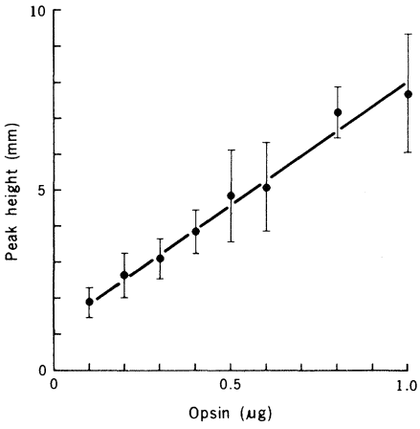

Fig. 3. Peak height as a function of the amount of opsin migrating during electrophoresis in the first dimension and subsequently into antiserum to ROS. One standard deviation (four measurements) is shown for each point.

membrane components may not be able to regenerate antigenic determinants after the native conformation or supramolecular interactions are disturbed by SDS solubilization. For such systems this procedure may not be applicable. However, the simplicity of the technique and the ease of immunization with antigens isolated from SDS polyacrylamide gels should permit a large variety of systems to be explored.

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4. Electrophoresis in the first dimension is a modification of the method of Fairbanks *et al.* (*1*), in a slab gel apparatus [F. W. Studier, *J. Mol. Biol.* **79**, 237 (1973)]. Lengthwise strips cut from tube gels may also be used. The SDS concentration was reduced from 1 to 0.1 percent in order to reduce the amount of SDS migrating into the second dimension. Reference is made to 1 percent SDS gels for definitive assignment of protein bands. The buffer in the SDS gel and the reservoir was a diluted (1 : 40) stock buffer (Fig. 1) to increase protein mobility in the second dimension. Samples were solubilized in a mixture of 2.5 percent SDS, 2.5 percent β-mercaptoethanol, 2.5 m*M* EDTA, 15 to 60 minutes at 37°C. Sucrose (4 percent, by weight) and pyronin Y (Eastman) (1.0 μg/ml) were added. Samples were subjected to electrophoresis at 100 volts, 35 ma, for 1 to 3 hours. The reference portion of the gel was immediately fixed and stained (*1*). The second dimension was run on the same day to minimize broadening of the unfixed protein bands.
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6. Proteins having a molecular weight of less than about 150,000 migrate readily from the standard 5.8 percent gel (*1*) and may be detected by fixing and staining after electrophoresis into agarose lacking antiserum. Gels with 4 percent or less are satisfactory as the first dimension for studying larger molecules. It appears that some SDS is still attached to the protein after migration through the Lubrol layer. This was shown by the migration, under electrophoresis, of opsin, IgG, and H and L chains into the SDS polyacrylamide gel, then in the second dimension through the Lubrol layer into buffered agarose lacking antiserum. All of the proteins migrated into the agarose approximately the same distance; if all SDS has been removed, the IgG and the H and L chains should have been retarded or should have reversed their direction of migration and not been seen at all. At *p*H 7.4, IgG in the agarose layer migrates slightly toward the cathode (that is, toward the entering antigens). Gels run at *p*H 7.4 and *p*H 8.6 are not significantly different.
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Enteropancreatic Circulation of Digestive Enzymes

Abstract. *Intact digestive enzymes can be absorbed by the intestine and resecreted by the pancreas. The pancreas, therefore, appears to be able to recycle proteins much as the liver recycles bile salts, although the magnitude of this process remains uncertain.*

At least some digestive enzyme undergoes an enteropancreatic circulation, analogous to the enterohepatic circulation of bile salts; that is, intact digestive enzyme molecules are absorbed by the intestine and subsequently resecreted by the pancreas. The existence of this cycle was shown directly with radioactive enzyme and inferred from the following observations: (i) The baso-lateral cell membranes of pancreatic tissue are permeable to at least some digestive enzymes; (ii) there is the potential for transpancreatic movement of these enzymes from blood to duct lumen through the secretory cells; and (iii) the small intestine is permeable to several digestive enzymes.

In studies on the kinetics of [^{3}H]chymotrypsinogen (bovine) uptake by pancreatic tissue in vitro (*1*) we found that at least a portion of the cell membrane of the acinar cell, the pancreatic secretory cell, was permeable to this and presumably other digestive enzymes that were added to the suspending medium. This exogenous enzyme equilibrated with endogenous secretory protein of the same enzyme species in the cytoplasm and zymogen granules, crossing both zymogen granule (*1, 2*) and cell membrane (*1*) in the process. Since in this study the investigators used slices of pancreatic tissue no more than 0.1 mm thick that expose both apical and baso-lateral cell membranes to the bathing medium (*3*), it was not clear which part of the cell membrane was permeable to the enzyme. As a control, the uptake of exogenous enzyme was monitored in the same manner (*1*) by using strips of pancreatic tissue, that is, unsliced segments of rabbit pancreas in which the apical membrane is not exposed to the bathing medium (*3*). In these strips, after correction for intercellular space (with albumin) and a substantial wash (sufficient to remove 90 percent of the albumin) with a medium containing a high concentration of unlabeled chymotrypsinogen (40 μ*M*), a considerable amount of [^{3}H]chymotrypsinogen was still associated with the tissue. Since the magnitude of uptake was substantial [unsliced uptake was about 20 percent less than the previously reported values for tissue (*1*)], in all likelihood uptake across the baso-lateral surface of the cell was at least partially involved in the attainment of equilibrium between exogenous enzyme and intracellular digestive enzyme pools.

If secretory protein can enter the cell from the "blood" side, and if once these molecules are in the cell they mix with secretory pools, then this protein should, of course, eventually be secreted. In order to test for this, an in vitro preparation of whole rabbit pancreas was used (*4*). After excision, the whole organ was suspended by means of attached intestinal tissue on a Lucite frame that was in turn placed in a bath containing a physiological salt solution and oxygenated with 95 percent O_2 and 5 percent CO_2 without recourse to vascular perfusion. The pancreatic duct was cannulated and secretion collected directly from it without contamination by bath water, thereby maintaining the polarity of the natural secretory process. [^{3}H]Chymotrypsinogen (bovine) and [^{131}I]albumin (human) (to control for the leakiness of the system) were added and

the secretion was collected. Under these conditions the chymotrypsinogen concentration [^{3}H precipitable by trichloroacetic acid (TCA)] continued to rise in secretion for 1 to 2 hours before a "steady-state" secretory concentration was reached (Fig. 1A). Approximately 4 μg of chymotrypsinogen was secreted per hour at the steady state when 8 μM (20 mg/100 ml) of this bovine enzyme was in the bath. This means that exogenous chymotrypsinogen accounted for 0.5 percent of the total protein as measured in secretion, or about 3 percent of the chymotrypsinogen was of exogenous origin. At the steady state, the chymotrypsinogen : albumin ratio was about 20 : 1 (range, 16 to 24). To rule out breakdown and reincorporation of label as a significant contributory factor, amylase in secretion from a pancreas exposed to [^{3}H]chymotrypsinogen for 6 hours, the longest incubation, was purified by the method of Schramm and Loyter (*5*) and was found to contain virtually no tritium (0.2 $\pm$ 0.1 percent, N = 3, a quantity comparable to the [^{131}I]albumin contamination found for the purification procedure). Both the tissue uptake studies and the specificity (20 : 1) of chymotrypsinogen flux suggested that the transpancreatic movement of this enzyme occurred at least in part via an intracellular route. This view was further supported by a significant reduction in the rate of secretion of [^{3}H]chymotrypsinogen when unlabeled chymotrypsinogen was added to the medium, raising the total enzyme concentration from 0.8 to 8.0 μM (–16 $\pm$ 5 percent, $P < .02$, N = 5, for the first 30 minutes after introduction of unlabeled material) (Fig. 1A). This reduction is apparently the result of competition between unlabeled and labeled enzyme for transport sites and is just the opposite of the change that would have been expected if only simple diffusion between (or through) cells was involved. The decrease was specific at least in that the albumin flux did not change (albumin concentration increased 1 $\pm$ 7 percent, N = 4, for the same period).

The transpancreatic flux of homologous (rabbit rather than cow) amylase, purified from pancreatic tissue (*5*) after first being labeled in vitro with [^{3}H]leucine, was also studied (Fig. 1B). In these experiments cholecystokinin-pancreozymin (*6*) [6 Ivy dog-units per 100 ml of bath (*7*)] , a gastrointestinal hormone that stimulates pancreatic amylase secretion, was added to the bathing medium after [^{3}H]amylase had achieved its steady-state concentration (at 1½ hours after the addition of the tritiated enzyme). After the addition of the hormone, the concentration of [^{3}H]amylase in secretion rose markedly (Fig. 1B).

Digestive enzyme in the blood can be derived from at least two sources—the acinar cell itself and from the intestinal lumen via the bloodstream. The intestinal epithelium is permeable to a variety of proteins (*8*); for digestive enzymes in particular, substantial elastase, chymotrypsin, and trypsin permeabilities have been reported (*9*). We examined chymotrypsinogen permeability by comparing the mucosal to serosal flux of [^{3}H]chymotrypsinogen relative to that for [^{131}I]albumin across gut sacs prepared from rabbit ileum. Warshaw *et al.* (*10*) recently demonstrated that the ileum is quite permeable to albumin as compared to the other proteins they tested. Nevertheless, we found that the permeability of the ileal membrane to chymotrypsinogen expressed per unit of concentration gradient was some nine times greater than that found for albumin for the concentrations used (percentage of equilibration per hour for chymotrypsinogen/percentage of equilibration per hour for albumin, 9 $\pm$ 1, N = 16) (fluxes compared are TCA precipitable ^{3}H and total ^{131}I after 60 minutes of incubation, a comparison that provides for a maximum bias against preferential chymotrypsinogen absorption).

Finally, to directly examine the circulation of enzyme, we placed [^{3}H]chymotrypsinogen (in a Krebs-Henseleit salt mixture) into the intestine just downstream from the intestinal opening of the pancreatic duct and collected pancreatic secretion from the cannulated duct. The ^{3}H peaked in secretion within 15 minutes after its instillation into the upper part of the small bowel (Fig. 1C). Some 67 percent of the secreted label (50- to 60-minute collection period) comigrated with bovine chymotrypsinogen in disc gel electrophoresis, a band that contained only about 5 percent of the total protein secreted by the pancreas (Fig. 2).

The existence of an enteropancreatic circulation for at least some digestive enzymes seems clear. However, it remains to be seen whether it is of physiological importance relative to the regulation of enzyme synthesis and secretion or as a conservation mechanism; nor is it clear in what form the enzyme is naturally circulated. There is some indication in the literature that the digestive enzymes may indeed regulate their own secretion, although the site of action is uncertain (*11*). In addition, the fluxes we observed across the pancreas and intestine are of sufficient magnitude to indicate the possibility of a conservation mechanism. In any event, the na-

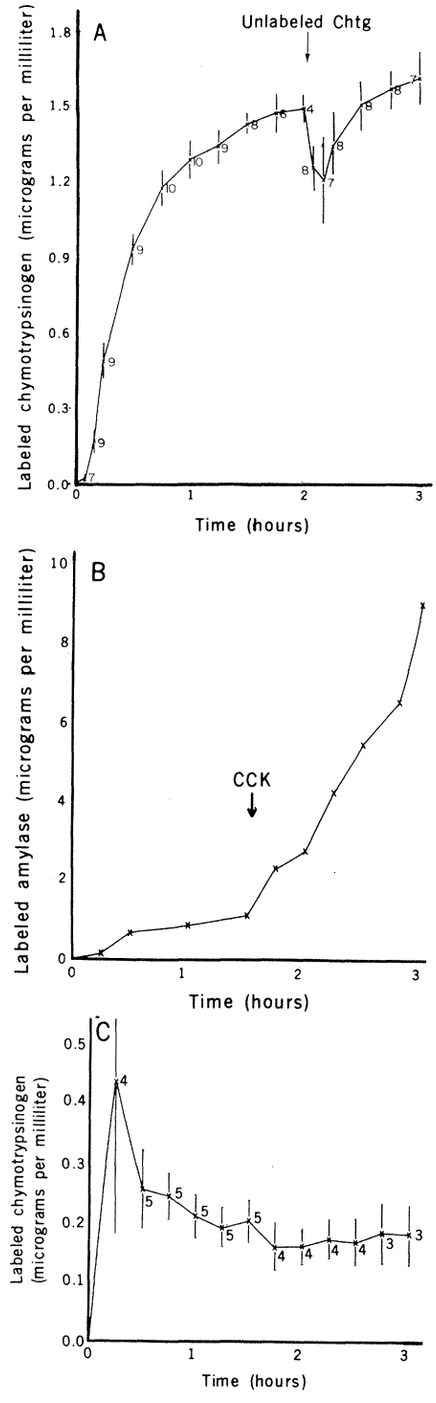

Fig. 1. (A) The mean concentration of TCA precipitable label in secretion from in vitro rabbit pancreas expressed as equivalent exogenous enzyme when 20 μg of α-[^{3}H]chymotrypsinogen (bovine) per milliliter was added to the bath at time 0; at 2 hours 180 μg of unlabeled α-chymotrypsinogen (bovine) per milliliter was introduced. Error bars indicate standard error of the mean (S.E.M.) and the number of experiments is given next to each sample. Concentration of [^{3}H]chymotrypsinogen plateaued in secretion after about 2 hours of incubation. (B) [^{3}H]Amylase (rabbit; 20 μg/ml) was added in a similar fashion. Values are the average concentration of exogenous amylase found in secretion for three experiments. Cholecystokinin (*CCK*) was added at 1½ hours. (C) The concentration of α-[^{3}H]chymotrypsinogen in pancreatic secretion after 2 mg of exogenous labeled enzyme was instilled into the upper intestine. Error bars are S.E.M.; the number of experiments is given next to each sample. *Chtg*, chymotrypsinogen.

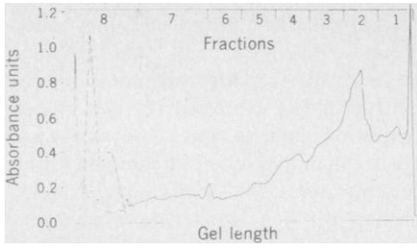

Fig. 2. Gel scan of secretion after the instillation of [^{3}H]chymotrypsinogen (bovine) into rabbit duodenum. The solid line represents a scan of gel prepared in the manner of Jones (*12*) and stained by the procedure of Ahlroth and Mutt (*12*) on which 25 μl of secretion collected 1 hour after the injection of labeled enzyme into the gut was layered. The gels were run for 25 minutes at 3.5 amp per tube toward the cathode. The dashed line represents a scan of 10 μl of the exogenous enzyme run in the same way at the same time. The number at the top represents the dividing line for gel fractions analyzed for tritium content. Fraction 8, the section of a gel of exogenous enzyme (dashed line) contained 70 percent of the label and virtually all of the protein; for secretion it contained 67 percent of the label and less than 5 percent of the total protein.

ture of the fluxes suggests that a wholly probabilistic or random circulation of molecules through a series of membranes with a finite but undifferentiated permeability cannot account for the circulation of digestive enzyme.

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Coexistence of Sparrows: A Test of Community Theory

Abstract. *A study of the coexistence of sparrows in grassland and woodland habitats of the Research Ranch in southeastern Arizona supports the predictability of current community theory. For each of the habitats studied, the production of seeds and the abundance of seed-eating, winter-resident sparrows were measured. The theory correctly predicts the number of species supported in each of the habitats. In both cases for which the prediction is that only one species could be supported, the theory correctly predicts which species should be present.*

Among the prime goals of theoretical ecology is the prediction of species abundance and species diversity. For this reason a predictive theory of species coexistence is central to the development of the science. In the last decade a sophisticated "community theory" has been developed to predict the composition of equilibrium communities and, thus, the criteria for coexistence. For this theory, ecologists are indebted to Levins, May, and, particularly, MacArthur (*1*, *2*).

This report is a result of an attempt to test predictions of community theory on populations of seed-eating sparrows inhabiting the Research Ranch in the plains grasslands of southeastern Arizona (*2*). The American sparrows comprise the bulk of the subfamily Emberizinae of the family Emberizidae. Although in many local areas 15 to 20 emberizinine species may occasionally be recorded, there are usually only three to eight winter resident species in the composite of all habitats of a local region.

The probable importance of competition for food among seed-eating sparrows is suggested by the empirical observation that there is an amazingly constant average ratio of bill sizes (culmen lengths) of adjacent pairs when the species are ranked by size. For the resident species near Turrialba, Costa Rica, this ratio is 1.11 ± 0.15 (S.D.); for the winter residents near Durham, North Carolina, the ratio is 1.10 ± 0.05; and for the winter residents at the Research Ranch in southeastern Arizona it is 1.09 ± 0.10 (*3*). This constant ratio allows one to generate a hypothetical sparrow guild, without reference to specific species, and then to predict which species will be present in a given habitat. To do this, let us start with the smallest North American sparrow, the Brewer's sparrow (*Spizella breweri*), with a culmen length of 8.8 mm. From the relation $C_{r+1} = (1.1)^r 8.8$, the vector of culmen lengths for the hypothetical guide is C = (8.8, 9.7, 10.6, 11.7, 12.9, 14.2, 15.6), and the hypothetical species are referred to as one through seven, respectively. The culmen length for the hypothetical species seven is 15.6 and roughly corresponds to the culmen length of the largest temperate North American sparrow.

Culmen length is important because

Table 1. Seed production in the three study sites. On the basis of these data and the utilization matrix for the six hypothetical species, the following vectors of carrying capacity were calculated: for site 1, k_1 = 505.4, k_2 = 512.9, k_3 = 515.3, k_4 = 512.8, k_5 = 500.4, k_6 = 489.3; for oak site, k_1 = 105.1, k_2 = 105.6, k_3 = 104.4, k_4 = 102.6, k_5 = 99.3, k_6 = 95.2; and for riparian site, k_1 = 84.3, k_2 = 84.9, k_3 = 84.8, k_4 = 85.8, k_5 = 83.4, k_6 = 82.1.

Organism	Seed production (mg/m²)		
	Site 1	Oak site	Riparian site
	Class 1 (0.0 to 0.5 mm)		
	Class 2 (> 0.5 to 1.0 mm)		
Aristida hamulosa	48.06	6.03	
Erichloa gracilis			105.76
Panicum sp.			26.15
Setaria macrostachya			40.55
Trichachne California	83.92		
	Class 3 (> 1.0 to 1.5 mm)		
Andropogon barbinoides	141.00	46.72	6.30
Aristida divaricata	484.22	31.46	
Bouteloua gracilis	246.51	30.39	6.67
Bouteloua hirsuta	156.26	24.22	
Bouteloua sp.	39.60	29.40	
Chloris virgata			15.48
Leptochloa dubia	24.28	228.40	48.52
Lycurus phleoides	112.72	1.51	3.34
	Class 4 (> 1.5 to 2.0 mm)		
Aristida ternipes	1036.56	43.76	67.20
	Class 5 (> 2.0 to 2.5 mm)		
Bouteloua curtipendula		30.93	151.33