these indicated comparable, approximately parallel curves for ACh and choline. Although we have not determined the transport number of choline during microiontophoresis, it is not likely to differ greatly from that of ACh [probably 0.1 to 0.4, according to K. Krnjević, J. F. Mitchell, J. C. Szerb, *J. Physiol. (London)* **165**, 421 (1963), V. E. Dionne, *Biophys. J.* **16**, 705 (1976)]. The ratios of equipotent currents are therefore likely to approximate the ratios of the equipotent amounts of choline and ACh released from the micropipettes.
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Endocrine Pancreas: Three-Dimensional Reconstruction Shows Two Types of Islets of Langerhans

Abstract. *Three-dimensional reconstructions of islets of Langerhans, based on immunofluorescent staining of successive serial sections with antiserums to insulin, glucagon, somatostatin, and pancreatic polypeptide reveal a marked difference in the number of cells containing glucagon and pancreatic polypeptide depending on the anatomical location of the islet in the pancreas. The two types of islets are situated in regions of exocrine tissue that are drained by different excretory ducts. This demonstration contradicts the assumption that all islets in the pancreas are similar in their endocrine cell content.*

Recent textbooks in histology (*1*) describe the endocrine pancreas as a collection of minute secretory masses, called islets of Langerhans, that are formed of insulin-containing cells (most abundant), glucagon-containing cells, and somatostatin-containing cells (least abundant). Islets of Langerhans are said to be dispersed randomly in the exocrine tissue and to be more numerous in the body and tail of the gland than in the head. This report demonstrates that this view is largely incomplete.

In the rat, the pancreas is an irregularly shaped, elongated organ extending between the duodenum and the spleen. It is drained by at least two main excretory ducts (*2*) which open into the biliary duct at various levels. Injection of India ink (*3*) into the main proximal (or dorsal) duct, which drains into the biliary duct closest to the liver, results in the blackening of at least two thirds of the gland including the tail, body, and upper part of the head (Fig. 1A). Injecting the main distal (or ventral) exocrine duct, which opens into the hepatic duct closest to the duodenum, stains the remaining third of the pancreas (the lower part of the head) (Fig. 1C). Figure 1B illustrates diagrammatically the paths of these ducts.

A rat pancreas was fixed by perfusion with Bouin's fluid, and a piece (1 by 0.5 by 0.3 cm) was removed from the region drained by the dorsal duct, dehydrated, and embedded in paraffin. Three hundred serial sections, each 3 μm thick, were cut from the block; one of every 25 was stained with hemalum-eosin to ascertain the presence of well-preserved islets of Langerhans (the islets in this region are referred to as dorsal islets). The sampled areas yielded several islets completely or incompletely cut by the serial sectioning. From these islets, one was selected for reconstruction on the basis of completeness and quality. Successive groups of four consecutive sections in this series (77 sections were needed to include the entire selected islet) were stained by the immunofluorescence technique of Coons *et al.* (*4*), with specific antiserums to insulin, glucagon, somatostatin, and pancreatic polypeptide (*5*) being used. Color photographs from each immunofluorescent stained section were copied to scale onto Lucite sheets (Fig. 2, A to D) (*6*). The numbers of each endocrine cell type on each sheet were counted. Of 3126 cells counted, 2063 (66 percent) contained insulin, 874 (28 percent) contained glucagon, 123 (4 percent) contained somatostatin, and 66 (2 percent) contained pancreatic polypeptide. Nine islets stained in the same sections showed qualitatively the same distribution.

A similar reconstruction procedure was applied to one of the 16 ventral islets present in serial sections of a block taken from the lower part of the pancreatic head (Fig. 2, E to H), which is drained by the main ventral exocrine duct. In 68 serial sections evaluated, 4136 cells were counted, of which 3061 (74 percent) contained insulin, 818 (20 percent) contained pancreatic polypeptide, 190 (4 percent) contained somatostatin, and 67 (< 2 percent) contained glucagon (*7*).

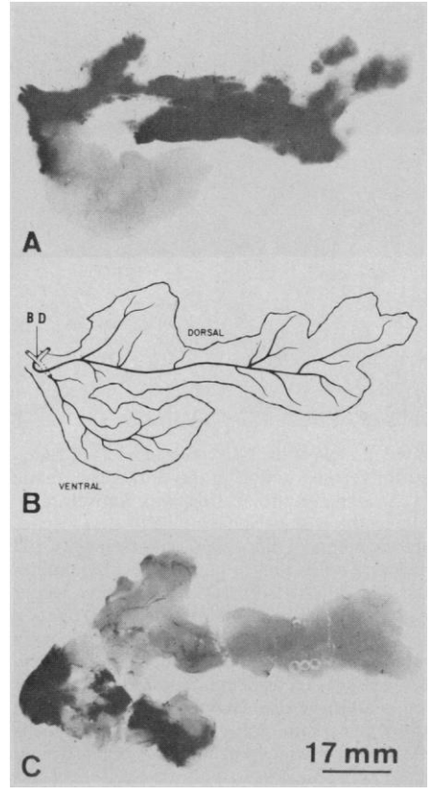

Fig. 1. (A) Thick longitudinal section in paraffin of an entire rat pancreas following injection of India ink into the main dorsal exocrine duct. Approximately two-thirds of the gland is stained, including the tail (right), body, and superior part of the head (left). The remaining third, the inferior part of the head, is not stained. The stained region yielded islets of Langerhans rich in glucagon-containing cells and poor in pancreatic polypeptide-containing cells (see Fig. 2, A to D) (×1.3). (B) Diagram of a pancreas showing pancreatic ducts injected with a mixture of India ink and latex (*3*). The main dorsal duct draining the part of the pancreas stained in (A) opens into the biliary tract at the point where the two hepatic ducts merge to form a single biliary duct (*BD*). The main ventral duct draining the lower part of the pancreatic head [see (C)] opens into the biliary duct below the entry of the main dorsal duct and close to the duodenum (not shown). (C) Thick longitudinal section in paraffin of an entire rat pancreas following injection of India ink into the main ventral exocrine duct. Only the lower part of the pancreatic head is stained. This part of the pancreas yielded islets of Langerhans rich in pancreatic polypeptide-containing cells and poor in glucagon-containing cells (see Fig. 2, E to H) (×1.3).

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The nonhomogeneous distribution of endocrine cell populations in islets from different pancreatic regions was further ascertained by performing a morphometric analysis (*8*) of the four endocrine cell types in 40 dorsal islets (glucagon-rich, pancreatic polypeptide–poor) and in 40 ventral islets (pancreatic polypeptide–rich, glucagon-poor) from two rats. The results of this evaluation confirm the data obtained by counting the four cell types individually in each reconstructed islet (*9*).

The pancreatic regions that provided the two different types of islets have been shown to be irrigated by different arterial systems (coeliac artery for the dorsal region; superior mesenteric artery for the ventral region) (*10*). The difference between the endocrine cell populations of the islets of the inferior region of the pancreatic head and those of the remainder of the pancreas can be explained from a developmental perspective. In mammals, the adult pancreas is formed by the fusion of two primordia, the dorsal and ventral pancreatic buds. The buds themselves are evaginations of the primitive gut; in the rat, the dorsal primordium appears at the 20-somite stage (11 days), whereas the ventral primordium develops a little later at the 28- to 30-somite stage (11½ days) (*11*). According to a review of pancreatic development (*12*), "the two growing pancreatic glands merge at about day 16–17 and are thereafter indistinguishable. In the fully developed organ each pancreas keeps its own duct. . . . In addition, in the rat, many accessory pancreatic ducts empty directly into the biliary duct. . . ."

By cannulating independently the two main ducts of the rat pancreas, we filled with India ink two well-defined pancreatic regions, each containing islets of Langerhans with a different endocrine cell composition. From a developmental perspective, the pancreatic polypeptide–rich, glucagon-poor islet would be characteristic of the ventral pancreatic primordium, while the glucagon-rich, pancreatic polypeptide–poor islet would be characteristic of the dorsal primordium. The reason for this difference is not understood; its existence, however, contradicts previous views that the islets have a similar cell composition throughout the pancreas.

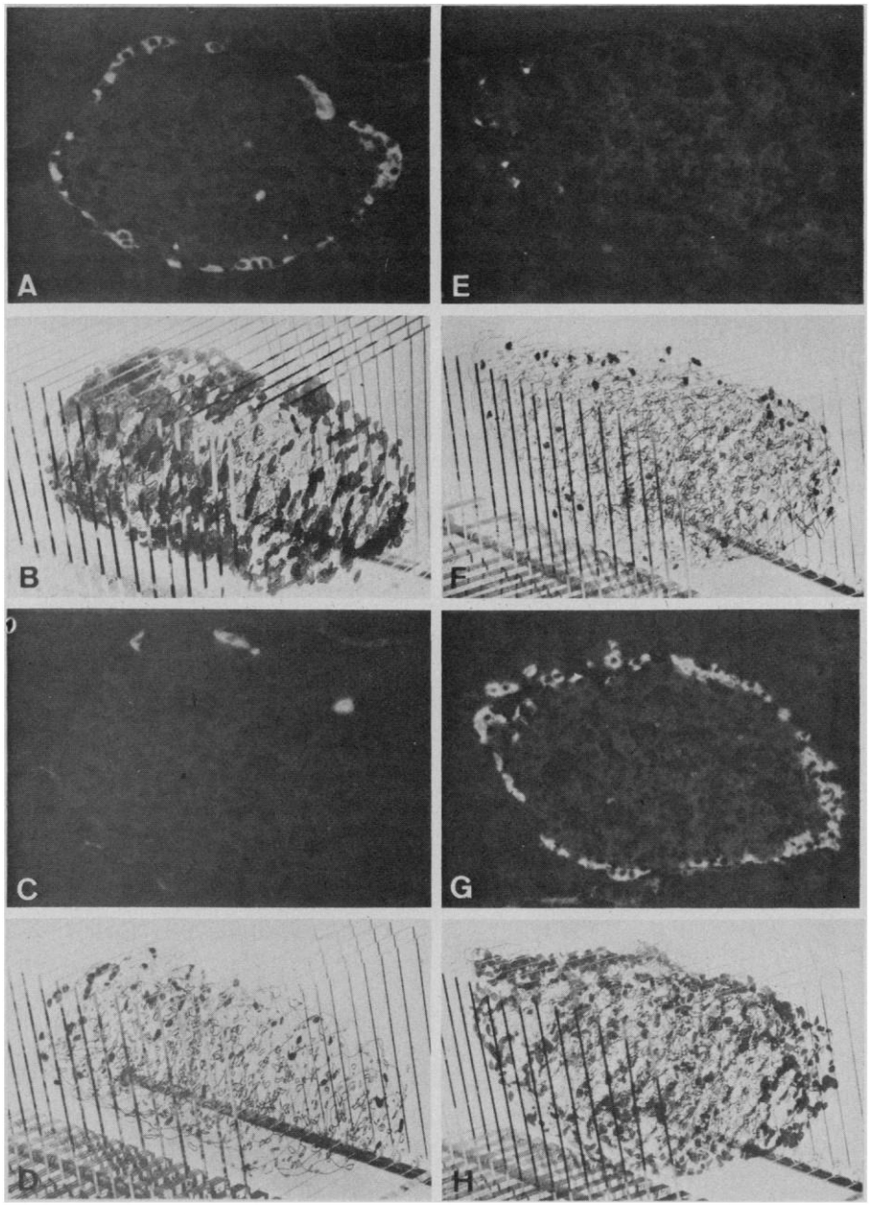

Fig. 2. Immunofluorescent sections and reconstructed models of the two types of islets of Langerhans in the rat. (A) Section of the islet selected for reconstruction in the dorsal pancreatic region drained by the main dorsal exocrine duct. Staining of the section with antiserum to glucagon reveals a rim of glucagon-containing cells at the islet periphery (×270). (B) Lucite-sheet reconstruction of the dorsal islet showing large numbers of glucagon-containing cells. (C) Section successive to the one shown in (A), stained with antiserum to pancreatic polypeptide. Note the scarcity of pancreatic polypeptide–containing cells (×270). (D) Lucite-sheet reconstruction of the dorsal islet showing scarce pancreatic polypeptide–containing cells. (E) Section of the islet selected for reconstruction in the lower part of the ventral head drained by the main ventral exocrine duct. Staining of the section with antiserum to glucagon demonstrates the scarcity of glucagon-containing cells (×220). (F) Lucite-sheet reconstruction of the ventral islet showing scarce glucagon-containing cells. (G) Section after the one shown in (E), stained with antiserum to pancreatic polypeptide. Note the rim of pancreatic polypeptide–containing cells (×220). (H) Lucite-sheet reconstruction of the ventral islet showing large numbers of pancreatic polypeptide–containing cells.

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3. India ink was injected with a syringe connected to a polyethylene catheter inserted in the biliary duct. To inject ink into the dorsal pancreatic duct, the catheter was secured by a ligature at the point where the two hepatic ducts merge to form the single biliary duct, and a second ligature closed the biliary duct distal to the opening of the dorsal pancreatic duct. To inject ink into the ventral pancreatic duct, the catheter was pushed distally to the opening of the dorsal duct,

secured by a ligature, and a second ligature closed the biliary duct just before its opening into the duodenum. Other experiments were conducted in which injections were made into the ducts only (not their respective regions of drainage) with a mixture of India ink and latex (50 : 50 by volume). Following the injections, the mixture was hardened with acetone; the resulting casts were revealed by digesting the exocrine tissue surrounding the ducts with concentrated HCl.

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6. After immunofluorescent staining with the specific antiserums, each section of the islet was photographed on Kodak color slide film at a final magnification of ×80 in a Zeiss photomicroscope (Zeiss, Inc., Ober Kochen, West Germany) equipped for fluorescence. Each color slide was projected onto a 245 by 330 by 4 mm Lucite sheet at a final magnification of ×1200. A colored pen was used to transfer the fluorescent image of each cell to the Lucite sheet. The same color was used to represent cells stained by a particular antiserum. In the reconstructed islet, every fourth Lucite sheet represented the staining by the same antiserum.
7. A morphometric analysis (*8*) of human pancreases obtained from autopsies and stained by the immunofluorescent techniques used for the rat pancreases has revealed similar heterogeneity among the populations of endocrine cells depending on their location within the gland. Of the endocrine cell volume in sections of the posterior part of the pancreatic head in normal adults, about 76 percent contained pancreatic polypeptide, 21 percent contained insulin, 2 percent contained somatostatin, and 1 percent contained glucagon. However, of cells in the body of the pancreas, about 85 percent contained insulin, 11 percent contained glucagon, 3 percent contained somatostatin, and only 1 percent contained pancreatic polypeptide. Moreover, the pancreatic polypeptide-rich region in the head of the pancreas was separated from the remainder of the gland by a plane of connective tissue [L. Orci, F. Malaisse-Lagae, D. Baetens, A. Perrelet, *Lancet* **1978-II**, 1200 (1978); F. Malaisse-Lagae, L. Orci, A. Perrelet, *N. Engl. J. Med.* **300**, 436 (1979); F. Malaisse-Lagae, Y. Stefan, J. Cox, A. Perrelet, L. Orci, *Diabetologia*, in press]. Independent evidence for a partition of the pancreas in pancreatic polypeptide-rich, glucagon-poor and pancreatic polypeptide-poor, glucagon-rich regions in human and canine pancreases was recently described by using radioimmunoassay to assess the hormone concentrations and immunoperoxidase to detect the respective endocrine cell populations [D. J. Gersell, R. L. Gingerich, M. H. Greider, *Diabetes* **28**, 11 (1979)]. These data correlate well wth ours, except for a few human cases in which high concentrations of pancreatic polypeptide were not accompanied by numerous pancreatic polypeptide-containing cells. This discrepancy is explained by the fact that in the human pancreas, the pancreatic polypeptide-rich islets are often contained only in the posterior part of the pancreatic head rather than in the entire head. If the anterior part of the head is radioimmunoassayed for hormonal concentrations and the posterior part for immunoperoxidase staining (or the reverse), then the discrepancy may arise.
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9. Series of four successive serial sections were obtained in dorsal and ventral regions of the pancreas of two male rats (250 g), stained sequentially with the four antiserums, and evaluated morphometrically as described in (*8*). Values pooled from 20 islets studied in each pancreatic region of each rat, expressed as volume density and percentage of each cell type, are:

Hormone	Volume density (by volume)	Percentage
Dorsal islets		
Insulin	0.618	82.5
Glucagon	0.111	15.0
Somatostatin	0.015	2.0
Pancreatic polypeptide	0.004	0.5
Ventral islets		
Insulin	0.600	82.0
Glucagon	0.010	1.3
Somatostatin	0.018	2.4
Pancreatic polypeptide	0.106	14.3

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13. Supported by Swiss National Science Foundation grant 3.120.77 and U.S. National Institutes of Health grant N01-AM-7-2213. We thank P. H. Wright, R. H. Unger, R. E. Chance, R. A. Donald, and S. Ito for providing the antiserums, and I. Bernard, I. Fuglister, A. M. Lucini, and M. Sidler-Ansermet for expert technical assistance.

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Sex Discrimination in *Gerris remigis*: Role of a Surface Wave Signal

Abstract. *Even when blinded with masks, adult male water striders* (Gerris remigis) *accurately ascertain the sex of other adult water striders in the laboratory. Freely moving females that were artificially made to play back computer-generated male surface wave and body-contact signals of about 90 waves per second were treated as males by the masked males and as females when no such playbacks were made. Thus, the males can use presence or absence of the male signal as the sole means for sex discrimination.*

Gerris remigis is a large riverine water strider (body length, 12 to 14 mm)—the most widely distributed North American species of the insect family Gerridae (*1*). Adults and nymphs produce intraspecific surface wave signals during spacing and mating behavior by vertical oscillations of their legs in a fashion similar to that used by adults of the Old World species *Rhagadotarsus kraepelini* (*2*). *Gerris remigis* males (third-instar nymph through adult stage) can produce high-frequency (HF) surface wave signals of 80 to 90 waves per second (Fig. 1) and low-frequency signals of 3 to 10 waves per second. Females (third-instar nymph through adult stage) produce only low-frequency signals. Previously, I demonstrated that, in *R. kraepelini*, signals from males attract receptive females and may induce oviposition (*2*). I now report experiments in which an unusual playback technique is used to show that adult *G. remigis* males can discriminate adult sex solely by whether or not other adults produce HF signals.

During many field trips in Kansas and New York from 1975 to 1978, I saw *G. remigis* males in their mating season repeatedly approach to within a few centimeters of other adults and apparently test each adult by producing HF surface wave signals or by grasping them. If a male was grasped, the two males exchanged HF signals while in contact, and then the grasping male disengaged and moved away. Males in close proximity (a few centimeters) generally exchanged HF surface wave signals, whereupon the approaching male would either grasp the other or move away. If the approaching male encountered a female, however, he usually attempted to copulate after testing her with an HF surface wave signal or grasping her (attempted copulations were easily distinguished from male-male encounters). Males also tested adults by approaching closely and remaining briefly without signaling. If the approached individual did not produce an HF signal, the approacher would usually attempt copulation. When approached, males generally maintained their position and, if grasped, raised their bodies and signaled immediately. However, females often moved away when approached and, if grasped, reacted with anything from passive acceptance of copulation to violent attempts to dislodge the male.

From these observations I hypothesized that males can use presence or absence of the HF signal to discriminate adult sex. In trial series 1 and 2, I tested this hypothesis by determining whether sighted and masked males discriminate sex in the laboratory during their mating season. Trial series 3 was designed to ascertain whether males can use only presence or absence of the HF signal to discriminate sex.

In trial series 1 and 2, three males were placed in an aquarium (surface area, 18 by 30 cm) (*3*) and were then required to discriminate between visitor males and females. Each visitor was introduced in random order with respect to sex at in-

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