[54] Hellman, B., Idahl, L-Å., Lernmark, Å., Sehlin, J., and Täljedal, I-B.: Iodoacetamide-induced sensitization of pancreatic B-cells to glucose stimulation. Biochem. J. *132*:775, 1973.

[55] Pagliara, A. S., Hover, B. A., Ellerman, J. E., and Matschinsky, F. M.: Iodoacetate and iodoacetamide-induced alterations of pancreatic α and β-cells. Endocrinology *97*:698, 1975.

ABSTRACTS

(All are verbatim summaries)

Grodsky, G. M.; Batts, A. A.; Bennett, L. L.; Vcella, C.; McWilliams, N. B.; and Smith, D. F. (Metab. Res. Unit and Depts. of Biochem. and Physiol., Univ. of California Sch. of Med., San Francisco): **Effects of Carbohydrates on Secretion of Insulin from Isolated Rat Pancreas. Am. J. Physiol. *205*:638, 1963.**[1]

The effect of carbohydrates on the secretion of immunochemically measurable insulin was studied in an isolated perfused pancreatic preparation from the rat. Degradation of circulating insulin (as measured by chromatographic examination of added insulin-I^{131}) was less than 15% during the 4-hr experimental period. Without the addition of glucose, or at glucose concentrations of less than 50 mg/100 ml, insulin secretion was not detectable. At glucose concentrations of 50–150 mg/100 ml, insulin secretion occurred immediately and persisted throughout the experimental period. Insulin secretion was further increased by increasing glucose concentration to 150–500 mg/100 ml. The incidence of islet cell degranulation increased with increasing insulin secretion, suggesting that glucose stimulated secretion of stored insulin faster than synthesis of insulin de novo. Galactose, xylose, L-arabinose, pyruvate, and 2-deoxyglucose in concentrations of 600 mg/100 ml did not stimulate insulin secretion. Mannose stimulated the pancreas equally as well as glucose. Fructose was also active, but was less effective than glucose. Neither 2-deoxyglucose nor galactose blocked the insulin secretion by glucose. The data suggest that secretion of insulin is stimulated by a metabolite or a product resulting from the metabolism of glucose which can also be supplied by other metabolizable sugars.

Coore, H. G.; and Randle, P. J. (Dept. of Biochem., Univ. of Cambridge): **Regulation of Insulin Secretion Studied with Pieces of Rabbit Pancreas Incubated in Vitro. Biochem. J. *93*:66, 1964.**[2]

1. The effect of various factors on the rate of release of insulin from pieces of rabbit pancreas incubated *in vitro* has been studied by estimating the insulin concentration in the medium after incubation by immunological assay with insulin-antibody precipitate, and the conditions necessary for eliciting consistent responses have been defined.

2. Insulin release was accelerated by D-glucose at concentrations above 0.35–0.7 mg./ml. and by D-mannose (3 or 6 mg./ml.), but not by D-galactose, 3-*O*-methyl-D-glucose, D-fructose, D-ribose or sodium D-gluconate (3 mg./ml.), D-2-deoxyglucose (3 or 6 mg./ml.), *N*-acetyl-D-glucosamine (15 mg./ml.) or D-mannoheptulose (3 mg./ml.). The stimulating effect of glucose (3 mg./ml.) on insulin release was abolished by mannoheptulose but not by 2-deoxyglucose, 3-*O*-methylglucose, ribose or *N*-acetylglucosamine.

3. Insulin release at a low glucose concentration (0.6 mg./ml.) was accelerated by tolbutamide (200 μg./ml.) but not by anoxia, 2,4-dinitrophenol, salicylate, *p*-phenylenediamine or phenazine methosulphate. The rate of insulin release at a high glucose concentration (3 mg./ml.) was markedly diminished by anoxia, 2,4-dinitrophenol (250 μM), salicylate (5 mM), *p*-phenylenediamine (1 mM) and phenazine methosulphate (100 μM), but not by malonate (10 mM). The stimulating effect of tolbutamide (unlike that of glucose) was not influenced by mannoheptulose.

4. The stimulating effect of glucose (3 mg./ml.) on insulin release was augmented by the presence in the medium of glutamate, fumarate and pyruvate. At a low glucose concentration (0.6 mg./ml.) neither these acids nor octanoate, acetoacetate or β-hydroxybutyrate influenced the rate of insulin release.

5. The stimulating effect of glucose (3 mg./ml.) on insulin release was abolished by adrenaline (200 μmg./ml.), and the effect of adrenaline was suppressed by ergotamine tartrate (2.8 μg./ml.).

6. Evidence is presented that the release of insulin by rabbit pancreas *in vitro* provides a suitable model for the behavior of β-cells *in vivo* and for studying the influence of various factors on insulin secretion.

7. The possible roles of glucose phosphorylation and of pathways of metabolism of glucose 6-phosphate in β-cells in the stimulation of insulin release induced by glucose are discussed.

Grey, N. J.; Goldring, S.; and Kipnis, D. M. (Metab. Div., Dept. of Med., Washington Univ. Sch. of Med., St. Louis, MO): **The Effect of Fasting, Diet, and Actinomycin D on Insulin Secretion in the Rat. J. Clin. Invest. *49*:881, 1970.**[9]

The present studies were performed to elucidate the mechanisms responsible for the impairment of glucose-stimulated insulin secretion observed in fasting. Rats fasted for 48 hr displayed marked impairment in their insulin secretory response to both oral and intravenous glucose. Glucose-stimulated insulin secretion was restored within 24 hr by refeeding; actinomycin D given before refeeding blocked the expected return of normal glucose-stimulated insulin secretion despite adequate food intake. Fasted rats refed a diet devoid of carbohydrate failed to display a return of normal insulin secretory responsiveness to oral glucose in contrast to rats fed isocalorically a high carbohydrate diet. Differences in insulin secretion in fed, fasted, and fasted-refed rats could not be attributed to changes in pancreatic insulin content. There was no significant difference in the insulin secretory response to aminophylline of fed, fasted, or fasted-refed rats. The intermittent pulsing of fasted rats with hyperglycemic episodes by the injection of small amounts of glucose (500 mg) intraperitoneally every 8 hr ameliorated the impairment of glucose-stimulated insulin secretion characteristic of the fasting state. These results suggest that the impairment of glucose-stimulated insulin secretion during fasting and its restoration by refeeding are regulated by changes in a glucose-inducible enzyme system in the pancreatic beta cell.

Ashcroft, S. J. H.; Hedeskov, C. J.; and Randle, P. J. (Dept. of Biochem., Univ. of Bristol, U.K.): **Glucose Metabolism in Mouse Pancreatic Islets. Biochem. J. *118*:143, 1970.**[16]

1. Rates of glucose oxidation, lactate output and the in-

tracellular concentration of glucose 6-phosphate were measured in mouse pancreatic islets incubated *in vitro*. 2. Glucose oxidation rate, measured as the formation of $^{14}CO_2$ from [U-^{14}C]glucose, was markedly dependent on extracellular glucose concentration. It was especially sensitive to glucose concentrations between 1 and 2 mg/ml. Glucose oxidation was inhibited by mannoheptulose and glucosamine but not by phlorrhizin, 2-deoxyglucose or *N*-acetylglucosamine. Glucose oxidation was slightly stimulated by tolbutamide but was not significantly affected by adrenaline, diazoxide or absence of Ca^{2+} (all of which may inhibit glucose-stimulated insulin release), by arginine or glucagon (which may stimulate insulin release) or by cycloheximide (which may inhibit insulin synthesis). 3. Rates of lactate formation were dependent on the extracellular glucose concentration and were decreased by glucosamine though not by mannoheptulose; tolbutamide increased the rate of lactate output. 4. Islet glucose 6-phosphate concentration was also markedly dependent on extracellular glucose concentration and was diminished by mannoheptulose or glucosamine; tolbutamide and glucagon were without significant effect. *Mannose increased islet fructose 6-phosphate concentration but had little effect on islet glucose 6-phosphate concentration.* Fructose increased islet glucose 6-phosphate concentration but to a much smaller extent than did glucose. 5. [1-^{14}C]Mannose and [U-^{14}C]fructose were also oxidized by islets but less rapidly than glucose. Conversion of [1-^{14}C]mannose into [1-^{14}C]glucose 6-phosphate or [1-^{14}C]glucose could not be detected. It is concluded that metabolism of mannose is associated with poor equilibration between fructose 6-phosphate and glucose 6-phosphate. 6. These results are consistent with the idea that glucose utilization in mouse islets may be limited by the rate of glucose phosphorylation, that mannoheptulose and glucosamine may inhibit glucose phosphorylation and that effects of glucose on insulin release may be mediated through metabolism of the sugar.

Hellman, B.; Idahl, L-Å.; Lernmark, Å.; Sehlin, J.; and Täljedal, I-B. (Dept. of Histol., Univ. of Umea, Sweden): **The Pancreatic B-Cell Recognition of Insulin Secretagogues. Comparisons of Glucose with Glyceraldehyde Isomers and Dihydroxyacetone. Arch. Biochem. Biophys. *162*:448, 1974.**[23]

D-Glyceraldehyde stimulated the release of insulin from pancreatic islets of Umeå-*ob*/*ob*-mice whether or not glucose was present in the medium. Like the action of glucose, that of D-glyceraldehyde was biphasic in time, exhibited a sigmoidal dose-response relationship, was potentiated by theophylline, arginine, iodoacetamide, or L-glyceraldehyde, and was inhibited by epinephrine, 2,4-dinitrophenol, or Ca^{2+} deficiency. Half-maximum and maximum stimulations were produced by about 3 mM and 10 mM D-glyceraldehyde. Positive interactions were observed between 5 mM D-glyceraldehyde and 5 mM glucose and between 10 mM D-glyceraldehyde and 10 mM leucine. Mannoheptulose (10 mM) or glucosamine (10 mM) did not inhibit but potentiated the effect of 10 mM D-glyceraldehyde. Dihydroxyacetone (2.5–20 mM) also initiated insulin release in the absence of glucose. On the other hand, 5–10 mM L-glyceraldehyde did not initiate secretion but potentiated the effects of 5 mM glucose or 5 mM D-glyceraldehyde. D-Glyceraldehyde or dihydroxyacetone reduced the production of $^{14}CO_2$ from D-[U-^{14}C]glucose; L-glyceraldehyde had a smaller and statistically insignificant effect. The results suggest that by being phosphorylated and entering glycolysis in the β-cells, D-glyceraldehyde and dihydroxyacetone act as functional analogues of glucose as secretory stimulus. Initiation of insulin release by glucose, D-glyceraldehyde, or dihydroxyacetone may thus depend on the production of a metabolic signal at or below the triose phosphate level.

Zawalich, W. S.; Dye, E. S.; Rognstad, R.; and Matschinsky, F. M. (Depts. of Pediatr. and Pharmacol., Washington Univ. Sch. of Med., St. Louis, MO): **On the Biochemical Nature of Triose- and Hexose-stimulated Insulin Secretion. Endocrinology *103*:2027, 1978.**[26]

The differential effects of several specific inhibitors of intermediary metabolism, mannoheptulose, 2-deoxyglucose, and iodoacetate, were studied with isolated perifused pancreatic islets stimulated with glucose, mannose, glyceraldehyde, dihydroxyacetone, or α-ketoisocaproate. Insulin release rates and/or capacities to metabolize these caloric stimuli served as indicators of the inhibitors' actions. Mannoheptulose and 2-deoxyglucose blocked hexose-stimulated hormone release and hexose metabolism concomitantly but left the functional and metabolic actions of trioses unaltered. Iodoacetate blocked hexose and triose-stimulated hormone release as well as their metabolism in a parallel fashion. The action of α-ketoisocaproate was not affected by any of these three inhibitory agents. The data are most easily explained by a theory that incorporates metabolic signals, arising during the degradation of insulin releasing fuel molecules, as an integral component in the process of β-cell stimulation.

Malaisse, W. J.; Sener, A.; Koser, M.; and Herchuelz, A. (Lab. of Exp. Med., Brussels Free Univ. Sch. of Med., Belgium): **Stimulus-Secretion Coupling of Glucose-Induced Insulin Release. Metabolism of α- and β-D-Glucose in Isolated Islets. J. Biol. Chem. *251*:5936, 1976.**[37]

α-D-Glucose is known to exert a more marked insulinotropic action than β-D-glucose. Both anomers are phosphorylated at the same rate by rat islet homogenates. The islet glucose-6-phosphate dehydrogenase displays a preferential affinity towards β-D-glucose 6-phosphate, and this coincides with a higher sorbitol content in the islets exposed to β-D-glucose. On the contrary, the islet phosphoglucose isomerase is stereospecific for α-D-glucose 6-phosphate and, hence, the concentration of glucose 6-phosphate is lower and that of further glycolytic intermediates higher in the islets exposed to α-D-glucose. The rate of conversion of the α-anomer to lactate and CO_2 is also higher than that of β-D-glucose. This increased glycolytic flux is associated with a more marked inhibitory action on ^{45}Ca efflux, a more pronounced stimulation of ^{45}Ca net uptake and a higher rate of insulin release in the islets exposed to α-D-glucose.

The more marked insulinotropic action of α- as distinct from β-D-glucose is thus compatible with the view that glycolysis represents the key component of the sensor device

through which glucose is identified in the pancreatic B-cell as a stimulus for insulin release.

Zawalich, W. S.; Rognstad, R.; Pagliara, A. S.; and Matschinsky, F. M. (Depts. of Pediatr. and Pharmacol., Washington Univ. Sch. of Med., St. Louis, MO): **A Comparison of the Utilization Rates and Hormone-Releasing Actions of Glucose, Mannose, and Fructose in Isolated Pancreatic Islets. J. Biol. Chem. *252*:8519, 1977.**[44]

The abilities of glucose, mannose, and fructose to serve as substrates for glycolysis were compared with the potencies of these hexoses to stimulate hormone secretion. Glucose and mannose induced insulin release at threshold levels of 4 and 10 mM and were effective half-maximally at 8 and 15 mM, respectively. Maximal release with glucose and mannose occurred at 15 and 20 mM, respectively. At their threshold levels for insulin release, glucose and mannose were both metabolized at rates of approximately 50 pmol/islet/h. At levels causing half-maximal release, the glycolytic rates were 80 to 85 pmol/islet/h. At substrate levels causing maximal hormone release, the glycolytic rates reached 100 to 110 pmol/islet/h for both hexoses. Fructose, even at 50 mM, did not stimulate insulin release. Half-maximal usage (15 pmol/islet/h) occurred with 16 mM fructose, while maximal usage (30 pmol/islet/h) was obtained at 27.5 mM fructose. Fructose and glucose usage were not interfered with by a 10-fold excess of glucose and fructose, respectively. Fructose was, however, capable of augmenting insulin secretion in the presence of substimulatory or stimulatory glucose. This potentiation could be predicted if one assumed that glycolytic rates of the two hexoses were additive. The results strongly support the concept that the glycolytic degradation of hexoses controls hormone secretion.

Zawalich, W. S.; Pagliara, A. S.; and Matschinsky, F. M. (Depts. of Pediatr. and Pharmacol., Washington Univ. Sch. of Med., St. Louis, MO): **Effect of Iodoacetate, Mannoheptulose, and 3-O-Methylglucose on the Secretory Function and Metabolism of Isolated Pancreatic Islets. Endocrinology *100*:1276, 1977.**[48]

The ability of iodoacetate, mannoheptulose, and 3-O-methyl glucose to alter islet cell metabolism and glucose-stimulated insulin secretion was examined. A method for the sequential analysis of the releasing and fuel function of glucose in isolated islets was applied. Insulin release was measured by radioimmunoassay and the metabolism of glucose by determining the rate of tritiated water production from [5-^{3}H]glucose and lactate accumulation. It was found that iodoacetate, in the range of 0.2–1.0 mM, inhibited the metabolism of glucose linearly while release was not blocked until metabolism was reduced by 30–40%. The K_I for both processes, release and metabolism, was the same. Pyruvate did not protect against or reverse the effects of iodoacetate. Mannoheptulose inhibited both release and metabolism half-maximally at about 5 mM when 27.5 mM glucose was used as the stimulatory agent. A mannoheptulose-resistant component of glucose metabolism, amounting to 30% of the maximal rate was observed. 3-O-Methyl glucose had no effect on insulin release but reduced glucose utilization and lactate production from low glucose. The results are discussed in light of the two prevailing hypotheses explaining glucose induced insulin release, *i.e.*, the receptor and the metabolism hypotheses.