rich prey [for instance, a mid-water shrimp, or selective feeding on its hepatopancreas (*8*)]. The low ^{210}Po family, the Gonostomatidae, must have a completely different, low ^{210}Po diet—copepods, perhaps. Sorting of fish according to genus and size before measurement of ^{210}Po could provide information on feeding behavior complementing that obtained by the standard technique of stomach content examination.

Finally, the measurement technique used also gives data for ^{210}Pb, the grandparent of ^{210}Po. These data are less reliable than those for ^{210}Po; levels of ^{210}Pb in marine organisms are much lower (*2, 6, 8*) and errors are much larger. In some of our mid-water shrimp and fish samples, a conservative assessment of errors led us to conclude that ^{210}Pb concentrations were frequently more than 1 pCi/g dry, a level rarely observed in surface shrimp and fish. In four samples of bathypelagic carid shrimp we found ^{210}Pb concentrations ranging from 0.09 to 1.17 pCi/g and in one penaeid 0.65 pCi/g; for surface carids and penaeids median ^{210}Pb concentrations of 0.04 and 0.20 pCi/g, respectively, have been reported (*8*). In benthic amphipods from the central Pacific ^{210}Pb ranged from 0.6 to 5.3 pCi/g (median, 1.1 pCi/g), while in surface amphipods [(*23*) and our unpublished results] the range was 0.2 to 1.5 pCi/g (median, 0.5 pCi/g). If confirmed, higher ^{210}Pb levels in mid-water and deep sea organisms could have implications for the overall geochemical balance of ^{210}Pb in the water column. In most cases where ^{210}Pb was measured there was a tendency for the ^{210}Po/^{210}Pb activity ratio to be lower than in the corresponding surface animals. The ^{210}Po/^{210}Pb ratio ranges over more than two orders of magnitude in oceanographic materials. Fecal pellets and oceanic particulates are rich in ^{210}Po and have a ^{210}Po/^{210}Pb ratio of about 2 (*19–22*); shrimp hepatopancreas have this ratio approaching 100 (*6, 8*). Measurements of ^{210}Po/^{210}Pb ratios in mid-water organisms might aid the assessment of the relative contribution of detritus to the animals' diet.

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Evidence That Glucose "Marks" β Cells Resulting in Preferential Release of Newly Synthesized Insulin

Abstract. *Studies of isolated islets labeled with radioactive leucine show that glucose at a critical time "marks" islets in such a way as to cause preferential release of newly synthesized insulin. The preferential release of insulin from marked islets is relatively independent of subsequent secretagogues or rates of insulin secretion. Previous kinetic studies have indicated that the critical time at which marking occurs is after proinsulin biosynthesis but before the secretory event. Thus, secretory cells may regulate the diversion of newly synthesized material for immediate release as it is approaching or transiting the Golgi apparatus.*

Investigators at several laboratories, including our own, have concluded that newly synthesized insulin is secreted preferentially from glucose-stimulated pancreatic slices (*1*) or islets (*2–9*). This conclusion was based on results from experiments with radioactively labeled islets that showed that secreted insulin has a higher specific activity than the average cellular insulin and that radioactive insulin is secreted at a higher fractional rate than immunoreactive insulin (IRI). The results were the same whether islets were selected from the entire pancreas or only from either the dorsal or ventral regions (*9*), which are known to contain islets that differ from each other in their hormonal storage and secretion characteristics (*10*). Similar observations of nonrandom secretion also have been made for placental lactogen (*11*), prolactin (*12*), parathyroid hormone (*13*), salivary amylase (*14*), pancreatic amylase (*15*), gonadotropin (*16*), vasopressin (*17*), thyroglobulin (*18*), and acetyl choline (*19*). However, it has not been established whether preferential secretion reflects an intrinsic process of secretory cells that can be regulated in response to physiologic conditions. In the study reported here we show that the same newly synthesized insulin can be secreted either preferentially or near-randomly. The determining event precedes secretion, is glucose-sensitive, and occurs during what we call the "marking" period.

The effect of glucose during the early period of a pulse-labeling experiment on the specific activity of subsequently secreted insulin is shown in Fig. 1. Groups of islets were identically labeled with [^{3}H]leucine, exposed to either 20 or 2 m*M* glucose from minutes 15 to 90 (the marking period), then similarly incubated again in a high concentration of glucose. Noncumulative samples of secreted insulin were collected for two consecutive 20-minute observation periods (windows) during this later period. Secreted and islet insulin were purified separately without carrier by a sequence

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Table 1. Effect of glucose concentration during the marking period on the rate of insulin secretion and the specific activity ratio between secreted and cellular insulin. The results show means ± standard error. Labeling, sampling, and purification methods were the same as in Fig. 1; SA, specific activity.

Secretagogue in the post–marking period	Number of experiments	Unmarked in 2 m*M* glucose		Marked in 20 m*M* glucose	
		Percentage secreted per 20 minutes	SA secreted / SA in islets	Percentage secreted per 20 minutes	SA secreted / SA in islets
Glucose (20 m*M*) plus IBMX (1 m*M*)*	11	7.72 ± 2.2	1.54 ± 0.18	5.81 ± 1.6	3.69 ± 0.32†
Glucose (2 m*M*) plus potassium (50 m*M*)*	8	2.54 ± 0.40	1.29 ± 0.12	2.76 ± 0.35	3.47 ± 0.44†
Glucose (5 m*M*) plus tolbutamide (100 μg/ml)*	8	0.50 ± 0.11	1.56 ± 0.12	1.60 ± 0.26‡	3.25 ± 0.42‡
Glucose (5 m*M*)§	8	0.13 ± 0.01	0.79 ± 0.29	1.40 ± 0.25†	2.60 ± 0.33†

*Samples taken during the second observation period (window B). Because of the increased secretion rate, 20 m*M* glucose plus 1 m*M* IBMX was used in a 7-minute rather than a 20-minute window. †$P < .001$. ‡$P < .005$, compared to unmarked islets. §Sample taken during the first observation period (window A).

of acid-ethanol extraction, isoelectric precipitation, affinity chromatography with antibody to insulin, and chromatography on Biogel P-30 (*9*). Insulin samples were eluted from the Biogel columns with a nearly constant specific activity (counts per minute per nanogram of IRI) throughout the peak, indicating relative homogeneity (*9*). For islets marked in 20 m*M* glucose, the secretion rate of IRI [reported as a percentage per 20 minutes, that is, (secreted IRI × 100)/(secreted IRI + islet IRI)] was higher in window A; however, the rate in window B was nearly identical to the secretion rate of unmarked islets that had been exposed to 2 m*M* glucose at the critical time. These results were anticipated because prior stimulation with glucose is known to potentiate insulin secretion transiently (*20*).

The rate at which unmarked islets secreted insulin in response to 20 m*M* glucose was near-random, as indicated by a specific activity ratio only slightly above unity. However, the specific activity of insulin secreted by marked islets exceeded that of the average cellular insulin by more than threefold; the same elevated glucose concentration, therefore, is able to stimulate either near-random or preferential secretion. This was observed in window B as well as window A, and it is interesting that 52 minutes of exposure to 20 m*M* glucose did not cause preferential secretion of the new insulin if marking had not occurred earlier.

We conducted other experiments with islets labeled in the same way and incubated in either 20 or 2 m*M* glucose during the critical marking period, and then exposed them to lower concentrations of glucose or other secretagogues during the post–marking period (Table 1). The other secretagogues were 1 m*M* 3-isobutyl-1-methylxanthine (IBMX), which increases the cellular concentration of adenosine 3′,5′-monophosphate (cyclic AMP), and potassium (50 m*M*) or tolbutamide (100 μg/ml), which both act by partially depolarizing the β cell. Regardless of these diverse conditions, islets that were marked by exposure to 20 m*M* glucose subsequently secreted insulin with a specific activity that was always significantly elevated above that of the near-randomly secreted insulin of unmarked islets. In addition, preferential secretion was not a function of the insulin secretion rate during the observation periods (windows A and B). Therefore, a glucose-regulated event occurred as the labeled hormone was moving through the cell, and this event, not the subsequent secretagogue, was the determinant of preferential or random secretion.

On the basis of our previous studies of this system, we conclude that three primary cellular events occur during the marking period. First, regardless of the glucose concentration, there is a lag time between minutes 15 and 45, before the conversion of proinsulin to insulin is observed (*9*). This lag time during the first half of the marking period is associated with proinsulin transport from the rough endoplasmic reticulum to the Golgi apparatus, a period in which vesicles form from the terminal elements of rough endoplasmic reticulum and migrate to-

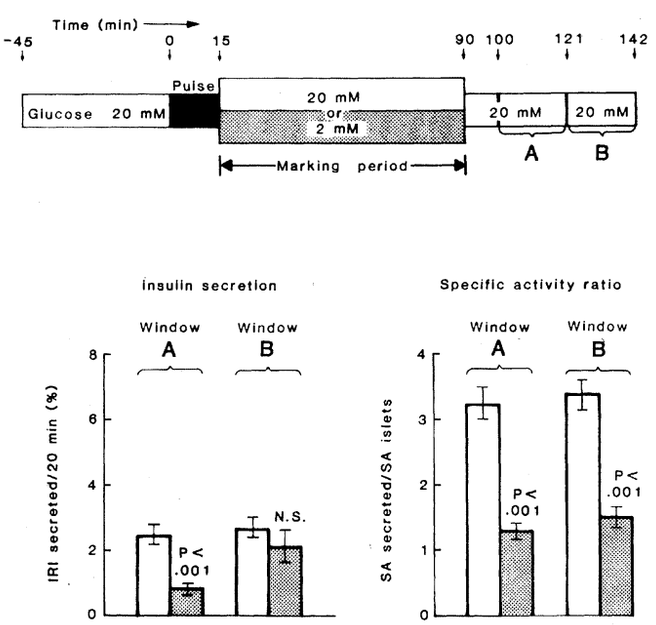

Fig. 1. The effect of glucose concentration during the marking period on the secretion rate of immunoreactive insulin (*IRI*) and the specific activity (*SA*) ratio between secreted and cellular insulin. Groups of 150 to 250 islets were incubated at 37°C in Krebs-Ringer bicarbonate buffer beginning at −45 minutes and exposed to [^{3}H]leucine (400 μCi/ml) from minutes 0 to 15 (pulse). The incubation buffer always contained 20 m*M* glucose from minutes −45 to 15. After this buffer was removed, groups of islets were washed twice and then either marked by incubation in 20 m*M* glucose or not marked by incubation in 2 m*M* glucose from minutes 15 to 90. Both groups of islets were washed twice at 90 minutes and identically incubated in 20 m*M* glucose for the remainder of the experiment. Secreted insulin was collected noncumulatively during two consecutive observation periods (windows A and B). Each window was preceded by removal of all previous incubation buffer and a single buffer wash. The wash buffers contained the same concentration of glucose or other secretagogue as the buffer for the next period of incubation. All buffer after the labeling period also contained 0.2 m*M* leucine. After purification of samples of the secreted insulin and the insulin from the islets by acid-ethanol extraction and by isoelectric precipitation and affinity chromatography, the radioactivity eluting from columns of Biogel P-30 in the insulin peak was used for the calculation of specific activity. The averages of the specific activities used to determine ratios were, in counts per minute per nanogram of immunoreactive insulin: 24.4 ± 2.1, 26.8 ± 2.4, and 8.08 ± 0.81 in window A, window B, and the marked islets, respectively; and 13.8 ± 2.8, 17.9 ± 3.5, and 12.0 ± 1.5 in window A, window B, and the unmarked islets, respectively.

ward the Golgi apparatus (*21*). Second, conversion of insulin begins midway into the marking period at both glucose concentrations and continues as a pseudo-first-order process for more than 2 hours (*9*). Thus, approximately 50 percent of the proinsulin is converted to insulin by the end of the marking period (*9*). Third, the later part of the marking period coincides with initial secretion of labeled proinsulin and insulin, which begins at about the same time as the conversion process (*9*) and is associated with formation of Golgi-derived secretory vesicles (*21*). Therefore, cellular events occurring during the marking period correspond to the time the newly labeled material is approaching and crossing the Golgi apparatus. Glucose during this period may mark for immediate release vesicles forming in either the rough endoplasmic reticulum or the Golgi apparatus, perhaps shunting new vesicles into a novel, glucose-activated secretory route.

How the cell both marks and recognizes marking, and how marking plays a role in the time-dependent modulation of the secretory rate of insulin in response to a given stimulus remain to be determined. Because nonrandom secretion occurs in other secretory cells, marking may prove to be a common mechanism in a variety of cell types responding to their physiologic regulators.

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Modulation of Striatal Dopaminergic Function by Local Injection of 5′-*N*-Ethylcarboxamide Adenosine

Abstract. *Rats rotated to the left when 5′-*N*-ethylcarboxamide adenosine (NECA) was injected into the left caudate nucleus and apomorphine was administered subcutaneously. The combination of NECA and apomorphine was more potent than L-(phenylisopropyl)adenosine and apomorphine in eliciting rotation, suggesting the involvement of adenosine receptors of the R_a type. The response was reduced when 2′,5′-dideoxyadenosine was injected along with NECA into the caudate nucleus or when theophylline was given intraperitoneally. Higher doses of apomorphine elicited a self-mutilatory response after the injection of NECA into the caudate nucleus. These results suggest that adenosine may be involved in the modulation of dopaminergic function in the striatum.*

The involvement of the basal ganglia in several neurological conditions such as Parkinson's disease, Huntington's chorea, and drug-induced dyskinesias has stimulated research on the molecular and physiological basis of striatal function (*1*). The recent report suggesting that striatal dopaminergic function is altered in patients with the Lesch-Nyhan syndrome implicates the basal ganglia in yet another neurological disease which is characterized by movement disorders (*2*). We report studies herein which suggest that adenosine plays a role as a neuromodulator in the striatum and that imbalances in the striatal "adenosine system" may be involved in the etiology of movement disorders.

There has been much recent interest in adenosine as a potential neuromodulator (*3*). The ability of adenosine to increase adenosine 3′,5′-monophosphate (cyclic AMP) in intact cells was first demonstrated in slices of cerebral cortex (*4*) and has since been demonstrated in several tissues and in numerous cells in tissue culture (*5*). There are multiple types of adenosine receptors (*6*). R_a (A_2) and R_i (A_1) receptors stimulate and inhibit adenylate cyclase, respectively, and are located on the cell surface. Both types of R receptors are blocked by methylxanthines such as theophylline or caffeine. Sites of the P type are believed to be intracellular and may be associated with the catalytic unit of all adenylate cyclase enzymes. P sites mediate inhibition of adenylate cyclase activity by adenosine and are not blocked by methylxanthines.

Adenosine may be a neuromodulator in the basal ganglia since adenosine and adenosine analogs stimulate adenylate cyclase activity in broken cell preparations of rat striatum (*7*). Furthermore, ligand binding studies with tritiated *N*-ethylcarboxamide adenosine and 2-chloroadenosine, and autoradiographic studies with tritiated cyclohexyladenosine, show the striatum contains both R_a and R_i sites (*8*). Since the functional significance of these sites has not been investigated, we have studied rotational behavior in rats to determine the effects of adenosine analogs injected directly into the caudate nucleus. This rotation model has been used extensively to study dopaminergic function in the striatum (*9*). After the dopaminergic innervation to the striatum is destroyed by the administration of 6-hydroxydopamine into the substantia nigra, the systemic administration of directly acting dopamine agonists such as apomorphine causes vigorous turning away from the lesioned side (*10*). This is believed to be due to postsynaptic denervation supersensitivity on the denervated side (*10*). Conversely, when one caudate nucleus is destroyed by kainic acid the systemic administration of apomorphine causes rotation toward the lesioned side (*11*). Theophylline and caffeine have been reported to potentiate the rotational response to apomorphine in rats with lesions of the substantia nigra (*12*). This potentiation may have been due to blockade of R site adenosine receptors rather than inhibition of cyclic nucleotide phosphodiesterase as suggested by the authors.

We examined the rotational behavior of rats after injecting adenosine analogs

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