vesicles, expressed in relation to cytoplasmic volume. As judged according to this method, the endocytotic activity over 60 minutes of incubation was three times greater in the presence of glucose (3.0 mg/ml) than in its absence (Table 1).

In a second series of experiments, islets were preincubated for 1 hour in the absence or presence of glucose, and then exposed to peroxidase for 15 more minutes in glucose-free media. As shown in Table 1, endocytosis was twice as great in beta cells of islets preincubated at a high glucose concentration, and further incubated with peroxidase in the absence of glucose, than in beta cells never stimulated by glucose (*8*).

Finally, islets were exposed to peroxidase at a high concentration of glucose (3.0 mg/ml) for 30 minutes and were, thereafter, further incubated for 60 minutes at the same glucose level but in the absence of peroxidase. In this condition, the reaction product was gone from the extracellular space, but could still be found in the beta cells, almost exclusively in large collections of vesicles in the Golgi area (Fig. 1c). The ultimate fate of the peroxidase taken up with beta cell vesicles has not yet been determined.

These findings indicate that the release of insulin by emiocytosis is not associated with the permanent loss of intracellular membranous material, since a relocation of beta cell plasma membrane via endocytotic vesicles occurs concomitantly, and consequently to glucose-stimulated insulin release. The physiological significance of endocytosis in the beta cell is at present highly speculative. Nevertheless, we wish to suggest that the endocytotic process may represent a mechanism by which Golgi-derived membranous material initially incorporated in the cell membrane at the time and site of emiocytosis would be restituted to the cytoplasmic compartment, and its components thus become again available for the elaboration of intracellular membranes. Similar recycling of excess surface membranes has been indicated by studies of other secreting cells (*9*). The possibility that endocytosis could also be a route for the uptake by the beta cell of substances otherwise restricted to the extracellular space remains to be investigated.

In summary, the present findings suggest that the emiocytotic release of insulin is coupled with an endocytotic process, leading to the relocation of membranous material from the cell membrane into an intracellular vacuolar system.

Table 1. Effect of glucose concentration in incubation medium or in preincubation and incubation media on peroxidase uptake by beta cells of isolated rat islets. Horseradish peroxidase (2.0 mg/ml) was present only during the incubation period. Results (mean ± standard error of the mean) are expressed as surface area of peroxidase-containing vesicles (in square micrometers) per cytoplasmic volume (in cubic micrometers) and are shown together with the number of individual determinations in each experiment (in parentheses) and the significance of observed differences.

Glucose concentration in media (mg/ml)	Surface density of peroxidase-containing vesicles
Incubation with peroxidase (60 min)	
0.0	0.135 ± 0.020 (9)
	$P < .0005$
3.0	0.429 ± 0.026 (9)
Preincubation without peroxidase (60 min) followed by incubation with peroxidase in glucose-free medium (15 min)	
0.0	0.125 ± 0.012 (3)
	$P < .0005$
3.0	0.282 ± 0.008 (3)

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Predator-Prey Interactions in Continuous Culture

Abstract. *The exploitation of a bacterial food supply by a protozoan predator does not necessarily lead to the extinction of either species. Even a "homogeneous" experimental system contains sufficient heterogeneity (the boundaries) for avoidance strategies to evolve, which allow the populations to presist.*

Many models of predator-prey interactions in simple environments predict the occurrence of damped reciprocal oscillations in the densities of the predator and its prey (*1, 2*). "These oscillations can only be produced in the laboratory with elaborate immigrations of both predator and prey, or with extremely complex experimental situations in which spatial heterogeneity is deliberately introduced into the population's environment" (*3*). Thus while Gause (*4*) regularly reinoculated his *Paramecium-Didinium* cultures to prevent elimination of one or the other of the components, it has been estimated that the protection from predation of at least 65 percent of the prey would have been required to stabilize the population (*5*). Similar limitations apply to other experimental populations (*6*). Utida (*7*), however, successfully maintained an experimental population of the azuki bean weevil (*Callosobruchus chinensis*) and its larval parasite (*Heterospilus prosopidis*) for 112 generations, with damped reciprocal oscillations of population densities.

Continuous culture of micoorganisms provides an ideal system for studying large populations under closely defined environmental conditions while permitting energy flow. Natural populations are subject to fluctuations due to the simultaneous operation of both physical and biotic factors. These fluctuations contradict the assumption that natural

populations reach a stable equilibrium or "steady state" (*8*). In continuous culture, however, organisms are maintained under closely defined physicochemical conditions, and fluctuations in population densities can be caused only by interactions between the organisms comprising the populations.

A perfectly mixed continuous culture appears to provide the most homogeneous environment that can be obtained for experimental studies. In fact it contains one major heterogeneity—the boundaries of the system provided by the glass walls of the culture vessel.

Cultures of *Klebsiella aerogenes* NCTC 418 were grown in continuous culture (*9*) at 22°C in mineral salts medium (*10*) with limiting sucrose (80 mg/liter). Bacterial cultures were allowed to equilibrate at 2.5×10^8 cells per milliliter for 7 days. A washed axenically grown suspension of *Tetrahymena pyriformis* W was added to give a final concentration of about 10^3 protozoa per milliliter. Samples (1 ml) were removed daily and assayed for viable bacteria (colonies on tryptone agar) and *Tetrahymena* (direct microscopic counts). All assays were performed in triplicate.

Addition of the predator to the "steady-state" bacterial culture led to a rapid increase in protozoan density at the expense of the bacterial population, the protozoa reaching a maximum density of 5.5×10^4 cells per milliliter within 48 hours. A subsequent slow decrease in predator numbers corresponded with an increase in bacterial density. Subsequent oscillations in bacterial numbers were mirrored by slightly out-of-phase oscillations in protozoan density (Fig. 1). Mixed populations of *Tetrahymena* and *Klebsiella* were maintained for more than 1000 hours (about 40 generations for *Tetrahymena* at the dilution rate of 0.04 per hour). During this period five oscillations were observed, each oscillation having both a lower amplitude and frequency than the preceding one; thus the oscillations were damped and the population appeared to approach an equilibrium.

There is evidence of considerable interaction between the two species; both appear to be adapting to the presence of the coexisting species. The experiment is therefore a model of both an ecological and an evolutionary system.

Tetrahymena undergoes gross changes in size according to the availability of prey. Rapidly growing protozoa show a size distribution of 40 to 200 μm, while starvation favors the survival of only the smallest predators. This reflects the changing ratio of bacteria to predator, since a small predator can be maintained by fewer prey than a large one. After 800 hours, all the protozoa present are small, few exceeding 60 μm in length.

Coexistence of a predator and its prey is usually due to environmental heterogeneity, the coexisting species being partially separated by their abilities to occupy separate niches. In continuous culture, however, the bacterial culture is homogeneous. The rapid increase in bacterial numbers exhibited between 140 and 200 hours is marked by the disappearance of normal mucoid (capsulate) colonies and the appearance of glassy (noncapsulate) colonies. The slight decline in bacterial numbers observed after 200 hours is due to the onset of slight growth on the walls of the culture vessel. The walls of this vessel were not scraped, but a similar culture showed only a slight increase in bacterial titer when the wall growth was scraped into the bulk of the culture. The loss of polysaccharide capsules by the bacteria may reflect an economy permitting more rapid growth or easier adherence to solid surfaces. As *Tetrahymena* feeds by creating currents which direct food particles to the cystostome, active movement of the predator is a necessary prerequisite for feeding. In a homogeneous liquid environment no refuge is available for the prey which is actively searched out and ingested. The more the predator eats the faster it divides and ultimately the prey will become extinct. If, however, the ecosystem is partitioned into a liquid phase in which predation occurs and a refuge

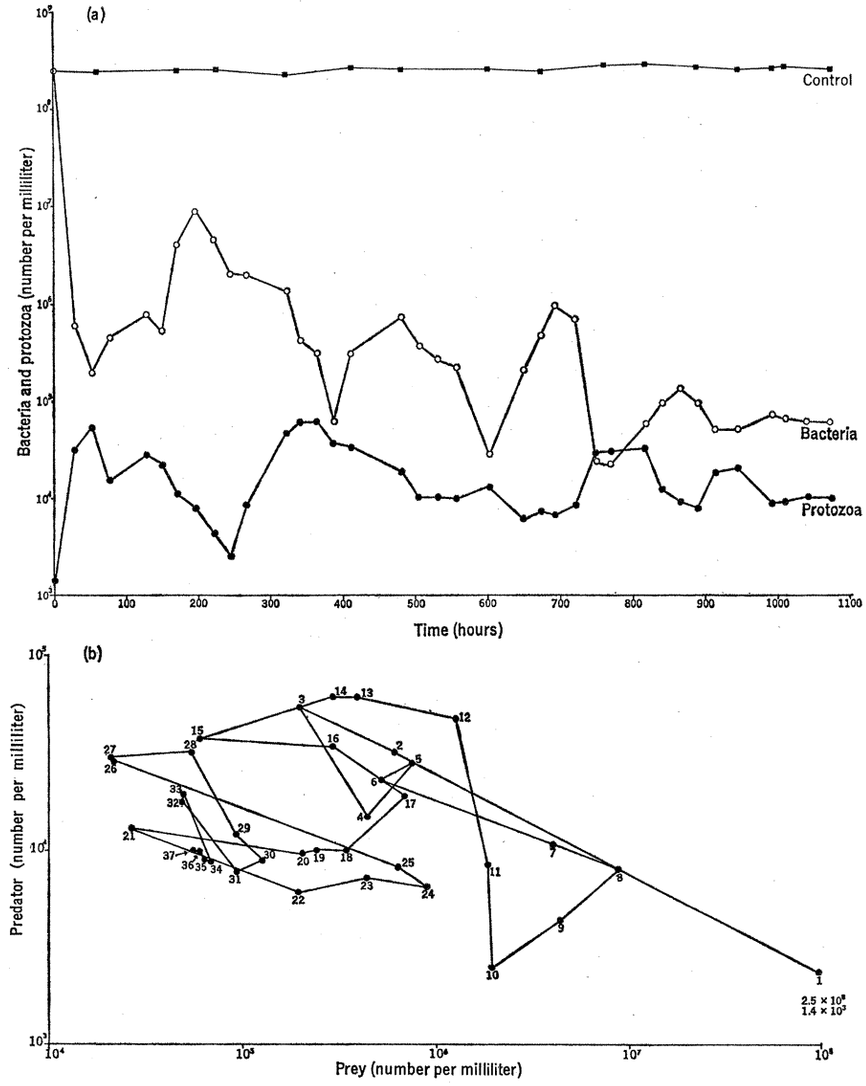

Fig. 1. (a) The history of population changes of *Klebsiella aerogenes* (prey) and *Tetrahymena pyriformis* (predator) in a sucrose-limited continuous culture. The control culture contained a steady-state bacterial population. (b) Predator population plotted against prey population.

which is relatively inaccessible to the predator, the prey can multiply in safety while releasing progeny into the hostile liquid phase. The wall of the culture vessel provides such a refuge. The onset of growth on the wall in the experimental populations was preceded by one complete oscillation of the population densities, that is, predation decreased the bacterial density from 2.5×10^8 cells per milliliter to a residual population of 2×10^5 cells per milliliter. Slight wall growth then appeared.

An electrostatic attractive force of about 10^{-8} dyne is required to hold a bacterium against the wall of a culture stirred at 300 rev/min (*11*). To dislodge adherent bacteria, *Tetrahymena* would have to increase the strength of its ciliary currents. Alternatively, attachment to the wall, plus an increase in ciliary action, might dislodge attached prey. Both adaptations would, however, involve major morphological and physiological changes akin to speciation.

The lag before wall growth first appears and the lack of wall growth in the control cultures strongly suggest that, in this particular system, adherence to the vessel wall is a specific adaptation avoiding predation. Although the control cultures did not exhibit significant wall growth for the duration of the experiments, the subsequent addition of *Tetrahymena* to these cultures resulted in the appearance of wall growth after about 250 hours. Bacteria isolated from the walls of the culture vessel on termination of the experiment showed a marked preference for growth on solid surfaces or in large cell aggregates. Attempts were made to measure the amount of wall growth by suspending glass slides in the culture vessel, scraping the adherent cells into buffer, and counting under the microscope. Densities of 10^5 to 10^6 cells per square centimeter were found.

Apparently genetic feedback (*12*) occurs within the populations ultimately stabilizing the predator-prey relationship at a level that ensures the survival of both species. Natural selection in predator-prey systems increases the efficiency of the predator in finding and eating its prey but it also favors those individual prey which escape being eaten. If adherence to the walls of the culture vessel enables a bacterium to survive and reproduce, these variants have significant selective advantage in the presence of the predator and eventually "take over" the bacterial population. This system provides a specific example of niche diversification, the creation and utilization of a separate niche in what was initially chosen to be a "homogeneous" experimental system.

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Intestinal Calcium Transport: Stimulation by Low Phosphorus Diets

Abstract. *Rats maintained on a low phosphorus diet supplemented with 25-hydroxyvitamin D_3 show high intestinal calcium transport activity as compared to rats similarly treated but fed a diet containing adequate phosphorus. This increased transport activity is correlated with an increased biosynthesis of 1,25-dihydroxyvitamin D_3, the probable metabolically active form of the vitamin in the intestine.*

Vitamin D must be hydroxylated in the C-25 position by the liver (*1*, *2*) and in the C-1 position by the kidney (*3*) before it can function to stimulate both intestinal absorption of calcium (*4*) and mobilization of bone calcium (*5*). In addition, strong evidence indicates that the resulting 1,25-dihydroxyvitamin D_3 [1,25-$(OH)_2D_3$] does not require further metabolic alteration before it initiates both intestinal calcium absorption and bone calcium mobilization (*6*). Therefore, it appears that 1,25-$(OH)_2D_3$ is at least one of the hormonal forms derived from vitamin D responsible for the mobilization of calcium from these two organs. This concept was strengthened by the find-

Table 1. Stimulation of intestinal calcium transport and accumulation of 1,25-$(OH)_2D_3$ in blood and intestine of rats fed a low phosphorus diet. Weanling rats were fed a vitamin D–deficient diet containing 1.2 percent calcium and either 0.1 or 0.3 percent phosphorus for 2 weeks. At that time the rats receiving 0.1 percent phosphorus weighed 76 ± 6 g (24 rats) and those receiving 0.3 percent phosphorus weighed 88 ± 10 g (20 rats). At this stage a third of the rats in each group received 130 pmole of 25-OHD_3 orally each day in cottonseed-soybean (Wesson) oil; another third received 130 pmole of 25-OH[^{3}H]D_3 orally each day in Wesson oil, and the other third received only Wesson oil each day. All rats were killed by decapitation 2 weeks after this treatment was begun. The tissues from three or four rats receiving 25-OH[^{3}H]D_3 were pooled for each group, extracted, and chromatographed (*13*) to determine the amount of 1,25-$(OH)_2D_3$, while the other rats (at least six per group) were used to measure intestinal calcium transport by the everted sac method (*12*). Serum calcium and phosphorus were measured in all animals. Standard deviations are given.

Treatment	Body weight (g)	Serum calcium (mg/100 ml)	Serum phosphorus (mg/100 ml)	Calcium transport (inside/outside)	1,25-$(OH)_2D_3$ in Intestinal mucosa (pmole/g)	1,25-$(OH)_2D_3$ in Serum (pmole/ml)
		0.1 percent dietary phosphorus				
Oil	104 ± 10	10.7 ± 0.4	1.7 ± 0.3	1.6 ± 0.1		
25-OHD_3	116 ± 11	13.1 ± 0.7	6.5 ± 0.8	7.9 ± 0.6*	0.760	1.07
		0.3 percent dietary phosphorus				
Oil	154 ± 9	11.6 ± 0.4	3.9 ± 0.3	2.0 ± 0.2		
25-OHD_3	165 ± 16	11.7 ± 0.4	9.8 ± 0.7	3.9 ± 0.3†	0.322	0.395

* Significantly different from † ($P < .001$).