory effect of an individual of species b on the growth of species a, and θ is the corresponding effect of adding an individual of species a on the growth of species b.

Let us assume a unit surface area such that

$$N_{max_a} = \phi N_{max_b} \qquad [4]$$

Substituting Equation [4] into [2] we have Equation [2a]

$$dN_a/dt = \gamma_a N_a \left(1 - \frac{N_b}{N_{max_b}} - \frac{N_a}{\phi N_{max_b}}\right) \qquad [2a]$$

which can be compared with an alternate form of Equation [3], namely [3a]:

$$dN_b/dt = \gamma_b N_b \left(1 - \frac{N_b}{N_{max_b}} - \frac{N_a}{\phi N_{max_b}}\right) \qquad [3a]$$

Since we are assuming that only the relative sizes of a and b are involved in inhibition and competition for the unit surface area, $\phi \cdot \theta = 1$, and the quantities within the parentheses of Equations [2a] and [3a] are identical. By dividing Equation [2a] by [3a] we have

$$\frac{dN_a}{N_a} = \frac{\gamma_a dN_b}{\gamma_b N_b} \qquad [5]$$

which, for initial populations of N_{o_a} and N_{o_b} can be integrated to give

$$N_a = N_{o_a} N_b^{\gamma_a/\gamma_b} / N_{o_b}^{\gamma_a/\gamma_b} = N_b^{\gamma_a/\gamma_b} e^c \qquad [6]$$

where c is a constant. This gives the population of N_a at any time in terms of the population of N_b.

When both populations are maximal, assuming that cells of neither population die, the total available surface is covered by cells of a and b, $dN_a/dt = dN_b/dt = 0$ and

$$1 - \frac{\hat{N}_b}{N_{max_b}} - \frac{\hat{N}_a}{\phi N_{max_b}} = 0$$

or

$$\hat{N}_a = \phi \hat{N}_{max_b} - \phi \hat{N}_b \qquad [7]$$

where N_a and N_b are the maximum populations obtained with a and b growing together, respectively.

Substituting Equation [6] into Equation [3a],

$$dN_b/dt = \gamma_b N_b \left(1 - \frac{e^c N_b^{\gamma_a/\gamma_b}}{\phi N_{max_b}} - \frac{N_b}{N_{max_b}}\right) \qquad [3b]$$

DISCUSSION

In order to compare growth of mixed populations with growth of populations of species a or b growing alone we can, by way of illustration, pick some reasonable numbers for N_a, N_b, γ_a, γ_b, etc. Choosing $\gamma_b = 1.4$ day^{-1}, $\gamma_a/\gamma_b = 1.1$, $\phi = 3$, $N_{max_b} = 10^6$ cells, $N_{o_b} = 10^5$ cells, $e^c = 1$ and therefore $N_{o_a} = N_{o_b}^{\gamma_a/\gamma_b} = 3.16 \times 10^5$ cells, and solving Equation [3b] by a numerical procedure (Smith 1968), we obtain the lower curve in Fig. 1, namely the population of species b growing in the presence of growing species a as a function of time. The population of species b growing in the absence of a is given by the logistic equation (the integrated form of Equation [1]), namely

$$N_b = \frac{N_{max_b}}{1 + \left(\frac{N_{max_b} - N_{o_b}}{N_{o_b}}\right) \exp - \gamma_2 t}$$

and is plotted for comparison. With $N_{max_a} = \phi N_{max_b} = 3 \times 10^6$ cells we may also plot the logistic growth of species a as the upper curve in Figure 1.

By inspection, Figure 1, $\hat{N}_b$ is approximately 4.5×10^5 cells and by Equation [7] $\hat{N}_a = 3 \times 10^6 - 3(4.5 \times 10^5)$ or 16.5×10^5 cells. The entire curve for competitive growth of species a may be obtained from the corresponding growth of b by means of Equation [6] and the result is also shown in Fig. 1.

It may be seen that with competitive growth the maximum populations are approached at lower rates than with simple logistic growth. If N_{max_b} is very large, Equation [3b] becomes simply

$$dN_b/dt = \gamma_b N_b \qquad [8]$$

and this equation seems to hold for populations of nitrite oxidizers between 10^4 and 10^7 cells per gram during re-perfusion of a Japanese soil (Nishio and Furusaka, in press).

Returning to Equations [2a] and [3a] we may ask under what conditions, if any, can dN_a/dt and dN_b/dt both equal zero with populations extant other than $\hat{N}_a$ and $\hat{N}_b$. An "equilibrium" exists if

$$N_a \left(\gamma_a - \frac{\gamma_a N_a}{\phi N_{max_b}} - \frac{\gamma_a N_b}{N_{max_b}}\right) = 0$$

and

$$N_b \left(\gamma_b - \frac{\gamma_b N_a}{\phi N_{max_b}} - \frac{\gamma_b N_b}{N_{max_b}}\right) = 0$$

Two equilibria are given by $N_a = 0$, $N_b = N_{max_b}$ and $N_b = 0$, $N_a = N_{max_a}$. The other solutions are an identity, namely $N_b = N_{max_b} - \theta N_a$, which is an equation for a straight line on N_b, N_a coordinates with intercepts at $N_a = 0$, $N_b = N_{max_b}$ and $N_b = 0$, $N_a = N_{max_a}$. For any population of a and b representable by a point on a line cutting axes with $N_b < N_{max_b}$ and $N_a < N_{max_a}$, the values of dN_a/dt and dN_b/dt are positive and the populations of each on the surface can increase. Populations represented by points on lines with intercepts $N_b > N_{max_b}$ and $N_a > N_{max_a}$ yield negative values for dN_a/dt and dN_b/dt, meaning that some cells of a and b will fall off the unit surface, since the populations exceed the carrying capacity of the surface represented by N_{max} for either species. Such a situation could exist if excess cells were deliberately spread on a surface. Negative rates can also imply death, as will be discussed below.

A made-up population consisting of, e.g., $N_a = 18 \times 10^5$ cells and $N_b = 4 \times 10^5$ cells, satisfies the solution $N_b = N_{max_b} - \theta N_a$, indicating no growth of either species, but in the situation described by Fig. 1 these populations do not occur at the same time, so growth can continue; i.e., dN_a/dt and dN_b/dt are both positive in the region of 1 to 3 days growth time.

If one or both species die , and Equations [2] and [3] are applicable, it may be shown that the two species cannot coexist indefinitely if they compete for the same limiting resource (Smith 1968). One species will win, and from a soil evolutionary point of view one can expect to find only one species in a given sample, say a cc of soil. Nitrifiers are observed to die even during perfusion experiments; in the presence of ammonium,

ammonium oxidizers may die but not nitrite oxidizers or vice versae (both situations have been observed (Chase et al. 1968, Morrill and Dawson 1967)). A perusal of the literature has failed to turn up examples of more than one organism oxidizing either substrate in a given soil sample. It would not be surprising to find one such species in preponderant numbers. A number of individuals of any species, i, each obeying Equation [8], can be represented as a total population, N_T, given by

$$N_T/N_{o_T} = \sum^{i} N_{o_i}/N_{o_T}\, e^{\gamma_i t}$$

where N_{o_T} is the sum number of individuals at the start of a period of observation t, and N_{o_i} is the initial number of any one species at the start. This equation does not plot as a straight line in semilog plot unless only one species is present or is present in overwhelming numbers as seems to be the case during the re-perfusion experiments of Nishio and Furusaka. Hence we should look at the influence of death on Equations [1], [2], and [3].

In the simple case the specific death rate k of a cell can be considered independent of N, then death will be given by

$$-dN/dt = kN \qquad [9]$$

and combining [9] with [1] we have

$$dN/dt = \psi N - \gamma N^2/N_{max} \qquad [10]$$

where $\psi = \gamma - k$. Upon integrating from N_o at $t = 0$ to N at t we find

$$N = \frac{N_o \psi e^{\psi t}}{\psi - \frac{\gamma N_o}{N_{max}} + \frac{\gamma N_o}{N_{max}} e^{\psi t}}$$

which shows that a lower maximum population, $\psi N_{max}/\gamma$, is reached than without death.

Perhaps a more likely tendency is for the specific death rate to depend upon N; Equation [1] then reads

$$dN/dt = N\left(1 - \frac{N}{N_{max}}\right) - kN\left(1 + \frac{\sigma N}{N_{max}}\right) \qquad [11]$$

where N^2 is the influence of N on k. To simplify, consider the case σ equals unity. Integration gives

$$N = \frac{N_{max}}{(\gamma + k)/(\gamma - k) + \left(\frac{N_{max} - [(\gamma + k)/(\gamma - k)]\, N_o}{N_o}\right) e^{-(\gamma - k)t}}$$

which shows again that a lower maximum population, $\frac{\psi N_{max}}{\gamma + k}$, is reached than without death.

Note that Equation [11] can also be written in the form

$$dN/dt = \psi N \left[1 - \left(\frac{\gamma + k}{\psi}\right) \frac{N}{N_{max}}\right] \qquad [12]$$

which is of the form of Equation [1], but Equation [12] provides for death. dN/dt can be negative for large values of N.

Returning to Equations [2a] and [3a], it is obvious that

$$-\left(\frac{\theta N_a}{N_{max_b}} + \frac{N_b}{N_{max_b}}\right)$$

cannot exceed unity and that dN/dt is never negative unless death terms, k, are included in these equations; this is not always clearly stated (Pielou 1969).

Expansion of Equation [12] to take into account competition between two species gives Equations [2b] and [3b], namely,

$$dN_a/dt = \psi_a N_a \left(1 - \psi'_a \frac{N_a}{N_{max_a}} - \frac{\phi N_b}{N_{max_a}}\right) \qquad [2b]$$

where $\psi'_a = (\gamma_a + k_a)/\psi_a$, and

$$dN_b/dt = \psi_b N_b \left(1 - \psi'_b \frac{N_b}{N_{max_b}} - \frac{\theta N_a}{N_{max_b}}\right) \qquad [3b]$$

where $\psi'_b = (\gamma_b + k_b)/\psi_b$. Although the <u>effective</u> population $N = N_a + \phi N_b$ is applicable to either equation, substitution into both [2b] and [3b] does not reduce them to simultaneous equations that can be explicitly solved. Nevertheless, as is well known (Smith 1968, Pielou 1969), it can be shown that with real values for k_a and k_b, population N_a or N_b will win, depending on the values of ψ_a, ψ_b, ϕ, θ, and N_{o_a} and N_{o_b}, and the other population will tend to die out.

The validity of these considerations depends, of course, on the constancy of γ_a and γ_b. The dependence of γ on substrate concentration is sometimes found to be of the form $\gamma = \gamma_\infty(S)/(K_g + (S))$, where γ_∞ is the maximum specific growth rate for large substrate concentrations (S), and K_g is a constant characteristic of the species, as discussed elsewhere (McLaren 1970). Powell (1967) has extended this relationship to read $\gamma = \gamma_\infty(S)/(K_g + L + (S))$, where L has the dimensions of a concentration and gives the influence of geometry and physical characteristics of the organisms and their environment on K, e.g., limiting diffusion rates of substrate at low (S) at cell membranes on the apparent saturation constant $(K_g + L)$. With so many adjustable parameters, $\gamma_a > \gamma_b$, $\gamma_a < \gamma_b$, $\gamma_a = \gamma_b$ are all possible, depending on (S). In the real lives of microorganisms where surface concentrations such as surface pH and surface (S) (McLaren and Packer 1970) can more directly influence growth than do bulk concentrations, the equations outlined can only be considered as suggesting possible courses of events and draw attention to the kinds of observations that might be made with profit.

The value of any such models as we have presented (cf. McLaren 1970, 1971) rests in whether or not existing data can be marshalled in order to predict the results of another experiment to be performed or whether large gaps in information exist that were not altogether obvious before, or in indicating how little we really understand some natural phenomenon. Nitrification is known to involve reduction of oxygen and fixation of carbon dioxide by an increasing long list of nitrifying organisms (Watson 1971), but little is known about microenvironmental influences in soil, about relative numbers of species taking part in any one site, about the influences of other organisms present or even about the variations of nitrogen and nitrifiers.

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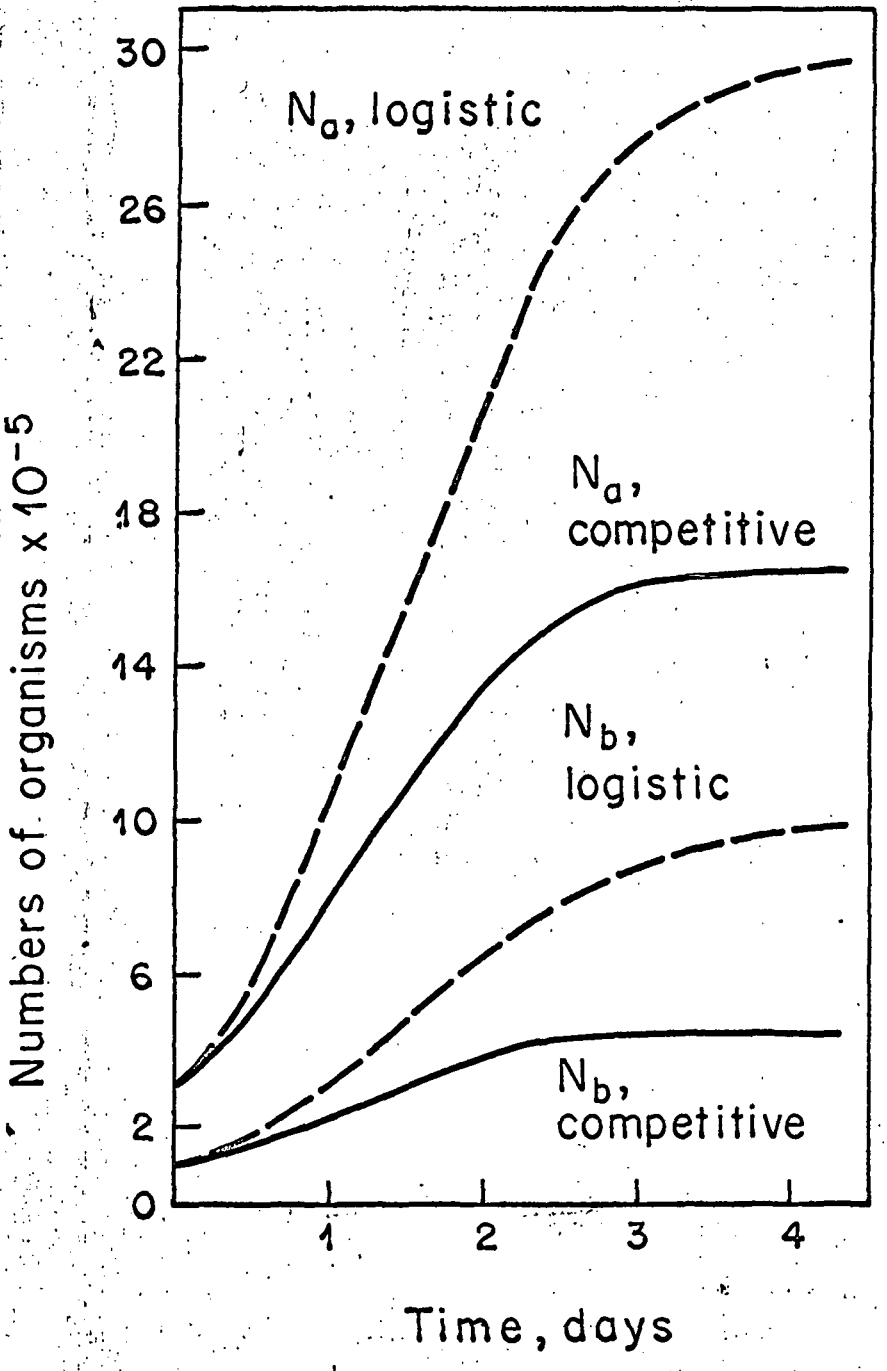
FIGURE LEGEND

Fig. 1. Logistic and competitive growth of two organisms, a and b. Upper broken curve is population of a and lower broken curve is population of b, both as functions of time, with maximum populations of 3 and 1 millions respectively, Equation [1]. The populations N_a increasing in the presence of multiplying b and of N_b increasing in the presence of a are indicated by solid lines, Equations [2] and [3].


30
26
22
18
14
10
6
2
0
1
2
3
4
N_a, logistic
N_a, competitive
N_b, logistic
N_b, competitive
Numbers of organisms x 10⁻⁵
Time, days