Finnigan-MAT 4023 quadropole mass spectrometer. The capillary column was a fused silica 4m BP1 (0.5-μm film, SGE). The conditions of the GC-MS were as follows: injector temperature at 290°C, source at 150°C, electron multiplier at −2200 volts. Temperature program was set at 45°C for 1 minute ramping to 290°C at 10°C per minute.

13. The NMR spectra were collected on a Varian 360-MHz spectrometer ($CDCl_3$, TMS standard, δ=chemical shift in parts per million) 0.85, triplet (CH_3), 1.24, broad singlet (CH_2–CH_2), 1.54, triplet ($CH_2CH_2COCH_3$), 1.88, quartet (CH_2CH=CH), 2.10, singlet (CH_3C=O), 2.38, triplet (CH_2COCH_3), 5.32, triplet (CH=CH).
14. A hexane solution (25 μl) containing 500 ng of material was treated with 50 μl of dimethyl disulfide and 5 μl of 0.06% iodine in ethyl ether and stirred overnight at room temperature. The sample was diluted with 200 μl of hexane and 50 μl of an aqueous solution of sodium thiosulfate (5%) stopped the reaction. The sample was reduced under nitrogen and analyzed directly by GC-MS.
15. To 100 μl of pyridine and 10 mg of *O*-methylhydroxylamine hydrochloride were added 500 μl of the dimethyl disulfide derivative of the methyl ketones in ether. The mixture was heated at 100°C for 15 minutes and analyzed by GC-MS directly.
16. For example, the bis thiomethyl derivative of the compound with molecular weight = 476 yielded two major ions in electron impact–mass spectrometry (EI/MS) at masses 397 and 173; its methoxime yielded a parent ion at mass-to-charge ratio of 599.
17. Methyl ketones were synthesized by reacting 100 mg of octadocosanoic acid (0.235 m*M*) or mellissic acid (0.221 m*M*) in hexane with methyl lithium (1.4*M*) under nitrogen overnight. The reaction was quenched with H_2O, extracted in ether and dried over $MgSO_4$.
18. A. Vagvolgyi and M. Halpern, *Can. J. Zool.* **61**, 1171 (1983).
19. Hexane extracts (20 ml) of the skin lipids of males and females were presented in a sequential choice design. Female skin lipids were placed on a paper towel and the percentage of males that courted in 5 minutes noted. Male skin lipids were then added on top of the female's skin lipids. A final test of female skin lipids alone shows no lasting inhibition of courting males. Twenty males were used in each experiment.
20. R. T. Mason and D. Crews, *Nature* **316**, 59 (1985).
21. E. S. Albone, *Mammalian Semiochemistry: The Investigation of Chemical Signals Between Mammals* (Wiley, Chichester, 1984).
22. N. F. Hadley, *The Adaptive Role of Lipids in Biological Systems* (Wiley-Interscience, New York, 1985).
23. F. W. Forney and A. J. Markovetz, *J. Lipid Res.* **12**, 383 (1971).
24. M. S. Blum, *Chemical Defenses of Arthropods* (Academic Press, New York, 1981).
25. M. Gellért *et al.*, *Herba Hungarica* **24**, 53 (1985).
26. R. Nishida *et al.*, *Experientia* **30**, 978 (1974); T. Sato *et al.*, *Agr. Biol. Chem.* **40**, 391 (1976).
27. F. M. Schell and P. J. Weldon, *Agr. Biol. Chem.* **49**, 3597 (1985); D. G. Ahern and D. T. Downing, *Lipids* **9**, 8 (1973).
28. N. Nicolaides, H. C. Fu, G. R. Rice, *J. Invest. Derm.* **51**, 83 (1968).
29. N. Nicolaides, *J. Am. Oil Chem. Soc.* **42**, 691 (1965).
30. R. Mykytowycz and B. S. Goodrich, *J. Invest. Derm.* **62**, 124 (1974).
31. Supported by an NIMH predoctoral fellowship NIMH NRSA 09310 to R.T.M. and NIMH Research Scientist Award 00135, NICHHD 16687, and a Texas Higher Education Coordinating Board Advanced Research Program grant to D.C. The authors wish to thank M. Shoesmith and B. Koonz of the Manitoba Department of Natural Resources, Wildlife Branch, for permits and many efforts on our behalf. We thank E. Sokoloski for obtaining the NMR spectra; J. Bull assisted in the statistical analyses and critical reading of the manuscript; J. Whittier, M. Mendonca, A. Tousignant, R. Krohmer, S. Macmillan, S. Miller, T. Spande, L. Tsai, and Q.-L. Pu assisted in the field and in discussion of ideas.

28 December 1988; accepted 12 April 1989

Pulmonary Blood Flow Regulation in an Aquatic Snake

HARVEY B. LILLYWHITE AND JOHN A. DONALD

Regulation of pulmonary blood flow was studied during voluntary diving in the aquatic file snake, *Acrochordus granulatus*. Measurements of pressure and blood flow in pulmonary and systemic vessels indicate that blood flow completely bypasses the lung for significant periods during prolonged and quiescent submergence (greater than 30 minutes). When the lung is ventilated, pulmonary blood flow increases to 36 milliliters per minute per kilogram of body mass (measured in the anterior pulmonary artery), and the cardiac output largely bypasses the systemic circulation. These reciprocating patterns of preferential blood flow reflect inverse relations between flow and vascular resistance, with the result that systemic and pulmonary arterial pressures remain virtually constant throughout repetitive dive cycles. Neuropharmacological studies of freely diving snakes and isolated, perfused lung preparations show that pulmonary blood flow is regulated by an interplay of adrenergic vasodilatation and cholinergic vasoconstriction within the densely innervated lung vasculature. The patterns of blood circulation shown by diving *Acrochordus* reflect an unusual lability of intracardiac shunts.

In amphibians and non-crocodilian reptiles, a single ventricle allows redistribution of cardiac output between systemic and pulmonary vessels by way of central cardiovascular shunts. Such shunts are especially pronounced in aquatic species in which perfusion of the lung closely matches its ventilation (*1*). Although patterns of cardiovascular shunts are documented in several diverse species, knowledge of their controlling mechanisms is rudimentary. We investigated the regulation of pulmonary blood flow as it relates to diving behavior in the aquatic file snake, *Acrochordus granulatus* (*2*). We report that both sympathetic and parasympathetic components of the autonomic nervous system play interactive roles in regulating pulmonary blood flow in precise correspondence with ventilation and intracardiac shunts. Although the sympathetic (adrenergic) innervation of pulmonary vascular beds has been studied in mammals, little is known concerning a possible antagonistic interplay of parasympathetic and sympathetic nerves as occurs in the heart (*3*). Such activity in *Acrochordus* profoundly changes pulmonary perfusion and the magnitude of intracardiac shunts during repetitive dive cycles.

The species we studied is particularly interesting because of its adaptations to prolonged submergence in shallow, aquatic habitats. These include low metabolic rate, comparatively large volume and oxygen capacity of blood, large pulmonary oxygen stores, high-affinity *p*H-sensitive hemoglobin, and cutaneous gas exchange (*4*). File snakes possess sufficient oxygen stores to sustain aerobic dives for 1.5 to 2 hours (minimally), whereas some individuals can remain submerged for as long as 3 to 5 hours (*4*). As in all three species of the genus and family, typical breathing episodes consist of two to four breaths spaced over several minutes, during which lung gases reequilibrate with air, CO_2 stores are released, and the blood is saturated with oxygen (*5*).

The most prominent cardiovascular event associated with the ventilatory period is a large increase of pulmonary blood flow ($\dot{Q}_p$) attributable to tachycardia, a decrease in pulmonary vascular resistance and a left to right shunt of the ventricular outflow (Fig. 1). Ventilatory tachycardia typically entails four- to sevenfold increases of heart rate over submergence values (mean maximum rate ± SD = 25.7 ± 3.3 beats per minute; n = 47 dives in seven animals) (Fig. 1). However, the increase of $\dot{Q}_p$ during ventilation entails a net left to right cardiac shunt as well, for systemic blood flow ($\dot{Q}_s$) measured in the dorsal aorta, carotid artery, or either aortic arch does not mirror the increases of $\dot{Q}_p$ during ventilation. More usually, $\dot{Q}_p$ and $\dot{Q}_s$ change in a reciprocating manner, and changes of stroke flow in pulmonary and systemic vessels are inversely related during ventilatory episodes as well as during diving when the cardiac output shifts predominantly to the systemic circuit (Fig. 1). Characteristically, $\dot{Q}_p$ increases more than tenfold while $\dot{Q}_s$ approaches zero during ventilation (*6*). Thus, the major fraction of ventricular outflow is directed to the lung during ventilatory episodes.

Both systemic and pulmonary arterial pressures are relatively constant throughout dive cycles, except for occasional, small changes of pulmonary pressure which are coincident with ventilatory tachycardia and

Department of Zoology, University of Florida, Gainesville, FL 32611.

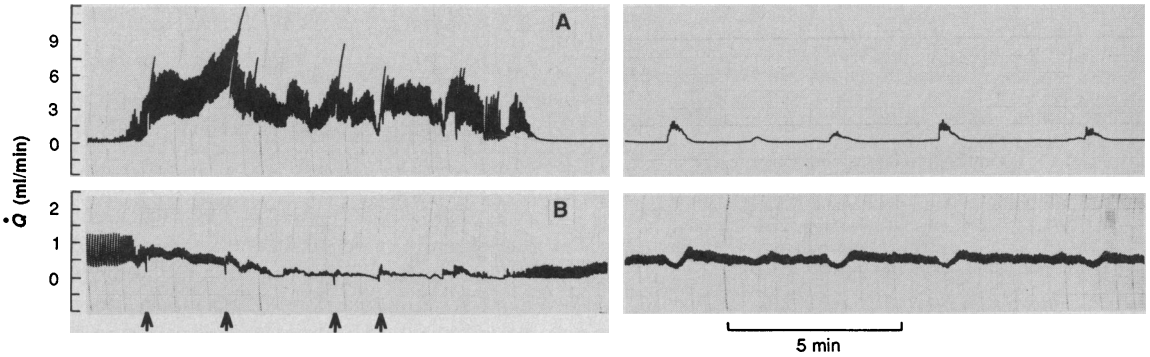

Fig. 1. Blood flow measured in (**A**) the anterior pulmonary artery and (**B**) dorsal aorta of an 80-g *Acrochordus granulatus* during breathing (breaths indicated by arrows) and diving (right half of records). Note that there is virtually complete pulmonary bypass of blood flow during submergence except for periodic, brief pulses of perfusion. The dramatic left-right shunt during breathing episodes and right-left shunt during diving are attributable, in part, to a regulated interplay of adrenergic vasodilatation and antagonistic cholinergic vasoconstriction within the pulmonary vasculature.

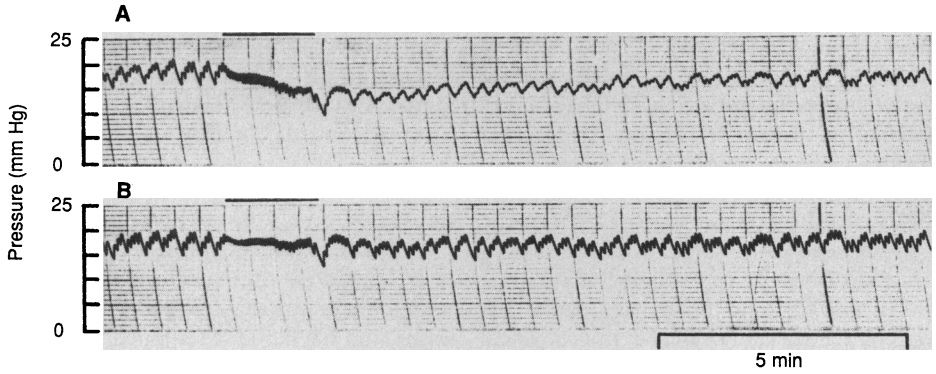

Fig. 2. Arterial pressures measured in (**A**) the distal extrinsic pulmonary artery and (**B**) the central dorsal aorta in a 96-g *Acrochordus granulatus*. The ventilatory period is indicated by the horizontal bar above each record in the left-hand portion of the panel. Mean pulmonary pressure falls about 28% during the ventilatory period, although this magnitude of change is unusual. Typically, pulmonary arterial pressure changes very little during the dive cycle, as illustrated for systemic pressure in (B).

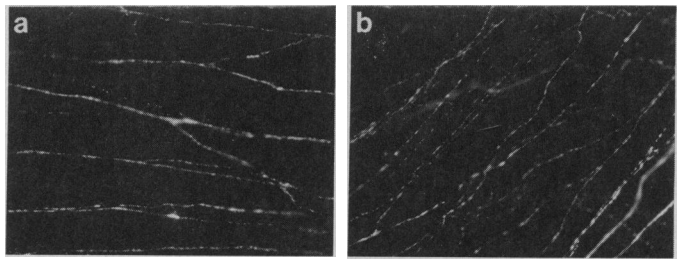

Fig. 3. Vasoactive intestinal polypeptide-like immunoreactive axons in (**a**) a small pulmonary artery within the lung parenchyma and (**b**) the anterior pulmonary vein (*12*) (×115).

the redistribution of blood flow during ventilation (Fig. 2). In view of the magnitude of $\dot{Q}_P$ during breathing, pulmonary vascular resistance decreases greatly and clearly reflects net vasodilatation in the lung (*7*). On the other hand, maintenance of pressure in the systemic circulation during breathing must be attributable to increased systemic resistance concomitant with the reduction of $\dot{Q}_S$.

During long (>30 min) dives, heart rate decreases and $\dot{Q}_P$ falls to very low levels that are coincident with increased flow in the systemic circuit. In quiescent animals there is complete pulmonary bypass during much of the dive, interrupted by brief 2- to 5-min pulses of $\dot{Q}_P$ spaced at variable intervals throughout submergence (*8*) (Fig. 1). There is always small but steady $\dot{Q}_P$ (<0.3 ml/min) for several minutes immediately preceding breathing and tachycardia.

Although large right to left shunts have been observed in some other diving reptiles (*9*), left to right shunts of comparable magnitude have not been noted. Because blood flow in major systemic vessels such as the dorsal aorta is nearly zero during periods of lung ventilation (Fig. 1), we conclude that there is nearly complete systemic bypass for relatively brief periods of lung ventilation, just as there is complete pulmonary bypass during diving. In these contexts, *Acrochordus* exhibits possibly the greatest functional lability of cardiac shunts that has been reported in any vertebrate.

Administration of atropine elevates $\dot{Q}_P$ and prevents its reduction during diving, whereas adrenergic blockade abrogates $\dot{Q}_P$ during ventilation (*10*). These findings suggest that pulmonary perfusion is regulated by antagonistic adrenergic and cholinergic innervation mediating vasodilatation and vasoconstriction, respectively. This conclusion was confirmed by in situ perfusions of isolated lung preparations: vasodilatory responses to nerve stimulation were adrenergic in nature, whereas vasoconstrictory responses were cholinergic (*11*). The bulk of the vasoconstriction appears to occur within the pulmonary microvasculature because normal levels of pulsatile pressures persist in the extrinsic pulmonary arteries of freely diving snakes, even while $\dot{Q}_P$ falls to zero. If, on the other hand, constriction were to occlude the proximal pulmonary outflow tract, pulmonary arterial pressures would be expected to equilibrate with pulmonary venous pressures.

The importance of autonomic regulation of $\dot{Q}_P$ is corroborated by neuroanatomical investigations (*12*) that demonstrate extensive perivascular plexuses of adrenergic and vasoactive intestinal polypeptide-like immunoreactive (VIP-LI) axons on arteries and veins along the length of the lung (Fig. 3). The density of VIP-LI axons in the pulmonary vasculature is exceptional, exceeding that in many pulmonary and systemic vessels of mammals. The VIP-LI immunoreactive material probably is localized in postganglionic parasympathetic neurons (*12*), but its role in neurogenic control of the pulmonary vasculature of *Acrochordus* is unclear because we found no evidence for nonadrenergic, noncholinergic neurotransmission to pulmonary vascular smooth muscle (*11*).

In reptiles that exhibit intermittent breathing, lung ventilation and perfusion remain closely matched in spite of large changes in breathing rate. Clearly, the left to right shunt and increased $\dot{Q}_P$ during lung ventilation in *Acrochordus* permit rapid oxygenation of the blood and reduce the breathing time at the water surface (*5*). The value of pulmonary bypass during diving is less clear. Current proposals that the condition saves cardiac energy or reduces plasma filtration in lungs are controversial (*13*). In the case of *Acrochordus*, reductions of $\dot{Q}_P$ may be

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.

REFERENCES AND NOTES

1. K. Johansen, in *Cardiovascular Shunts: Phylogenetic, Ontogenetic and Clinical Aspects*, K. Johansen and W. W. Burggren, Eds. (Alfred Benzon Symposium 21, Munksgaard, Copenhagen, 1985), pp. 17–32; W. W. Burggren, *ibid.*, pp. 121–136.
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.
3. J. R. McLean, in *Contemporary Issues in Pulmonary Disease, Abnormal Pulmonary Circulation*, E. H. Bergofsky, Ed. (Churchill, New York, 1986), vol. 4, pp. 27–81.
4. H. Heatwole and R. S. Seymour, *Comp. Biochem. Physiol.* **51A**, 399 (1975); R. S. Seymour and M. E. D. Webster, *J. Exp. Zool.* **191**, 169 (1975); M. E. Feder, *Physiol. Zool.* **53**, 394 (1980); H. B. Lillywhite and A. W. Smits, *Physiologist* **28**, 283 (1985); unpublished data; H. B. Lillywhite and J. A. Donald, *Physiologist* **30**, 190 (1987); ———, M. Wheatly, unpublished observations.
5. M. Glass and K. Johansen, *Physiol. Zool.* **49**, 328 (1976); R. S. Seymour *et al.*, *J. Comp. Physiol.* **144**, 215 (1981). Despite cutaneous losses of CO_2, blood CO_2 tension increases during diving because of the long submergence times.
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.
8. Similar blood flow patterns were reported in a small percentage of turtles investigated by W. Burggren *et al.* (*Physiol. Zool.*, in press).
9. F. N. White and G. Ross, *Am. J. Physiol.* **211**, 15 (1966); F. N. White, *Copeia* **1969**, 567 (1969); R. S. Seymour, *Physiol. Zool.* **51**, 388 (1978).
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.
12. J. A. Donald and H. B. Lillywhite, *Cell Tissue Res.* **255**, 585 (1989); *ibid.* **256**, 113 (1989).
13. W. W. Burggren, *Am. Zool.* **27**, 5 (1987).

14. We thank W. Burggren and M. Wheatly for comments on the manuscript. The care and experimental use of animals were with approval of, and within, institutional guidelines. Supported by NIH grant HL 33821.

13 February 1989; accepted 17 May 1989

Localization of the Pancreatic Beta Cell Glucose Transporter to Specific Plasma Membrane Domains

LELIO ORCI, BERNARD THORENS,* MARIELLA RAVAZZOLA, HARVEY F. LODISH

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-

L. Orci and M. Ravazzola, Institute of Histology and Embryology, University of Geneva Medical School, 1221 Geneva 4, Switzerland.
B. Thorens, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142.
H. F. Lodish, Whitehead Institute for Biomedical Research, Nine Cambridge Center, Cambridge, MA 02142, and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02139.

*To whom correspondence should be addressed.