regulated to avoid over-filling of the pulmonary veins which necessarily accumulate a substantial volume of blood during the ventilatory period. Following submergence, $\dot{Q}_s$ is then expected to draw largely from the pulmonary venous reservoir of oxygenated blood, and $\dot{Q}_p$ increases only intermittently as the venous volume is reduced. The phasic pattern of $\dot{Q}_p$ evident during submergence conceivably "meters out" the lung oxygen store, as demonstrated recently in a turtle (*8*), while allowing the pulmonary venous volume to be reduced to preventilatory levels. Alternatively, patterns of $\dot{Q}_p$ during diving might be related to CO_2 exchange or chemosensory monitoring of circulating blood (*12*). Clearly, the ability to adjust the parallel perfusion of pulmonary and systemic tissues—a situation unattainable by birds and mammals—has been favored by natural selection almost universally during evolution of the lower tetrapods (*13*). The dramatic expression of this capability in *Acrochordus* may provide a novel system for studies of pulmonary blood flow regulation, as well as the performance of cardiovascular shunts.

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2. Snakes were collected from mangrove swamps in the central Philippines and returned to the University of Florida where they were maintained in a seawater tank at 27°C. Cardiovascular measurements were recorded from snakes prepared with indwelling catheters or blood flow transducers, or both, and allowed to dive freely within a 36 by 45 cm plastic tub with a substrate of shell fragments and 12- to 15-cm depth of seawater. A piece of gray plastic pipe (4 cm in diameter and 23 cm long) was provided to simulate burrows that snakes naturally occupy in swamps. Surgical procedures were conducted while the snake was cooled to below 5°C on chipped ice; analgesic injections of 2% lidocaine were given locally at sites of incisions. Each snake was allowed 48 to 72 hours of postoperative recovery during which it became accustomed to anchoring itself on the plastic pipe and hiding within it. Blood pressures were measured from indwelling, saline-filled polyethylene catheters (0.58 or 0.28 mm internal diameter) tied into the posterior segment of dorsal aorta or the posterior segment of the pulmonary artery. For measurements of blood flow, miniaturized Doppler blood flow cuffs (Valpey-Fisher; 2 to 2.5 mm internal diameter) were fitted around the proximal anterior or posterior pulmonary artery, the proximal left carotid artery, the left aortic arch, or the proximal dorsal aorta. Catheters and flow cuffs were installed in various combinations in 12 different snakes (weighing 51 to 126 g); it was impractical to monitor more than three or four vessels successfully in any individual. All leads were secured externally with suture and cyanoacrylate cement. Blood pressures were monitored with Gould P23 ID transducers, and blood flow signals were conditioned with a Directional Pulsed Doppler Flowmeter (University of Iowa Bioengineering). Data were recorded on a Grass model 7D polygraph during daylight periods of 6 to 8 hours on each of between two and four successive days. The data reported are from quiescent, undisturbed animals in water maintained at 27° to 28°C.
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6. Peak blood flow measured in the pulmonary artery during 20 breathing episodes ranged from 15 to 57.5 ml/min·kg (mean ± SD, 35.6 ± 13.3) in three animals in which $\dot{Q}_p$ was quantified reliably throughout diving. During 46 dives recorded in seven snakes, the mean percentage reduction of pulmonary blood flow from peak flow during breathing to minimum flow during diving was 94.0 ± 7.8% (range 60 to 100%), with flow decreasing to zero in 18 cases.
7. In six dive cycles monitored in a single snake, pulmonary vascular resistance varied from 6.77×10^5 to 6.46×10^6 Pa·s·cm^{-3} while diving and from 4.36×10^4 to 5.92×10^4 Pa·s·cm^{-3} during lung ventilation. Calculations disregard the effects of pulsatile flow, fluid inertia, and vessel compliance and were not possible in the many instances where pulmonary blood flow was reduced to zero during diving. It is clear, however, that vascular resistance varies by orders of magnitude between submergence and lung ventilation.
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10. All of the blocking drugs (Sigma and Burroughs-Wellcome) were effective at doses of 1 to 2 mg per kilogram of body mass, injected in 100-μg boluses by an arterial catheter.
11. Five snakes were treated with heparin (2000 U/kg) for 30 min, anesthetized with pentobarbital, and the heart, central lung, and associated tissues exposed by a ventral midline incision. The pulmonary artery was cannulated through the pulmonary outflow tract of the truncus arteriosus, with the cannula extending to the end of the truncus. Left and right vagi were exposed 6 to 10 cm anterior of the heart and harnessed with cotton threads, then passed through platinum electrodes. All tissues were left in situ and kept moist with physiological saline. Pulmonary vasculature was perfused with Mackenzies salt solution bubbled with 95% O_2 to 5% CO_2. The preparation was perfused at a constant pressure of 20 mmHg, which approximates the in vivo pulmonary pressure of this species. Input pressure was monitored from a T-junction close to the cannula using a Gould P23 ID pressure transducer recording on a Grass model 7C polygraph. The perfusion flow rate was measured at a drop chamber in the inflow line using a Grass PTTL photoelectric pulse transducer monitored with a 7P4F tachograph and recorded on the polygraph. The vagus nerves were stimulated with 1-ms pulses at 1 to 20 Hz for 30 or 60 s at 10-min intervals with a Grass S44 stimulator. Constant pressure perfusion of the pulmonary vasculature resulted in flow rates between 4 and 7 ml/min. Stimulation of the vagal nerves resulted in a marked vasoconstriction which was abolished by atropine (1 μ*M*), indicating that it was cholingergic in nature. In addition, a post-stimulus vasodilatation was observed which was abolished by bretylium or propranolol (each 1 μ*M*), indicating that it was adrenergic in nature. No nonadrenergic, noncholinergic responses to vagal nerve stimulation were observed following cholinergic and adrenergic blockade.
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Localization of the Pancreatic Beta Cell Glucose Transporter to Specific Plasma Membrane Domains

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Immunocytochemical techniques revealed that the "liver-type" glucose transporter is present in the insulin-producing beta cells of rat pancreatic islets but not in other islet endocrine cells. Ultrastructural analysis of the transporter by the protein A–gold technique showed that it is restricted to certain domains of the plasma membrane, its density being sixfold higher in microvilli facing adjacent endocrine cells than in the flat regions of the plasma membrane. These results support a possible role for this glucose transporter in glucose sensing by beta cells and provide evidence that these cells are polarized.

PANCREATIC β cells secrete insulin in response to an increase in blood glucose concentration, and both glucose uptake and metabolism by the β cell are required for signaling to occur (*1*). A member of the facilitated glucose transporter (GT) family has recently been characterized by molecular cloning (*2, 3*). This GT, pre-

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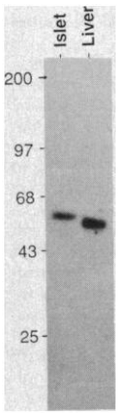

Fig. 1. Protein-blot analysis of the glucose transporter (GT) in 20 μg of membrane proteins prepared from liver or islets (*13*). The blots were probed with the antibody to the COOH-terminal peptide of the liver-type GT that was used for the immunocytochemical study. The position of the molecular mass standards is indicated on the left (values are in kilodaltons).

sent in liver, intestine, and kidney, is also expressed in pancreatic islets (*2*) and is thought to have a high Michaelis constant (K_m) (15 to 20 m*M*) for glucose compared to that of the erythroid GT (1 to 2 m*M*) (*4, 5*). It has been suggested that, because of its kinetic properties, the "liver-type" GT may be partly responsible for glucose sensing by β cells (*2*). We now report the localization of the liver-type GT in pancreatic islets and show that it appears to be present exclusively in insulin-secreting cells. Moreover, immunofluorescence and immunoelectron microscopic studies localize the GT at the surface of β cells and show that its distribution is not homogeneous.

For the immunocytochemical study, an antibody was raised against the COOH-terminal peptide (amino acids 513 to 522) of the liver GT as described (*2*), which was then affinity-purified. This antibody was used in a protein-blot analysis of membrane proteins isolated from liver and islets (Fig. 1). The antibody recognized a single protein of 53 kD in liver and predominantly a 55-kD protein in islets; the same results were obtained when an antibody raised against a peptide corresponding to amino acids 47 to 60 was used (*2*). With the anti–COOH-terminal antibody, a protein of lower molecular mass (~51 kD) in the islet membrane preparation was detected upon longer exposure of the film. The intensity of the minor component was ~5% of that of the major band as assessed by densitometric scanning of the autoradiogram, and its nature is unknown; this protein probably does not account for the immunocytochemical staining observed, because the same antibody applied to sections of liver generates strong immunofluorescence on the plasma membrane and recognizes a single band on protein blots (*6*).

Immunofluorescence detection of the liver-type GT and of insulin (or proinsulin), glucagon, somatostatin, and pancreatic polypeptide (Fig. 2, B to H) on consecutive semithin sections showed that the GT was present in the insulin-containing β cells but not in the three other types of endocrine cells. The distribution of the transporter on the plasma membrane of β cells appeared nonhomogeneous (Fig. 2A); at higher magnification (Fig. 2B), it was evident that some segments of the plasma membrane were strongly labeled, in particular those facing adjacent endocrine cells, but other segments were poorly labeled or not labeled at all.

Electron microscopic detection of the GT by the protein A–gold technique revealed that immunoreactivity was greatly concentrated on domains of the β cell plasma membrane that contain microvilli, whereas flat surfaces were only weakly labeled (Fig. 3). Quantitation of these data (Table 1) revealed the density of the transporter to be sixfold as great on microvilli as on the flat surfaces that face either the capillary or the spaces between endocrine cells. Microvilli face spaces adjacent to other endocrine cells (Fig. 3), and a probable consequence of this is that glucose uptake will preferentially proceed in these regions of the plasma membrane and not in membrane regions in close proximity to the capillary lumen.

The expression of the liver-type GT in β cells but not in other types of islet endocrine cells is consistent with the particular sensitivity of β cells to changes in the concentration of extracellular glucose. Glucose-stimulated insulin release displays a sigmoidal dose-response curve with a threshold concentration of 5 m*M* and a maximal effect at concentrations above 15 m*M* (*1*). Glucose transport across the liver plasma membrane has a K_m of between 15 to 20 m*M* (*4*), and because the cloned liver GT is localized to the plasma membrane of hepatocytes (*6*) it can be assumed that the K_m of transport

Table 1. Protein A–gold labeling of the glucose transporter (GT) of the β cell plasma membrane.

Membrane domain	GT density*	Number of gold particles	Membrane length (μm)
Flat membrane (facing the capillary)	0.36 ± 0.05	48	138
Flat membrane (facing the intercellular space)	0.38 ± 0.05	87	224
Microvilli	2.13 ± 0.14	839	379

*Densities are expressed as the number (mean ± SEM) of gold particles per unit length (1 μm) of membrane; 30 different β cells were evaluated. The density of labeling on the plasma membrane of other endocrine cells was 0.10 ± 0.02 (134 μm evaluated) and of exocrine acinar cells was 0.08 ± 0.02 (161 μm evaluated).

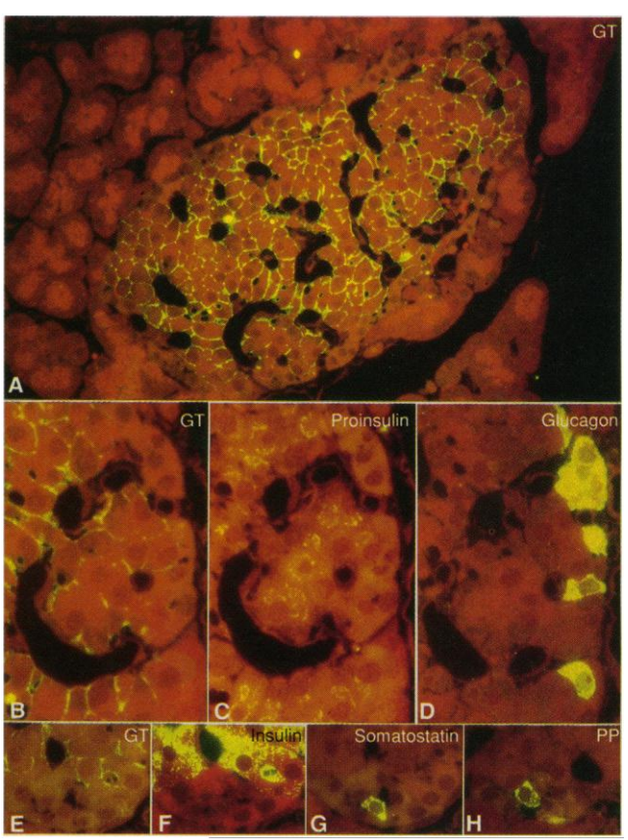

Fig. 2. Localization of the GT on semithin sections of pancreatic islets by immunofluorescence (*14*). (**A**) Islet labeled with the anti-GT antibody, showing the cell profiles outlined by a discontinuous line of immunofluorescence. (**B** through **H**) Serial semithin sections showing that the GT (B and E) is present on β cells identified by the presence of proinsulin (C) or insulin (F) but not on cells containing glucagon (D), somatostatin (G), or pancreatic polypeptide (PP) (H). Magnification: (A) ×240; (B through D) ×490; (E through H) ×380.

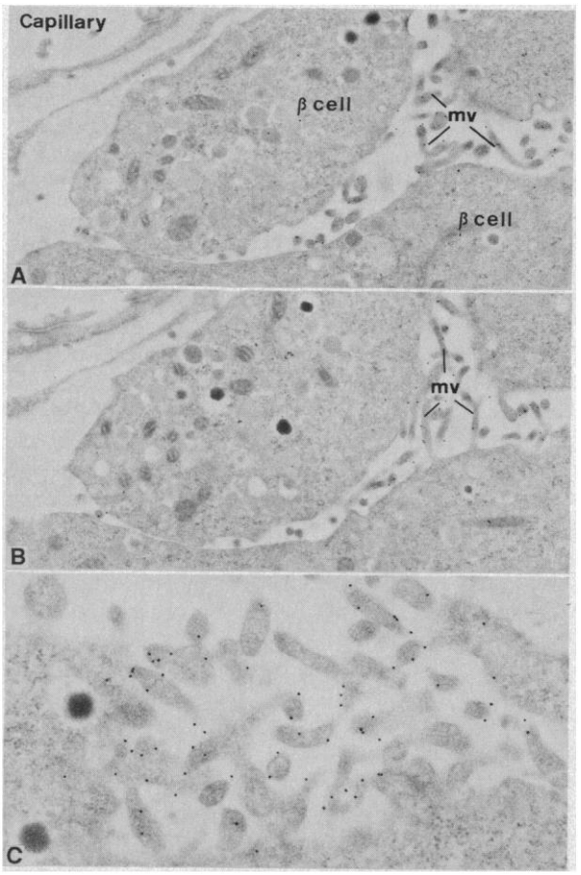

Fig. 3. Ultrastructural localization of the GT on β cells by the protein A–gold method (*15*). (**A** and **B**) Serial thin sections showing numerous gold particles labeling the microvilli (mv) projecting into the intercellular space. The labeling density on the various parts of the cell is shown in Table 1. (**C**) Higher magnification showing the association of the GT immunoreactive sites with microvilli. Magnification: (A and B) ×11,000; (C) ×21,000.

applies to this GT species. In support of this, we have shown that the liver GT is also present in the basolateral plasma membrane of absorptive cells of the small intestine (*6*). The K_m of glucose transport across this membrane is similar to that for liver (*5*). Moreover, several insulinoma cell lines have an abnormal sensitivity to glucose, with insulin secretion being maximal at 1 to 3 m*M* glucose (*7*). All these cell lines express large amounts of the GT found in erythrocytes and brain (K_m = 1 to 2 m*M*) and little or none of the liver-type GT (*2, 8*). Also, RINm5F insulinoma cells have a reduced rate of glucose equilibration across their plasma membranes as compared to normal β cells (*9*). These observations are thus consistent with the hypothesis that the β cell GT may be part of the glucose sensor (*2*).

Glucose sensing in pancreatic β cells has been attributed to one rate-limiting enzyme of glucose metabolism, a hexokinase, usually referred to as glucokinase, which has a K_m for glucose of 5 to 6 m*M* (*10*). For glucokinase to function as a glucose sensor, the concentration of intracellular glucose would need to change as the concentration of extracellular glucose changed from the normal concentration of 5 m*M*. This is possible if the GT exhibits a K_m for glucose of 15 to 20 m*M* but not if the K_m is 1 to 2 m*M*. The rate of glycolysis in the former case would thus be dependent on the relative velocities of both glucose transport and glucose phosphorylation. In this way, variations in glucose metabolism would reflect changes in blood glucose concentrations and eventually trigger insulin secretion (*1*).

Beta cells are not thought to be polarized, and, because of the lack of reliable surface markers, no specializations of any segments of their plasma membrane have been described. However, when placed in tissue-culture dishes, β cells sort budding vesicular stomatitis and influenza viruses to opposite regions of the cell (*11*), indicating that some polarization of the plasma membrane does exist. The preferential localization of the liver-type GT to the microvilli may thus define a domain of the β cell plasma membrane and suggests an interaction with the underlying cytoskeleton (*12*), possibly through binding of the GT to an ankyrin-like molecule.

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