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Predator-Prey Interactions of *Dictyostelium discoideum* and *Escherichia coli* in Continuous Culture[1]

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Dictyostelium discoideum and *Escherichia coli* were aerobically propagated in mixed continuous culture in a predator-prey relationship, and the effects of temperature and holding times were examined. Oscillations developed in the concentration of glucose, the limiting substrate for *E. coli*, and in the densities of the two populations, but eventually steady-state populations were reached. The experimental data were analyzed according to the Lotka-Volterra model for prey-predator relationships and by the Monod model for saturation kinetics. A comparison of the adequacy of the two models in describing predation is given.

Much is known qualitatively concerning food chains and food webs. However, relatively little quantitative information is available on the processes whereby organic matter and the mineral components of the environment are incorporated into the protoplasm of the prey and of how these components are upgraded to "better" proteins of the predator. Among some standard texts that treat on the phenomena of predation (10, 15, 18), only Gause worked with microorganisms occupying a common environment. This is not to say that microbiologists have ignored symbiotic associations (1, 3, 4). Bungay (25), Garver (21), Hamilton (12), and ourselves (9) have initiated work in the area of microbial prey-predator systems. Canale (5) has treated theoretically the same problem by stability analysis of the type introduced into biological problems by Ramkrishna et al. (22).

An aspect of symbioses that has hitherto not been appreciated is the significant role that the environment plays in these relations. Emphasis has been given instead to the symbionts. The use of mathematical models based on the saturation kinetics of Monod insists that the environment be recognized explicitly, instead of implicitly, as has been the case previously.

Finally, it should be noted that the kinetics of the predation phenomenon is the same as that of the parasitism phenomenon. The predator and the parasite play essentially the same role.

This paper deals with the phenomenon of predation of *Dictyostelium discoideum* on *Escherichia coli*. *E. coli* was grown in a minimal medium in which glucose was the limiting substrate. *E. coli* itself served as the limiting substrate for *D. discoideum*. Although *D. discoideum* has been studied primarily with respect to its life cycle and morphogenesis (24), the vegetative amoebae are indistinguishable from many small, free-living soil amoebae, and the periods of growth and morphogenesis are clearly separated (24). Because the vegetative amoebae are impermeable to glucose (29), and can be easily cultivated in liquid culture (13, 26) and easily be switched from one bacterial host to another (23), *D. discoideum* provides a convenient source of amoebae for submerged culture studies.

MATERIALS AND METHODS

Organisms. *E. coli* strain B/r was maintained on tryptone-glucose-yeast extract-agar slants. *D. discoideum* strain NC-4, a haploid strain (ATCC #11735), was maintained on Bonner's agar plates with *E. coli* B/r as the bacterial associate. Mature sorocarps were transferred at biweekly intervals. Both cultures were kindly supplied by K. B. Raper, University of Wisconsin, Madison. *D. discoideum* remains in the ameboid form in submerged culture; hence, we call it "amoeba" here.

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Medium. Minimal medium contained in g per liter: $(NH_4)_2SO_4$, 1.25; KH_2PO_4, 1.50; K_2HPO_4, 3.67; $MgSO_4 \cdot 7H_2O$, 0.10; NaCl, 0.010; $Fe_2(SO_4)_3$, .001; Na_2ethylenediaminetetraacetate (EDTA) $\cdot 2H_2O$, 0.003; glucose, 0.50 (added after autoclaving), *p*H 7.2 ± 0.1. The medium was filtered through hot, distilled water pre-washed membrane filters (Millipore Filter Corp., type RA, 1.2-μm pore size) before autoclaving. Glucose was the limiting substrate for *E. coli*.

Analytical methods. Bacteria were counted by using a model B Coulter counter with a 30-μm aperture tube; amoebae were counted using a model A Coulter counter with a 100-μm aperture. Dilutions were made in saline solution (0.6% NaCl; 0.02% $Na_2EDTA \cdot 2H_2O$) to give 10,000 to 30,000 counts for bacteria by using a 0.05-ml sample volume. At these levels, coincidence corrections are less than 10%.

Glucose was determined using Glucostat reagent (Worthington Biochemical Corp.).

Growth conditions. Batch and continuous culture experiments were carried out in 50- or 100-ml culture vessels similar to the original design of Novick and Szilard (17). Water jackets were added to provide temperature control (19, 22, 25 ± 0.1 C). Nutrient supply for continuous culture was regulated by a capillary feed system (19) by using lengths of stainless-steel hypodermic tubing. Flow rates were measured by weighing the collected overflow. Flow rates were constant to 1 to 2%.

For experiments in which the temperature and holding time were varied, three cultures were fed from the same reservoir of medium. *E. coli* was inoculated and grown continuously for from 5 to 7 days. During day 5 to 7 the flow rates, culture purity, and steady-state operation of the chemostats were verified. Culture purity was checked microscopically and by plating periodically thereafter. Spores of *D. discoideum* were then added to give initial counts of about 10^4/ml. Spores germinated after a lag of about 12 hr (6), and only vegetative amoebae were seen after 2 to 3 days of operation except as noted below. The time of spore inoculation was considered "zero" time for the mixed culture.

Operation of the continuous culture is characterized by the holding time, θ, which is the ratio of the culture volume to the volumetric feed rate. The holding time represents the average length of time a particle remains in the growth vessel. It is the reciprocal of the dilution rate, D. Holding times of 8, 16, and 32 hr correspond to steady-state doubling times of 5.6, 11.2, and 22.4 hr. During non-steady-state growth the doubling times can range from zero to the maximum for the species.

RESULTS

Continuous culture. A representative set of data for 25 C and three holding times are shown in Fig. 1 through 3. Other experiments at 25, 22, and 19 C gave qualitatively similar results. An induction period was often seen in the growth of the predator. This appears as a minor peak, or extended lag, early in the experiment. Presumably, the amoebae had to adjust to the environment before maximum utilization of the bacteria was possible. This behavior was usually most pronounced at the shorter holding times and the lower temperatures.

At a given temperature, as the holding time increased, the oscillations generally increased in period.

After 3 to 4 weeks the cultures began to damp out. It has been our experience in many similar experiments that, after three to five weeks of operation, oscillations were damped. The amoebae persisted at low levels and the bacteria returned to their initial, high density, steady-state level.

Stability of damped oscillations. Once the oscillations had damped out, significant amounts of amoebae began to accumulate on the chemostat walls just above the air-liquid interface. Clumps of three to ten amoebae and small numbers of spores were found upon microscopic examination of the culture. The majority of amoebae in the culture liquid, however, existed as single cells.

Attempts to reinitiate growth of the amoebae at this stage were not successful. Large inocula of either spores or vegetative amoebae simply washed out and did not consume significant amounts of bacteria. Stopping the feed and letting the chemostats run as a batch culture resulted in a slow drop in both bacteria and amoebae levels. Transfer to a clean chemostat to remove the effects of wall growth did not result in renewed oscillations. One such experiment is shown in Fig. 4.

DISCUSSION

The interactions between predator and prey are difficult to analyze without recourse to some kinetic model of the interaction. It is not intuitively clear how changes in the growth parameters of prey or predator will affect the system. An adequate kinetic model is useful both as a description and an aid in interpreting the data as well as in predicting and suggesting new lines of approach. Such models must be designed to reflect the biological realities of the organisms and of the environment involved. Models based on unrealistic assumptions are undesirable, and any correspondence between observed and predicted behavior may be fortuitous. Any mathematical model of a biological system must be a rather abstract analogue. Complex organisms are treated as "chemical" entities, and elaborate interactions

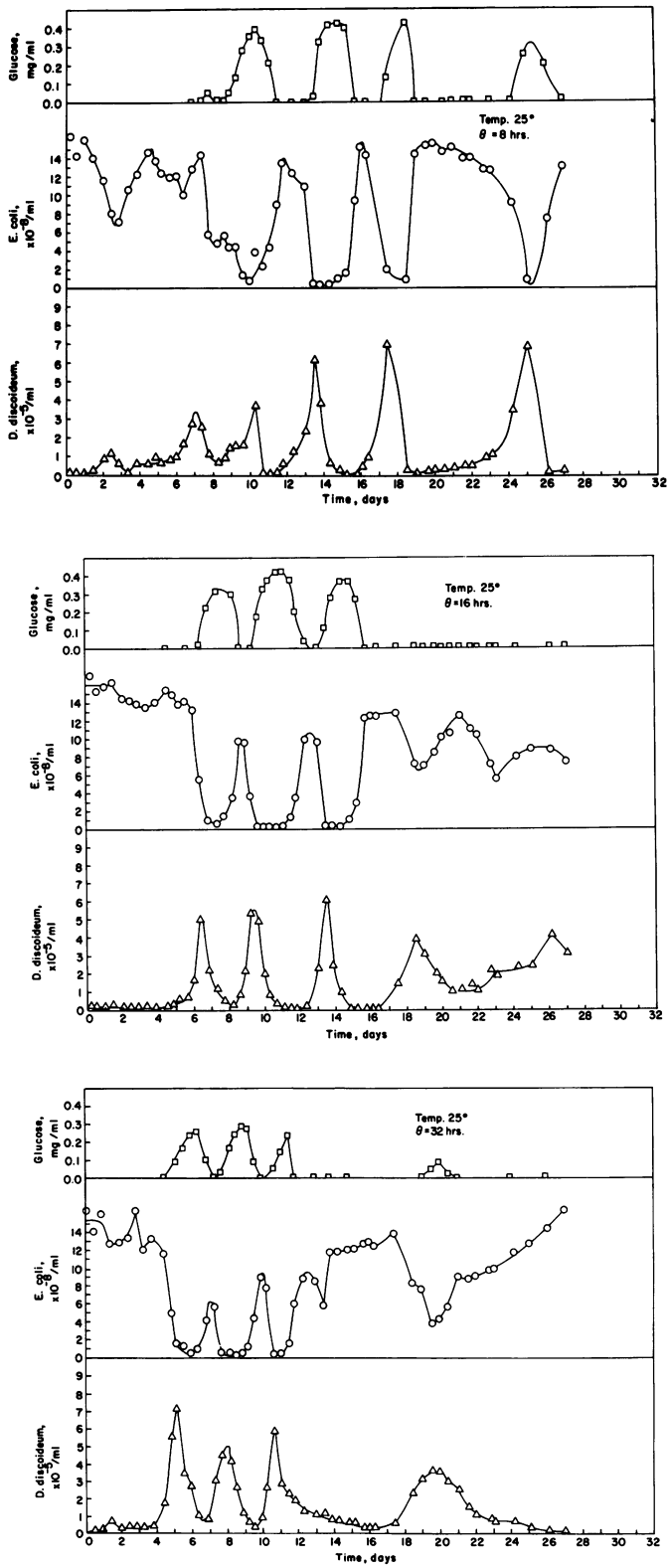

FIG. 1-3. *Changes in numbers of amoebae and bacteria and concentration of glucose in continuous culture at 25 C. Holding time, θ, as shown.*

are reduced to a manageable number of terms. If the major features of the biological system have been recorded in the model without severe distortion, the model serves as a convenient description of the system.

Data suitable for testing the validity of quantitative models describing prey-predator interaction are limited in the earlier microbiological literature. The present experiments were designed to provide such data. The intent of our experiments is not to "prove" a particular model. We are examining to what extent the rational formulation of some known properties of predator and prey can account for the observed interactions in a mathematical model.

A model proposed by Lotka and Volterra (14, 28) predicts regular oscillations in the prey and predator densities and has been analyzed by various investigators (8, 20). Several modifications of the model have been proposed in order to increase its usefulness (7). Unfortunately, its limitations apparently have not been appreciated. The Lotka-Volterra model for the batch case is as follows: $dN_1/dt = aN_1 - bN_1N_2$; $dN_2/dt = cN_1N_2 - dN_2$, where N_1 is the prey density and N_2 is the predator density and a, b, c, d are rate constants.

The saturation model has been described elsewhere (9) and is summarized here. For a continuous culture, the equations are:

$$\frac{dC_A}{dt} = \left(\frac{\mu_A C_B}{K_B + C_B}\right) C_A - \frac{C_A}{\theta}$$

$$\frac{dC_B}{dt} = \left(\frac{\mu_B C_S}{K_S + C_S}\right) C_B - a_B \left(\frac{\mu_A C_B}{K_B + C_B}\right) C_A - \frac{C_B}{\theta}$$

$$\frac{dC_S}{dt} = a_S \left(\mu_B \frac{C_S C_B}{K_S + C_S}\right) + \frac{C_{sf} - C_S}{\theta}$$

where θ = holding time; for batch culture, $\theta = \infty$; C_A, C_B, C_S = concentration of amoebae, bacteria, and glucose, respectively; C_{sf} = concentration of glucose in feed solution; μ_A, μ_B = maximum specific growth rate of amoebae and bacteria, respectively; K_B, K_S = saturation constant for bacteria and glucose, respectively; a_S, a_B = stoichiometric coefficient for glucose and bacteria.

Death and maintenance of the amoebae and bacteria, although undoubtedly factors, are not considered here. In continuous culture, washout acts as a constant nonspecific "death" process. Neither the amoebae nor the bacteria are subject to high death rates by starvation under our experimental conditions, and the major sources of loss of organisms are washout and predation. In the model, biomasses are converted to number densities with the simplifying assumption of fixed mean size for predator and prey.

A comparison of the model and the experimental data is shown in Fig. 5. The experimental data are generally more irregular in period than predicted, although the more regular experimental oscillations are matched reasonably well by the model. The constants, obtained from batch experiments, are shown in Table 1.

The cause of the irregular periods of oscillations is not clear. Precautions to maintain temperature and flow rates at constant levels seem adequate. Comparison of earlier experiments, with less stringent temperature and flow-rate controls than in later experiments, does not indicate that improved control of the environmental conditions leads to a greater regularity in the oscillations.

Events occurring at the minima of either population are a possible source of irregularity. The bacteria often recover slowly from the lower level even though the amoebae have decreased and considerable substrate has accumulated. Bacterial growth in minimal medium is often preceded by a lag even if exponentially growing cells are used as an inoculum. Possibly, the medium must be "conditioned" before maximum growth rates are possible. Low numbers of cells faced with the continued influx of fresh medium may exhibit a varying degree of lag from cycle to cycle and account for part of the irregularity.

The decay of the oscillations after 3 to 5 weeks of operation is a regularly observed experimental fact not predicted by the model. If "mutation" of the bacteria to forms more resistant to predation is assumed, the model can easily be modified to exhibit damped oscillations during which selection of the resistant bacteria is strongly favored. Bacterial variations affecting either the maximum amoebae growth rate (μ_A) or the saturation constant, or both could be responsible for the damping. Other possibilities exist and more experimental data are required to justify such changes.

Two differences between the saturation, or Monod, kinetics model and the Lotka-Volterra model are apparent. (i) The Lotka-Volterra model does not include the effects of the environment in the form of a limiting substrate for the prey, and (ii) the saturation growth kinetics for the predator and prey are omitted. The consequences of these omissions are

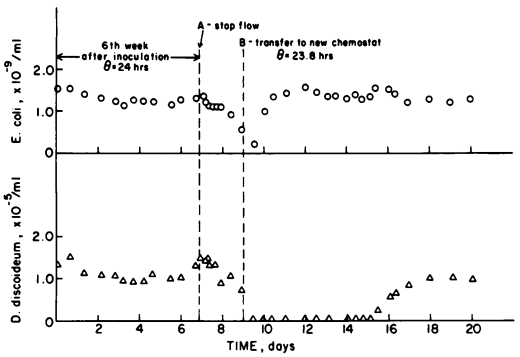

FIG. 4. *Stability of damped oscillations. Chemostat operated for five weeks prior to sampling. At point A, nutrient feed was stopped and run as batch culture for two days. Densities of both bacteria and amoebae decreased. At point B, 10 ml of culture was transferred to a clean chemostat of the same volume (100 ml) and nutrient flow resumed.*

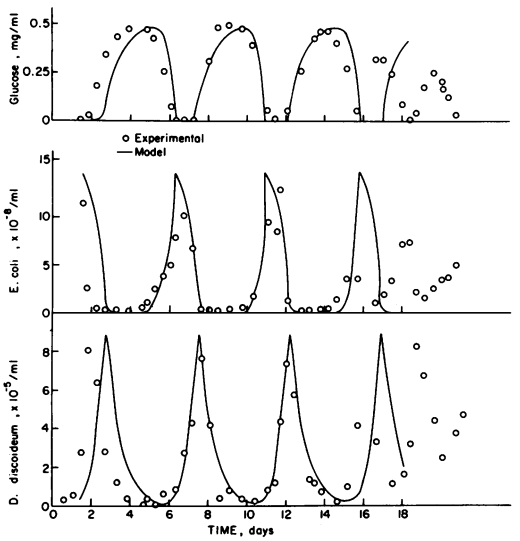

FIG. 5. *Comparison of experimental data and saturation model. Model predictions, (—), superimposed on data, (O), for continuous culture with a 16 hr holding time at 25 C.*

TABLE 1. *Experimental values of growth constants at 25 C*

Organism	Max specific-growth rate (μ)	Saturation constant (K)	Stoichiometric coefficient (a)
D. discoideum	0.24 hr^{-1}	4×10^8 bacteria/ml	1.4×10^3 bacteria/amoebae
E. coli	0.25 hr^{-1}	5×10^{-4} mg of glucose/ml	3.3×10^{-10} mg of glucose/bacteria

shown in Fig. 6 and 7. In Fig. 6, the saturation model is numerically integrated for two different initial inocula A and A' of the prey and the predator. From both initial conditions, the regular oscillations are reached *independently* of the initial inocula. Figure 7 shows the Lotka-Volterra model integrated from two different initial conditions. Two different regular oscillations are obtained. Both the periods and amplitudes of the Lotka-Volterra oscillations are dependent on the initial conditions.

The studies of Gause (10) on model prey-predator systems showed that, when oscillations do occur, they are not dependent upon the initial inocula but seem to be character-

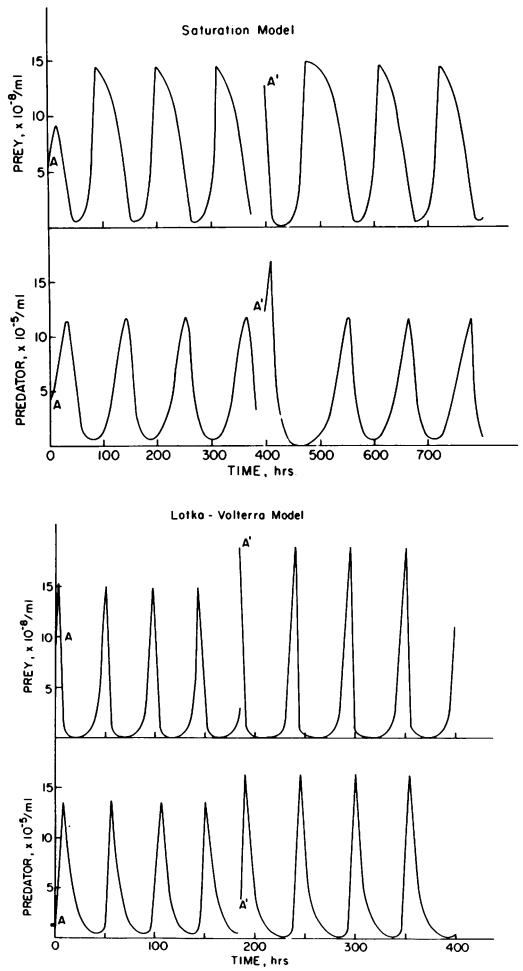

FIG. 6-7. *Comparison of saturation model and Lotka-Volterra model. Predicted numbers of predator and prey for the initial numbers at point A. At point A' the number of predator and prey are suddenly changed to new values.*

istic of the particular system. The ability to change the character of a prey-predator system by the single import or export of predators or prey is not a realistic prediction of a kinetic model. That is, a single "pulse" of predator (or prey) should not have a lasting effect on the populations.

Continuous oscillations of prey and predator are always predicted by models of the Lotka-Volterra type. The controversy among population ecologists over the "inherent oscillations" of prey and predator has been discussed by Slobodkin (27). Our model adds some clarification to the question. For a given prey-predator system at constant temperature, the values of the growth constants are fixed. The behavior of the system is not fixed, however, and depends upon (i) the holding time and (ii) the concentration of substrate in the feed.

These represent environmental factors which can be varied. Figure 8 shows the type of behavior predicted for various holding times and substrate concentrations. The system will show no oscillations (normal node), damped oscillations (normal focus), or regular oscillations (normal limit cycle), depending upon the choice of the holding time and substrate concentration. For a given holding time, the concentration of limiting substrate in the feed determines the behavior. Conversely, for a given substrate concentration, the holding time becomes critical in determining the behavior. The curves were constructed using the experimental values for the growth constants at 25 C given in Table 1. Numerical evaluation of the stability criteria were performed for a range of holding times and substrate concentrations on a Control Data Corp. 6600 digital computer (2).

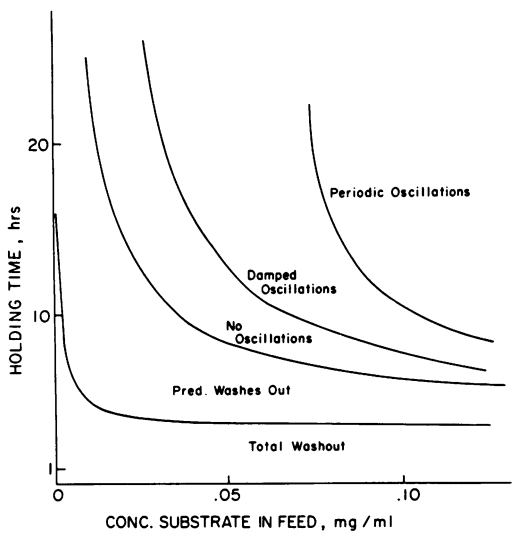

FIG. 8. *Stability regions of the saturation model. Predicted behavior of a continuous culture with respect to holding time and concentration of substrate in the incoming medium.*

The principal point is that specification of the growth constants is not sufficient to predict even the gross behavior of the system. Whether the populations will oscillate, or not, depends upon the environment. In this sense, oscillations are not inherent in the interaction of predator and prey, but are conditional upon a certain range of environmental conditions.

The model represents an idealized predator-prey system and only the essential features of the growth and environment of the organisms are included. In some cases the model corresponds reasonably well to the observed behavior over an initial period of about two or three weeks. Effects of lags and the development of a resistant bacterial population destroy the correspondence after several cycles.

We are currently examining the basis for both the irregular nature and eventual damping of the oscillations. Progress in these areas should be enlightening both with respect to microbial prey-predator systems and the growth physiology of *D. discoideum*.

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