ment of neuromuscular synapses (*3*). However, in other experimental situations—for example, in studies of the effects of neural tissue on muscle in organ culture (*9*), the effects of colchicine treatment on motor nerves (*10*) and the control of ACh sensitivity of frog muscles (*11*)—the participation of a neural trophic factor may be required.

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Exocytosis-Endocytosis Coupling in the Pancreatic Beta Cell

Abstract. *The stimulation of the release of insulin by glucose is accompanied by an enhanced uptake of cytochemically demonstrable horseradish peroxidase into endocytotic vesicles within the beta cells. An exocytosis-endocytosis coupling might represent a mechanism by which membrane constituents are recycled within the beta cells under conditions of increased secretory activity.*

As first described by Lacy and Hartroft (*1*), emiocytosis (or exocytosis) probably represents the physiological route by which insulin is released by the pancreatic beta cell into the extracellular space (*2*). In such a process, the Golgi-derived membranous sacs surrounding the insulin secretory granule cores fuse with and are eventually incorporated into the plasma membrane. However, the ultimate fate of this membranous material remains unknown. The present report aims at demonstrating that emiocytosis in glucose-stimulated beta cells is coupled with a compensatory movement of membrane from the cell surface into the cytoplasm.

Batches of 30 to 40 islets of Langerhans isolated from the pancreas of fed rats by collagenase digestion (*3*) were incubated for 60 to 90 minutes at 37°C in bicarbonate-buffered medium containing albumin (0.5 percent, weight to volume). Glucose (3.0 mg/ml) and horseradish peroxidase (2.0 mg/ml) (*4*), a macromolecular marker of endocytotic activity (*5*), were incorporated into the incubation medium at various periods of incubation. After incubation, the islets were centrifuged into a pellet, fixed in 2 percent glutaraldehyde in phosphate buffer (0.1*M*, *p*H 7.2) for 90 minutes, rinsed in the same buffer, embedded in agar gel, and sliced in sections 40 μm thick. The slices were treated according to the procedure of Graham and Karnovsky (*5*) to demonstrate sites of peroxidase activity. The fragments of islets were then postfixed with osmium tetroxide in phosphate buffer, dehydrated in graded dilutions of alcohol, and embedded in Epon 812. Thin sections, obtained with a diamond knife on a LKB microtome, were mounted on uncoated copper grids and examined with a Philips EM 300 electron microscope. Islets not exposed to peroxidase at any stage of incubation and processed in order to reveal endogenous peroxidase (*5*, *6*) served as controls. Such preparations contained no peroxidase-tagged structures.

In the first series of experiments, islets were incubated with peroxidase for 1 hour in the absence (Fig. 1a) or presence (Fig. 1b) of glucose. In both conditions, the macromolecular marker easily penetrated the extracellular space, the reaction product delineating sharply the islet cells. Reaction product could also be found in flask-shaped invaginations of the plasma membrane of beta cells, as well as in membrane-bounded vesicles free in the cytoplasm. A comparison of a and b in Fig. 1 clearly shows that the number of such peroxidase-containing vesicles was dramatically increased in beta cells incubated at a high concentration of glucose, a condition known to promote insulin release. However, because of the cell to cell variability of such a phenomenon, we have applied the stereological principles of morphometry (*7*) for the measurement of the surface density of peroxidase-positive

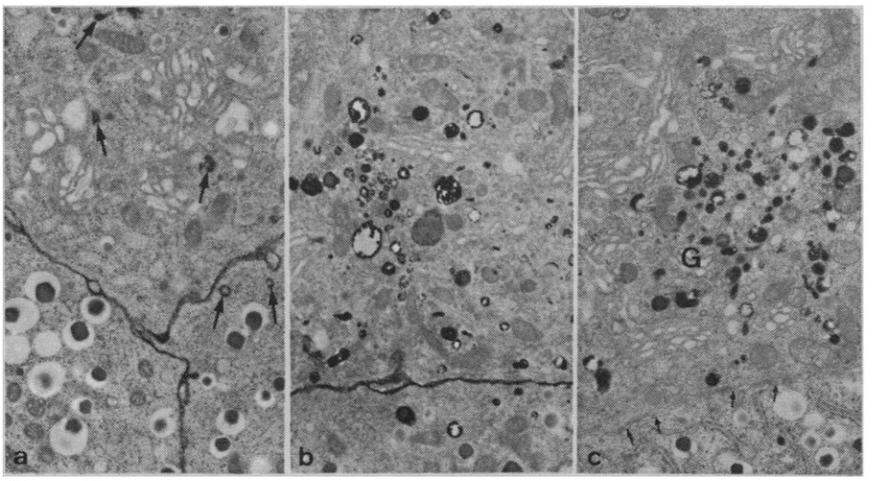

Fig. 1. Electron micrographs of beta cells incubated for 60 minutes in the presence of horseradish peroxidase (2.0 mg/ml). (a) Islet incubated in the absence of glucose. The intercellular space is filled with reaction product. Deposits of reaction product are also present in a few vesicles (arrows) (× 9500). (b) Islet incubated at a high glucose concentration (3.0 mg/ml). The reaction product is present in the intercellular space as well as in many vesicles of varying size (× 9500). (c) After incubation for 60 minutes with peroxidase at a high glucose concentration (3.0 mg/ml), the islet was further incubated for 30 minutes at the same glucose concentration but in the absence of peroxidase. Vesicles with the reaction product are present in large numbers in the Golgi area (*G*), but no reaction product is found in the extracellular space (arrows) (× 9500).

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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4. Two batches of horseradish peroxidase were used. The first batch (Fluka AG) was found to interfere with the measurement of insulin secretion in vitro by isolated islets of Langerhans. However, the second preparation (type II, Sigma Chemical Co.) failed to significantly affect the stimulant action of glucose (3.0 mg/ml) upon insulin release, the output of insulin averaging 296 ± 40 and 289 ± 16 microunits per islet per 60 minutes ($N = 14$ in both cases), in the absence and presence of peroxidase, respectively.
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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