Insulin secretion from beta cells in the islets of Lang-erhans can be stimulated by a number of metabolic fuels, including glucose and glycerol, and is thought to be mediated by metabolism of the secretagogues and an attendant increase in the ATP:ADP ratio. Curiously, glycerol fails to stimulate insulin secretion, even though it has been reported that islets contain abundant gly- cerol kinase activity and oxidize glycerol efficiently. We have reinvestigated this point and find that rat islets and the well differentiatied insulinoma cell line INS-1 contain negligible glycerol kinase activity. A recombin-ant adenovirus containing the bacterial glycerol ki- nase gene (AdCMV-GlpK) was constructed and used to express the enzyme in islets and INS-1 cells, resulting in insulin secretion in response to glycerol. In AdCMV- GlpK-treated INS-1 cells a greater proportion of glycerol is converted to lactate and a lesser proportion is oxi- dized compared with glucose. The two fuels are equally potent as insulin secretagogues, despite the fact that oxidation of glycerol at its maximally effective dose (2–5 mM) occurs at a rate that is similar to the rate of glucose oxidation at its basal, nonstimulatory concentration (3 mM). We also investigated the possibility that glycerol may signal via the glycolytic ATP:ADP ratio, and that “glycolytic” ATP more effectively regulates KATP channels than “mitochondrial” ATP is a concept originally introduced from studies in isolated myocytes (6). However, since other recent studies have provided data that seem to affirm the importance of mitochondrial ATP production by allowing membrane lipid turnover during granule exocytosis, or by gen-eration of bioactive byproducts such as diacylglycerol or inositol 1,4,5-trisphosphate. Second, because of the very high activities of mitochondrial glycerol phosphate dehydrogenase and fructose-1,6-bisphosphatase in islets, it has been suggested that transfer of reducing equiva-lents from the cytosol to the mitochondria may occur with high efficiency in such cells, and that FADH produced as a by-product of the mitochondrial glycerol phosphate dehydrogenase reaction can serve a signaling function by entering site II of the electron transport chain, resulting in generation of ATP (11–13).

According to the fuel hypothesis of insulin secretion, release of the hormone is stimulated only by metabolic fuels that are actively metabolized in islet beta cells. In support of this notion, glucose and glycerol are the most effective stimulators of insulin secretion in proportion to their rates of metabolism (1). A working model of stimulus-secretion coupling in the beta cell is that fuel metab-olism leads to an increase in the ATP:ADP ratio, which causes closure of ATP-sensitive K⁺ channels (KATP) and activation of voltage-gated Ca²⁺ channels, influx of extracellular Ca²⁺, and activation of the exocytotic machinery (reviewed in Refs. 1 and 2). Early studies in which mitochondrial uncouplers were found to block glucose-stimulated insulin secretion led to the assumption that fuel oxidation and mitochondrial ATP production were key events in insulin release (reviewed in Refs. 1–3). In the context of this model, the poor insulinotropic effect of pyruvate, despite its efficient oxidation in islets, has never been adequately explained. Subsequently, several lines of in-vestigation have suggested that ATP production from glycogenolysis constitutes the critical signal for KATP channel closure and initiation of insulin secretion, while ATP produced via mito-chondrial metabolism plays a secondary role of maintaining sufficient energy to drive secretory granule exocytosis (4, 5). That “glycolytic” ATP more effectively regulates KATP channels than “mitochondrial” ATP is a concept originally introduced from studies in isolated myocytes (6). However, since other recent studies have provided data that seem to affirm the importance of mitochondrial ATP in beta cell stimulus-secretion coupling (7), the matter remains unresolved.

In addition to fuel signaling via ATP produced by glycolysis or fuel oxidation, two other pathways for glucose-stimulated insulin secretion have been considered. First, a link between glucose and fatty acid metabolism has been implicated as a critical component of the glucose sensing response (2, 8–10). Glucose has been shown to increase the level of malonyl-CoA, which causes inhibition of carnitine palmitoyltransferase I and diversion of long-chain acyl-CoAs away from oxidation and into esterification (8–10). Phospholipids derived in this way could participate in stimulation of insulin secretion by allowing membrane lipid turnover during granule exocytosis, or by gen-eration of bioactive byproducts such as diacylglycerol or inositol 1,4,5-trisphosphate. Second, because of the very high activities of mitochondrial glycerol phosphate dehydrogenase in islets cells, it has been suggested that transfer of reducing equiva-lents from the cytosol to the mitochondria may occur with high efficiency in such cells, and that FADH produced as a by-product of the mitochondrial glycerol phosphate dehydrogenase reaction can serve a signaling function by entering site II of the electron transport chain, resulting in generation of ATP (11–13).
of insulin secretion that can be used to determine which of the four signaling pathways named above are actually operative in fuel-stimulated beta cells. Despite the potent effects of glyceraldehyde on insulin secretion, islets are unable to respond to another simple triose, glycerol. This is puzzling in light of another simple triose, glycerol. This is puzzling in light of

**MATERIALS AND METHODS**

**Construction of AdCMV-GlpK**—A plasmid pWT165 containing the gene encoding *Escherichia coli* glycerol kinase (glpK) was obtained from Dr. Donald Pettigrew, Texas A&M University (19). The intact glycerol kinase insert was amplified by polymerase chain reaction using oligonucleotides 5'-GAAGGTACCTTCATGACTGAAAAAAAATATATCG-3' and 5'-TGCAAGCTTTTATTCGTCGTGTTCTTCCCACGCCAT-3'. The oligonucleotides were used for amplification contained the 5' KpnI and 3' HindIII endonuclease sites, which allowed ligation into similarly digested pACCMV.pLpA (20). The new recombinant virus (AdCMV-glpK) was produced by homologous recombination of the PACCVM.pLpA plasmid containing the glycerol kinase insert with pJM17 (21) in cotransfected 293 cells and purified by CsCl density equilibrium ultracentrifugation as described previously (16).

**Culture and Viral Treatment of INS-1 Cells**—INS-1 rat islet cells were obtained from Drs. Claes Wollheim and Philippe Halban, University of Geneva (22). Cells were cultured in RPMI 1640 with 10% heat-inactivated fetal calf serum, 2 mM glutamine, 100 μg/ml penicillin, 100 μg/ml streptomycin, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol (22). Experiments were conducted by plating the cells into 12-well dishes and treating them at approximately 75% confluence with AdCMV-GlpK virus or, as a control, the AdCMV-βgal virus containing the bacterial β-galactosidase gene (23). Cells were exposed to one or the other of the recombinant viruses for 3 h at room temperature, after which 300–600 islets were transferred into the cup, and the cup was sealed within a 20-ml borosilicate glass scintillation vial by means of the seal vial. Vials were incubated at 37 °C for 2 h, after which 100 μl of HClO4 were added to the cells in the cup, followed by addition of 300 μl of methanolic hyamine hydroxide (1 N) to the bottom of the vials (both compounds were injected into the sealed vials through the seal vial). Following a further 2 h of incubation at 37 °C, vials were unsealed, cups were removed and discarded, and 10 ml of BioSafe II scintillation mixture were added. Vials were allowed to equilibrate overnight, prior to liquid scintillation counting.

**Glycerol Incorporation into Cellular Lipids**—Following a 1 h preincubation in culture medium containing 2 mM glucose with or without 10 μM Triacsin-C, cells were incubated in culture medium with added [2-14C]glycerol or [U-13C]glycerol for 3 h. The reaction was terminated by aspiration of the culture medium and addition of 20% methanol in phosphate-buffered saline. Cells were collected, washed with phosphate-buffered saline, resuspended in 250 μl of 0.2 N NaCl and snap-frozen in liquid nitrogen. 750 μl of chloroform:methanol (2:1) were added to the thawed cell suspension, and the mixture was vortexed and centrifuged for 5 min at 6,000 × g. The top (aqueous) fraction was transferred to new tubes, and the bottom (lipid) layer was washed once with methanol:chloroform:water (48:3:47) [2-14C]Glycerol incorporated into these fractions was quantified by liquid scintillation counting in 10 ml of BioSafe II scintillation mixture.

**Islet Perfusion**—Pancreatic islets were isolated from 150–200-g Wistar rats, placed in culture, and treated with the AdCMV-GlpK and AdCMV-βgal recombinant adenoviruses as described previously (17, 18). After exposure to virus, islets were cultured for an additional 48 h, after which 300–600 islets were transferred into Swinnex chambers and submerged in a 37 °C water bath. Islets were perfused at a flow rate of 0.8 ml/min, beginning with a 15-min equilibration with BioSBSS supplemented with 2 mM glucose, and followed by perfusion with HSBS supplemented with various secretagogues as indicated in the legend to Fig. 2. Fractions were collected at 1-min intervals, and 200 μl of each sample were used for insulin radioimmunoassay as described above.

**Statistical Analysis**—Two-tailed, two-sample Student's *t* test with pooled estimator of common variance was used for statistical comparison of experimental groups.

1. The abbreviations used are: HBBS, HEPES, bicarbonate balanced salt solution; m.o.1., multiplicity of infection.
The capacity of AdCMV-GlpK to direct expression of glycerol kinase in INS-1 cells, even at relatively low levels, is effective as the highest in conferring enhanced responsiveness to the combination of fuels. We conclude that expression of glycerol kinase in INS-1 cells, even at relatively low levels, confers glycerol-stimulated insulin secretion in normal islets as it does in INS-1 cells.

Glycerol-stimulated Insulin Secretion from AdCMV-GlpK-treated Rat Islets—To determine whether glycerol kinase expression confers glycerol-stimulated insulin secretion in normal islets as it does in INS-1 cells, pancreatic islets were isolated from normal Wistar rats and treated with AdCMV-GlpK or AdCMV-βGAL. Four days later, islet secretion was studied by perifusion (Fig. 2). Basal insulin secretion during perifusion with 1 mM glucose was the same in AdCMV-GlpK- and AdCMV-βGAL-treated islets. Addition of 5 mM glycerol to this basal perifusate resulted in an approximate 2-fold increase in insulin secretion over the base line from AdCMV-GlpK-treated islets (data not shown), as we have previously shown for other cell lines (29).

Results and Discussion

Adenovirus-mediated Expression of Glycerol Kinase in INS-1 Cells—The capacity of AdCMV-GlpK to direct expression of glycerol kinase mRNA in INS-1 cells was evaluated by RNA blot hybridization analysis and measurement of enzyme activity. A radiolabeled probe prepared from the GLUT-2 glucose transporter cDNA hybridized to a single 2.6-kilobase pair transcript in both AdCMV-βGAL- and AdCMV-GlpK-treated INS-1 cells. In contrast, a probe prepared from the bacterial glycerol kinase gene hybridized to a 2.2-kilobase pair transcript only in the AdCMV-GlpK-treated cells (data not shown). The level of glycerol kinase enzymatic activity was proportional to the multiplicity of infection (m.o.i.) of AdCMV-GlpK (Fig. 1A). Thus the lowest m.o.i. tested (2.5 plaque forming units/cell) caused an approximate doubling of glycerol kinase activity above the background level measured in AdCMV-βGAL-treated cells. Treatment of cells with higher viral titers caused a progressive increase in glycerol kinase activity, such that at an m.o.i. of 25 and staining of the background level measured in AdCMV-βGAL-treated cells. An m.o.i. of 25, falling in the middle of the range studied, was chosen for all subsequent experiments.

Glycerol-stimulated Insulin Secretion from AdCMV-GlpK-treated INS-1 Cells—Insulin secretion in response to the combination of 3 mM glucose and 5 mM glycerol was assayed in cells treated with AdCMV-GlpK or AdCMV-βGAL over the same range of m.o.i. values as studied for glycerol kinase activity. We chose to include 3 mM glucose because this concentration is itself not stimulatory for insulin secretion, but does provide a basal level of the hexose (22) (see also data of Fig. 3). All INS-1 cells treated with the control virus AdCMV-βGAL secreted the same amount of insulin (approximately 35 microunits/mg of protein/h) regardless of the m.o.i. used (Fig. 1B). Treatment of cells with AdCMV-GlpK increased insulin secretion by approximately 4-fold relative to AdCMV-βGAL-treated cells, to around 150 microunits/mg of protein/h. Interestingly, for the range of m.o.i. chosen, the lowest viral titer used was equally effective as the highest in conferring enhanced responsiveness to the combination of fuels. We conclude that expression of glycerol kinase in INS-1 cells, even at relatively low levels, confers a glycerol-stimulated insulin secretion response that is absent in control cells. An m.o.i. of 25, falling in the middle of the range studied, was chosen for all subsequent experiments.

Glycerol-stimulated Insulin Secretion from AdCMV-GlpK-treated Rat Islets—To determine whether glycerol kinase expression confers glycerol-stimulated insulin secretion in normal islets as it does in INS-1 cells, pancreatic islets were isolated from normal Wistar rats and treated with AdCMV-GlpK or AdCMV-βGAL. Four days later, islet secretion was studied by perifusion (Fig. 2). Basal insulin secretion during perifusion with 1 mM glucose was the same in AdCMV-GlpK- and AdCMV-βGAL-treated islets. Addition of 5 mM glycerol to this basal perifusate resulted in an approximate 2-fold increase in insulin secretion over the base line from AdCMV-GlpK-treated cells (data not shown), as we have previously shown for other cell lines (29).
Glycerol-stimulated Insulin Secretion in Islet Beta Cells

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**Insulin Secretion in Response to Glucose and Glycerol**—We next determined the glycerol concentration dependence of insulin secretion from AdCMV-GlpK-treated INS-1 cells. These studies were conducted in the presence and absence of 3 mM glucose and are summarized in Fig. 3. In the absence of glucose, 0.5 mM glycerol had no effect on insulin secretion, but 1 mM glycerol caused a 1.7-fold increase in insulin secretion relative to the base line level of 53 microunits/mg of protein/h, while 5 mM glycerol caused the maximal 3.6-fold enhancement (Fig. 3A). In other experiments that included glycerol concentrations between 1 and 5 mM, 2 mM glycerol was found to be sufficient to stimulate maximal insulin release (see Fig. 5). Addition of 3 mM glucose in the absence of glycerol had no effect on insulin secretion relative to cells incubated in the complete absence of substrates. However, inclusion of this nonstimulatory concentration of glucose caused a shift in the glycerol dose response, such that 0.5 mM glycerol now caused a 1.8-fold increase in secretion over the baseline of 67 microunits/mg of protein/h achieved by 3 mM glucose alone ($p < 0.001$). Furthermore, maximal insulin secretion, equivalent to that achieved with 5 or 10 mM glycerol alone, was achieved at 1 mM glycerol when 3 mM glucose was present (Fig. 3A). In parallel experiments, a maximal stimulatory effect of glucose on insulin secretion of approximately 4-fold above base line, similar to the maximal glycerol-stimulated response, was observed at concentrations of $\geq 10$ mM glucose (data not shown). A clear illustration of the left shift in the glycerol response curve that is elicited by the inclusion of a glucose concentration (3 mM) that is itself not stimulatory is provided by plotting the data as relative insulin secretion versus glycerol concentration (Fig. 3B). Thus, glucose potentiates insulin secretion at low glycerol levels, but mixing of the two substrates does not increase maximal insulin output beyond that achieved with high levels of glucose or glycerol alone.

**Comparison of Metabolic Fates of Glucose and Glycerol in AdCMV-GlpK-Treated Cells**—Expression of glycerol kinase in islet beta cells allows glycerol to function as a secretagogue with a potency similar to that of glucose (see Figs. 1–3). To learn more about the signal transduction pathways involved, we carried out a series of experiments in which we compared the metabolic fate of glucose and glycerol in these cells. As shown in Fig. 4A, at the doses that are maximally effective for stimulation of insulin secretion, more [U-14C]glucose is oxidized than [U-14C]glycerol in AdCMV-GlpK-treated INS-1 cells, expressing the data as total nanomoles of CO$_2$ produced/mg of cellular protein/h. Thus, glucose was oxidized to produce 75 nmol of CO$_2$/mg of protein/h at basal glycerol levels (3 mM) and 162 nmol of CO$_2$/mg of protein/h when glucose was raised to 10 mM (a further increase in glucose to 20 mM did not result in any further enhancement in glucose oxidation). In comparison, glycerol was oxidized at a rate of 44 nmol of CO$_2$/mg of protein/h at basal glycerol concentrations (0.5 mM), and at a rate of around 64 nmol of CO$_2$/mg of protein/h when glycerol was raised to a fully stimulatory concentration of 2–5 mM. Thus, glucose oxidation at 10 mM glucose was approximately 2.5 times that of glycerol oxidation at 5 mM glycerol, regardless of whether the data were expressed as nanomoles of CO$_2$ produced, as in Fig. 4A, or as triose equivalents oxidized (54 nmol of triose equivalents/mg of protein/h for 10 mM glucose versus 21 nmol of triose equivalents/mg of protein/h for 5 mM glycerol). Interestingly, the rate of glycerol oxidation at 2 or 5 mM glycerol (maximally stimulatory for insulin secretion) was approximately equal to the rate of glucose oxidation at 3 mM glucose (nonstimulatory for insulin secretion). Further, little change in the rate of glycerol oxidation was observed as glycerol was raised from a nonstimulatory concentration (0.5 mM) to a fully stimulatory level (5 mM). These data strongly suggest...
that fuel stimulation of insulin secretion is not strictly linked to a specific threshold of fuel oxidation.

The pattern for lactate production was opposite to that of oxidation (Fig. 4B). Thus, lactate output from AdCMV-GlpK-treated INS-1 cells incubated at basal glycerol (0.5 mM) was 223 nmol/mg of protein/h, while raising the glycerol concentration to 2–5 mM raised lactate output in these cells to 718 nmol/mg of protein/h. Note that more glycerol is converted to lactate than glucose, the inverse of the oxidation data. For both panels, data represent the mean ± S.E. of three independent groups of experiments, each performed in triplicate.

**Fig. 4. Glucose and glycerol metabolism in AdCMV-GlpK-treated INS-1 cells.** A, oxidation of varying concentrations of [U-13C]glycerol (left) and [U-13C]glucose (right) by AdCMV-GlpK-treated INS-1 cells. Data are expressed as total nanomoles of CO2 produced/mg of protein/h for each substrate. Note that CO2 production at concentrations of glycerol that are maximally stimulatory for insulin secretion (2–5 mM) are similar to the CO2 produced at a concentration of glucose that is nonstimulatory for insulin secretion (3 mM). B, lactate produced at varying concentrations of glycerol (left) and glucose (right) from the same AdCMV-GlpK-treated INS-1 cells studied in A. Note that more glycerol is converted to lactate than glucose, the inverse of the oxidation data. For both panels, data represent the mean ± S.E. of three independent experiments, each performed in triplicate.

Is Accumulation of Acyl-CoA and Its Esterification to Glycerol Phosphate Necessary for Glucose-stimulated Insulin Secretion?—It has been proposed that stimulation of insulin secretion by glucose is mediated in part by accumulation of long-chain acyl-CoAs, which in turn act directly on the exocytotic machinery or are esterified to form lipid by-products with known signaling properties such as diacylglycerol or inositol, 1,4,5-trisphosphate (8–10). A potential consequence of expansion of the glycerol phosphate pool in AdCMV-GlpK-treated beta cells could be to enhance such a lipid esterification-mediated signaling pathway. To test this idea, we evaluated the incorporation of [2-3H]glycerol into bulk lipids and measured glycerol-stimulated insulin secretion in AdCMV-GlpK-treated cells in the presence and absence of Triassicin-C, a potent inhibitor of long-chain acyl-CoA synthetase (32). In AdCMV-GlpK-treated cells, the incorporation of [2-3H]glycerol into the lipid

sated for by reoxidation of the surplus NADH in the lactate dehydrogenase reaction. Despite this diversion of pyruvate produced from glycerol away from mitochondrial oxidation and into lactate formation, glycerol retains full secretory potency. These results suggest that fuel-mediated insulin secretion is not dictated simply by the absolute rate of fuel oxidation, since rates of glycerol oxidation at maximally stimulatory concentrations of the fuel are equivalent to the rates of glucose oxidation observed at basal, nonstimulatory concentrations of the hexose. These data suggest that the common signal derived from glycerol and glucose metabolism is generated via enhanced flux through the glycerol shuttle or the distal portion of glycolysis and not from mitochondrial oxidation of pyruvate.

The FAD-linked mitochondrial glycerol phosphate dehydrogenase is one of several Ca2+-dependent mitochondrial dehydrogenases (12, 31). In an effort to estimate the contribution of the glycerol phosphate shuttle to overall glucose and glycerol utilization in AdCMV-GlpK-treated INS-1 cells, we studied the effect of removal of Ca2+ on glycerol and glucose metabolism. Cells were incubated either in the standard secretion buffer containing 2.5 mM Ca2+ or in that buffer with Ca2+ ommitted and with the addition of 2 mM EGTA. Removal of Ca2+ resulted in no significant change in the rate of glucose or glycerol oxidation (oxidation of 5 mM glycerol was 64 ± 7 and 56 ± 12 nmol of CO2/mg of protein/h and oxidation of 10 mM glucose was 161 ± 22 and 179 ± 39 nmol of CO2/mg of protein/h in the presence and absence of Ca2+, respectively). Removal of Ca2+ did, however, cause a 132% increase in lactate production in glycerol kinase expressing cells incubated with 5 mM glycerol (from 545 to 1263 nmol/mg of protein/h) and a 79% increase in lactate production from cells incubated with 10 mM glucose (from 314 nmol/mg of protein/h to 562 nmol/mg of protein/h). We suggest that this increase in lactate production reflects the carbon that would normally flow through the glycerol phosphate shuttle, but that is prevented from doing so in the absence of Ca2+ activation of the mitochondrial glycerol phosphate dehydrogenase. Unfortunately, most fuel-stimulated insulin secretion is dependent upon the presence of extracellular Ca2+, preventing us from linking these metabolic changes to the secretory response. Nevertheless, these experiments suggest that flux through the glycerol phosphate shuttle is actively occurring in INS-1 cells, particularly when glycerol is the substrate in AdCMV-GlpK-treated cells. Since entry of carbon into the lower half of glycolysis is also very efficient, it is not yet possible to distinguish the relative importance of the glycerol phosphate shuttle and the lower half of glycolysis for fuel-stimulated insulin secretion. These conclusions are similar to those made by Dukes and colleagues in a recent study employing pharmacologic agents for evaluation of insulin secretion from mouse islets (5).
phase was found to increase as a function of glycerol concentration, rising from a value of 3.2 ± 0.6 nmol/mg of protein/h at 0.2 mM glycerol to a maximum of 12.5 ± 1.0 nmol/mg of protein/h at 5 mM glycerol. Half-maximal glycerol incorporation occurred at approximately 0.5 mM glycerol (Fig. 5A). Incubation of these cells with 10 μM Triacsin-C resulted in reduction of glycerol incorporation at 0.2 mM glycerol to 1.1 ± 0.1 nmol/mg of protein/h, from which it rose to a maximum of 2.7 ± 0.4 nmol/mg of protein/h at 5 mM glycerol. This sharp reduction in glycerol incorporation is presumably due to the deficit in long-chain acyl-CoAs required for esterification to the glycerol phosphate backbone. Despite the large inhibition of fatty acid esterification induced by Triacsin-C, the drug had no effect on glycerol-stimulated insulin secretion, with a 4-fold stimulation induced by 2 or 5 mM glycerol in both Triacsin-C-treated and control groups (Fig. 5B). Still, it remained possible that the lack of secretory impact of Triacsin-C could be explained if only a small rate of fatty acid esterification was sufficient for signaling. This possibility appears unlikely, based on replotting of the data in Fig. 5, A and B, to generate Fig. 5C. Here all insulin secretion values for each of the individual wells studied in Fig. 5B are plotted against all of the individual values for glycerol incorporation into lipid summarized in Fig. 5A. The resulting plot clearly shows that insulin secretion from glycerol-stimulated AdCMV-GlpK-treated INS-1 cells is independent of the incorporation of glycerol into lipid. Furthermore, when studied at nonstimulatory glycerol levels (0.2 mM), cells incubated in the absence of Triacsin-C exhibited higher rates of glycerol incorporation than cells treated with the drug, with no effect on insulin release. Finally, Triacsin-C had no effect on the rate of [3H]glycerol usage in INS-1 cells (data not shown), consistent with the notion that entry of glycerol phosphate into the lower half of glycolysis and/or the glycerol shuttle are the major pathways for glycerol signaling in these cells.

Note that, in the absence of Triacsin-C, the amount of labeled glycerol incorporated into lipid was far less than the amount metabolized to lactate or oxidized to CO2 (at 5 mM glycerol, 12.5 nmol glycerol/mg of protein/h are incorporated into lipid compared with production of 718 nmol of lactate/mg of protein/h and 64 nmol of CO2/mg of protein/h). One concern with such a comparison is that use of [2-3H]glycerol might have caused us to underestimate the amount of glycerol converted to lipid, to the extent that unlabeled glycerol phosphate is reformed from dihydroxyacetone phosphate. We chose to use [3H]glycerol because it is available at much higher specific activity than 14C-labeled material. However, in one series of experiments with [U-14C]glycerol, we obtained a qualitatively similar estimate of glycerol incorporation into lipid (6.8 nmol of glycerol/mg of protein/h incorporated at a glycerol concentration of 2 mM and in the absence of Triacsin-C). Furthermore, Triacsin-C potently inhibited incorporation of the 14C-labeled material as it did the 3H-labeled glycerol. Thus, we conclude that incorporation of glycerol phosphate into lipid is a minor pathway for glycerol metabolism in AdCMV-GlpK-treated INS-1 cells and that insulin secretion is effectively stimulated even when this incorporation is markedly reduced. It is important to point out, however, that this result does not argue against the essentiality of lipids for stimulus/secretion coupling, as recently demonstrated by Stein et al. (33) in rodents rendered hypolipidemic by treatment with nicotinic acid.

Comparison of Previous Findings—Our findings in islets and INS-1 cells agree only partially with two previous studies on glycerol metabolism in islet cells. Similar to us, Malaisse and co-workers reported that glycerol had no effect on insulin secretion from either isolated rat islets or the rodent insulinoma cell lines RINm5F (14, 15). These investigators also reported,
however, that islets and RIN cells contain high levels of glycerol kinase activity, and also presented data suggesting that [U-14C]glycerol is oxidized in islets at the same rate as glucose. Using a radioisotopic assay similar to that employed by Mala-
isse and co-workers, we found glycerol kinase activity to be undetectable in islets or INS-1 cells, but easily detectable in fresh liver extracts, indicating that the assay method employed by our group was sufficiently sensitive to detect endogenous glycerol kinase activity in tissues known to express the en-
zyme. Yilmaz et al. (14) reported that islets contain 1.3 nmol/ 
m/min/100 mg of protein. Consistent with our measure-
ments of enzyme activity, we could demonstrate only very low levels of glycerol metabolism in AdCMV-GlpK tested (100), glycerol kinase activity is only 0.016 nmol/min/µg of protein. Consistent with our measure-
ments of enzyme activity, we could demonstrate only very low levels of glycerol metabolism in AdCMV-βGAL-treated INS-1 cells, such that their rate of [U-14C]glycerol oxidation was only 1.2% of the rate observed in AdCMV-GlpK-treated cells (data not shown). That normal islets lack glycerol kinase is also supported by our findings that the lowest m.o.i. of AdCMV-
GlpK tested in our studies, which caused a small rise in gly-
cerol kinase activity, was sufficient to confer near-maximal in-
sulin secretion in response to glycerol. Thus, our data are 
consistent with a model in which the explanation for the lack of glycerol-stimulated insulin secretion in normal islets or INS-1 cells is a lack of sufficient glycerol kinase activity to allow significant metabolism of the substrate.

Concluding Remarks—Our studies demonstrate that INS-1 or islet cells are not normally responsive to glycerol as an insulin secretagogue, but become so when the enzyme responsible for its phosphorylation is expressed. Glycerol is a highly effective secretagogue, despite the fact that it is less readily oxidized and more readily converted to lactate than glucose. Glycerol does not appear to signal insulin secretion via its conversion to glycerol phosphate and esterification with fatty acids to form triglycerides and other complex lipids. Rather, it appears that glycerol and glucose stimulate insulin secretion via a common signal that is produced in the distal reactions of glyco-
lysis or via entry into the glycerol phosphate shuttle, or both.

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