cellular concentration of glucose, appears to be mediated by a coordinated set of cellular events. These include the perception of the environmental change, its translation into a coupling mechanism and the eventual modification in the rate of insulin release. Such a sequence of events may be viewed as the process of stimulus-secretion coupling. It is often assumed that, in the B-cell, the information generated by distinct sensor systems is integrated to regulate, in a coordinated manner, a single and terminal process of insulin release, which corresponds to the exocytosis of secretory granules. Thus, distinct regulatory factors may feed in at distinct levels in the secretory sequence.

It is a demanding and occasionally frustrating ambition to provide a cartesian explanation for the cause-to-effect links between distinct, though almost simultaneous, events involved in the process of stimulus-secretion coupling. Indeed, as knowledge progresses in this field and the progress is obvious as judged for instance from successive multi-authors reviews on this topic[1-5], it became evident that almost any cellular process is affected by or participates in stimulus-secretion coupling. For instance, when the extracellular concentration of glucose is increased, changes in the B-cell involve the metabolism of nutrients, the consumption of O_2 and redox state, the heat production, the intracellular pH, the biosynthesis of proteins, the fluxes of several ions, the electrical activity, the turnover of phospholipids, the (de)phosphorylation of proteins, the fluidity of membranes, the production of cyclic AMP, the contractile activity of the cell web, the movements of secretory granules, the coupling of endocytosis to exocytosis and the intercellular communication between adjacent cells. Which of these changes occurs first, and how are they interconnected? It is the very aim of this series of reports to answer such questions. Each contributor to this review would probably accept the blame that it presently looks as an impossible dream to reach an unambiguous answer.

It seems mandatory to underline several restrictions in the scope and content of this review. First, the topic is restricted to the insulin-producing B-cell. The cytophysiology of adjacent non-B endocrine cells in the islets is not discussed. Second, the emphasis is given to the cytophysiology of insulin release. Other aspects of the anatomic and functional organization of the B-cell, e.g. the synthesis and conversion of proinsulin, are only considered as far as they are immediately relevant to the secretory process. Third, this review is conceived from the standpoint of cellular physiology, and not in the perspective of the regulation of insulin release in vivo. Hence, such topic as the neural and hormonal control of insulin release, e.g. by gastrointestinal factors, is not reviewed. Fourth, emphasis is given on the cellular events involved in the rapid process of stimulus-secretion coupling. The long-term regulation of islet function by ontogenic, nutritional and endocrine factors is not dealt with. Last, this review concerns the physiology, and not the pathology, of insulin-producing cells. No information is provided on the anomaly of B-cell function found in experimental or spontaneous diabetic syndromes or tumoral insulin-producing cells. I can only apologize for these restrictions. Needless to say, they do not reflect any lack of interest for the topics omitted from this review, but instead were imposed by considerations on both the homogeneity and length of this contribution. It is nevertheless hoped that potential readers may be interested by the information provided in this review.

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Nutrient metabolism in islet cells

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Key words. Pancreatic B-cell; nutrient metabolism.

1. Introduction

D-glucose and a variety of other nutrients are able, under suitable experimental conditions, to stimulate insulin release from the pancreatic B-cell. For some time, it had been proposed that the stimulant action of these nutrients upon insulin release may be mediated through stereospecific (membrane) receptors, the binding of each nutrient to its specific receptor initiating the sequence of events eventually leading to the exocytosis of

insulin secretory granules[14, 43, 76, 77, 88]. Today, however, it is generally believed that the capacitiy of nutrients to stimulate insulin release reflects their capacity to increase the rate of nutrient catabolism and O_2 uptake in the islet cells[2, 25, 49, 64]. In this respect, the semantic distinction between a regulator site and a substrate site hypothesis, as first formulated by Randle et al.[89], may no more represent an essential issue. It is indeed established that nutrient secretagogues may act in the islet cells either as a substrate undergoing intracellular metabolism and/or as an activator of key regulatory enzyme(s). For instance, the insulinotropic action of a nonmetabolized analog of L-leucine, 2-aminobicyclo-[2, 2, 1]heptane-2-carboxylic acid (BCH) is thought to be attributable primarily to activation of glutamate dehydrogenase, resulting in the facilitated conversion of endogenous amino acids to their corresponding 2-keto acids, which are then further oxidized[72, 95, 98]. However, the insulin-releasing pyruvate analog, 3-phenylpyruvate, which is poorly oxidized in the islets, acts as a transamination partner, as distinct from enzyme activator, and, nevertheless, also facilitates the conversion of endogenous amino acids to 2-keto acids and their further oxidation[71, 105].

The view that the process of nutrient-induced insulin release coincides with and is actually caused by an increase in the rate of nutrient utilization in the islet cells has been formulated as the fuel hypothesis[64], or fuel concept[49], for insulin release. The latter concept raises several important questions, as follows.

First, which are the nutrients utilized by the islet cells to cover their energy expenditure. Second, which are the major regulatory factors involved in the control of nutrient catabolism in the islet cells. Third, which are the ATP-requiring processes participating in the balance between ATP production and breakdown, whether in resting or stimulated B-cells. Last, which are the links between an increase in nutrient catabolism and more distal processes in the sequence of cytophysiological events leading to the release of insulin. The present report deals mainly with the first three of these questions, whilst the last one will be considered separately in the next report in this series[61].

2. *Nutrient catabolism in islet cells*

2.1 *O_2 uptake*

The rate of O_2 consumption by pancreatic islets deprived of exogenous nutrient was measured by several investigators[19, 28, 29, 34, 87, 112]. The reported values are rather variable. Incidentally, for the sake of comparison, the data on O_2 consumption, as well as all other data presented in this report, were recalculated by reference to a pancreatic islet with a protein content close to 0.8 µg/islet or a dry weight close to 1.0 µg/islet. On such a basis, the reported values for basal islet respiration range from 1.6 to 8.1 pmoles/min per islet (table). One of the factors which could account for the discrepancy between such values may consist in the fact that most measurements were performed in a bicarbonate-free buffer equilibrated against ambient air, whilst a few other measurements were performed in a bicarbonate-buffered solution equilibrated against a mixture of CO_2/air (5/95, v/v). It was indeed reported by Hutton and Malaisse[34] that both basal and nutrient-stimulated O_2 consumption by rat pancreatic islets is decreased in the absence or at low concentrations of bicarbonate. All authors agree on the fact that exogenous nutrient secretagogues increase O_2 consumption by the islets. As a rule, and with one exception, however[28], the absolute value for such an increment in respiration does not exceed the basal respiratory rate. Since isolated islets deprived of exogenous nutrient are able to maintain, for at least one or two hours, a stable respiratory rate, these data point to an important contribution of endogenous nutrients in islet respiration.

Oxygen consumption by pancreatic islets deprived of exogenous nutrient or exposed to D-glucose

Species	O_2 uptake (pmoles/min per µg dry wt or per islet)		
	Basal	D-glucose (17–20 mM)	Reference
Obese hyperglycemic mice	4.2	6.4	Hellerström[29]
Normal mice	1.6	4.0	Hedeskov et al.[28]
Normal rats	8.1	11.0	Hutton and Malaisse[34]
Obese hyperglycemic mice	ND	15.2	Frankel et al.[19]
Normal mice	3.6	8.0	Panten and Klein[87]
Normal mice	5.7	10.9	Welsh[112]

ND, not determined.

2.2 *Catabolism of endogenous nutrients in islets deprived of exogenous nutrient*

Figure 1 provides a schematic and tentative model for the utilization of endogenous nutrients by islets deprived of exogenous nutrient[50]. This model is based mainly on the measurement of O_2 uptake, NH_4^+ production, triglyceride and amino acid content, and the output of several organic acids (pyruvic, lactic and acetoacetic acid) in rat islets incubated in the absence of exogenous nutrient[52]. Three attributes of this model should be underlined.

First, endogenous triglycerides and amino acids represent the two major sources of fuel in the islets incubated in the absence of exogenous substrate. The contribution of endogenous carbohydrates and glycolytic intermediates is negligible. This feature relies on the knowledge that islet removed from normoglycemic animals do not contain any detectable amount of glycogen[66]. It should be stressed that a different situation prevails in islets cultured for 20 h at a high concentration of extracellular glucose[66] or removed from either obese-hyperglycemic mice[30] or rats rendered hyperglycemic by a prolonged infusion of glucose[57] or administration of diabetogenic dosis of triamcinolon[22]. In such circumstances, glycogen accumulates in the B-cell, more than in the other, non-B, pancreatic endocrine cells.

Second, the O_2 consumption in this model amounts to 6.8 pmoles/min per islet, with a respiratory quotient close to 0.751.

Last, amino acids are the principal precursors of pyruvic and lactic acid released by the islets deprived of exogenous nutrient. The model implies a rather elevated flow rate through the reaction catalyzed by the cytoso-

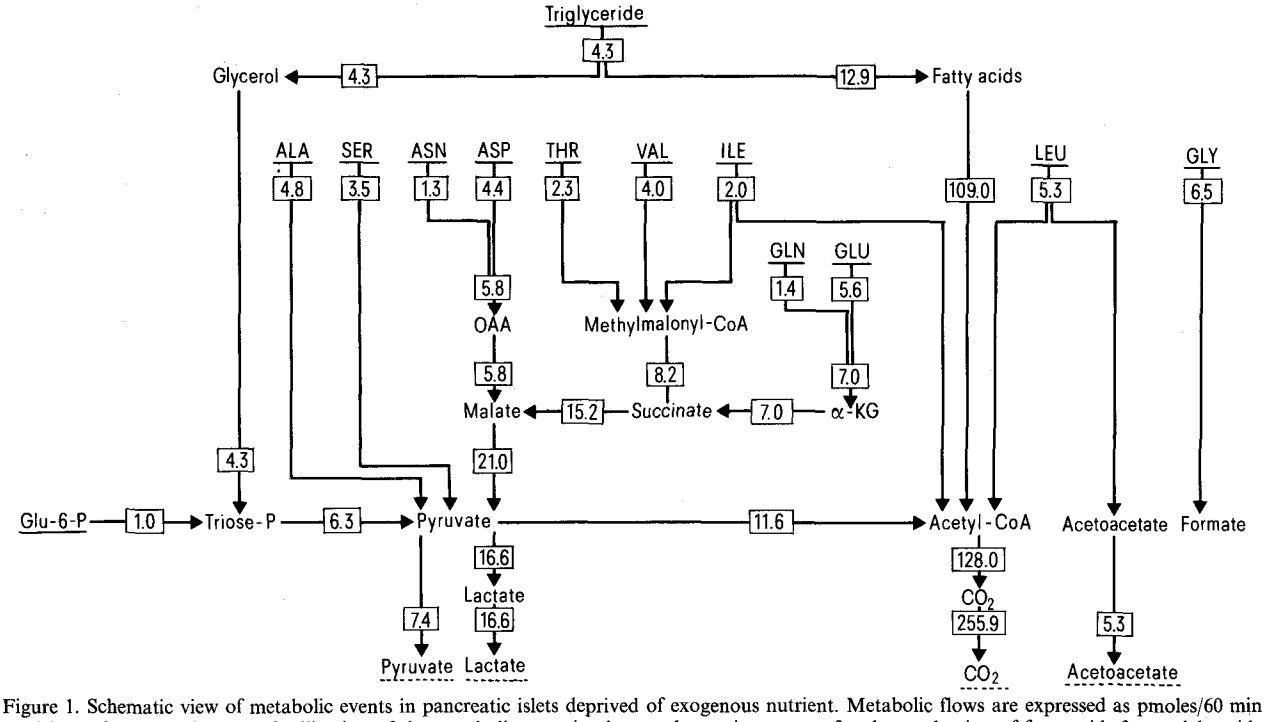

Figure 1. Schematic view of metabolic events in pancreatic islets deprived of exogenous nutrient. Metabolic flows are expressed as pmoles/60 min per islet and refer to the rate of utilization of the metabolite entering into each reaction, except for the production of fatty acids from triglyceride and their conversion to acetyl-CoA in which cases the flow rate refers to the metabolite emerging from the reaction. The parent nutrients and some of their metabolic end products are underlined by solid and dashed lines, respectively.

lic malic enzyme. We have recently validated such a concept, by measuring the output of ^{14}C-pyruvate by islets exposed to low concentrations of exogenous L-[U-^{14}C]glutamine or L-[U-^{14}C]leucine[99].

It is obvious that the situation depicted in figure 1 would eventually lead to an exhaustion of all endogenous nutrients, if the islets were maintained for a prolonged period in the absence of exogenous nutrient. The question must be raised, therefore, to which extent the catabolism of endogenous nutrients, as depicted in figure 1, is modified when the islets are exposed in vivo to the normal constellation of circulating nutrients. The latter question itself raises a number of further interrogations. For instance, which is the relative contribution of distinct circulating nutrients in covering the energy expenditure of islet cells in vivo? When fatty acids are oxidized by the pancreatic B-cell in vivo, are such fatty acids directly derived from circulating fatty acids or from endogenous triglycerides? Are the fatty acids in endogenous glycerides derived from circulating fatty acids or synthetized de novo in the B-cell from other precursors (e.g. D-glucose)? Several of these and other questions still remain unanswered. The limited information available is summarized in the following section of this report.

3. *Regulation of nutrient catabolism in islet cells*

It would be beyond the scope of this report to review in a systematic and exhaustive manner all the information available on the transport and catabolism of distinct nutrients in the islet cells. Because D-glucose is currently viewed as the major physiological substrate involved in the nutrient-dependent regulation of insulin release in vivo, emphasis will be given here to the regulation of glucose metabolism in islet cells[94].

3.1 Glucose metabolism in islet cells

3.1.1 Glucose transport

D-glucose, but not L-glucose which does not penetrate into the B-cell, is rapidly and uniformly equilibrated over the B-cell membrane[32, 75]. The uptake of D-glucose is saturable, but with a Km as high as about 50 mM[32]. In one study, it was claimed that the β-anomer of D-glucose is transported preferentially in islet cells[82]. Several observations do not support such a view[78]. On the contrary, the finding that the α-anomer of 3-O-methyl-D-glucose protects islet cell against alloxan more efficiently than the corresponding β-anomer could point to a preferential α-anomeric specificity for the transport of D-glucose and sterically similar molecules in islet cells[90].

3.1.2 Sorbitol pathway

The islet homogenates display aldose reductase activity, with a preference for α-D-glucose[16, 17]. In the presence of D-glucose, sorbitol accumulates in the islets and incubation medium[68, 84]. Incidentally, more sorbitol accumulates in islets exposed to β-D-glucose than α-D-glucose, suggesting a possible role for the availability of NADPH (e.g. as generated in the pentose phosphate pathway) in the regulation of metabolic flow through the sorbitol pathway[65]. Such a pathway apparently does not contribute significantly to the conversion of glucose

to triose phosphates[56]. The possible long-term deleterious effect of sorbitol accumulation in the islets in conditions of sustained hyperglycemia remains to be explored. It should be noted, however, that a major fraction of sorbitol formed by the islets is apparently released in the incubation medium[68].

3.1.3 Glucose phosphorylation

When the extracellular concentration of glucose is raised, a rapid and sustained increase in the glucose 6-phosphate content of the islet is noticed[4, 21, 35, 83]. Under steady-state conditions, the islet content in glucose 6-phosphate is related to the extracellular concentration of D-glucose by a sigmoidal curve (fig. 2). More precisely, a first increase is seen at low glucose concentration (< 5 mM), which reaches its saturation at a very low glucose level ($\leqslant 1.7$ mM). At higher glucose concentration (> 5 mM), a reascension in glucose 6-phosphate content is noticed, this second component of the dose-response relationship reaching saturation at very high glucose levels ($\geqslant 27.8$ mM). This type of relationship, with a dual saturation phenomenon, also characterizes the changes in several other variables of glucose metabolism (e.g. glucose utilization, lactic acid output or glucose oxidation) in islets exposed to increasing concentrations of extracellular glucose (fig. 3). It may reflect, in part at least, the kinetics of glucose phosphorylation in islet cells. Indeed, at least two enzymes participate in the phosphorylation of glucose in islet cells: a high-affinity hexokinase and a low-affinity glucokinase, which display Km for glucose close to , respectively, 0.05 and 10.0 mM or more[6, 21, 67, 79]. In islet homogenates, the low-Km hexokinase, which is indeed present in a population of purified B-cells, accounts for about 80–90% of the total rate of glucose phosphorylation measured at a physiological concentration of glucose (8.3 mM). Yet, in intact islets, the rate of glucose utilization at low glucose concentrations, sufficient to saturate hexokinase, represents only one third of that found in the presence of 8.3 mM glucose. This apparent discrepancy between enzymatic and metabolic data can be accounted for by the fact that hexokinase is severely inhibited in intact islet cells by such metabolites as glucose 6-phosphate and glucose 1,6-bisphosphate (fig. 4). For instance, glu-

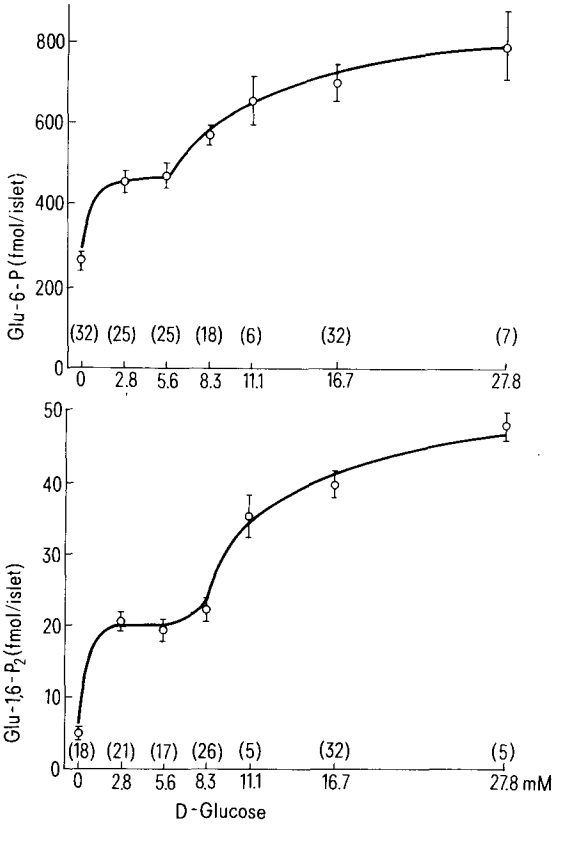

Figure 2. Effect of increasing concentrations of D-glucose upon the steady-state content of glucose 6-phosphate and glucose 1,6-bisphosphate in rat pancreatic islets. The number of individual observations is shown in parentheses.

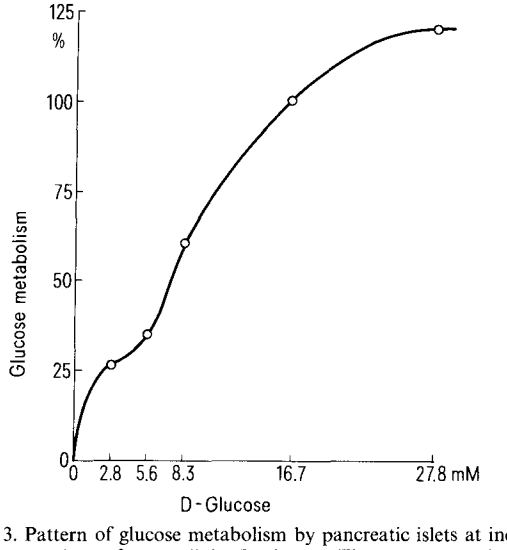

Figure 3. Pattern of glucose metabolism by pancreatic islets at increasing concentrations of extracellular D-glucose. The curve was calculated from mean values for the glucose-induced increment in O_2 uptake and lactate output, and the rate of $^{14}CO_2$ and ^3H_2O production from D-[U-^{14}C]glucose and D-[5-^{3}H]glucose (or D-[2-^{3}H]glucose), respectively, all data being expressed in percent of the control value found in the presence of 16.7 mM-glucose.

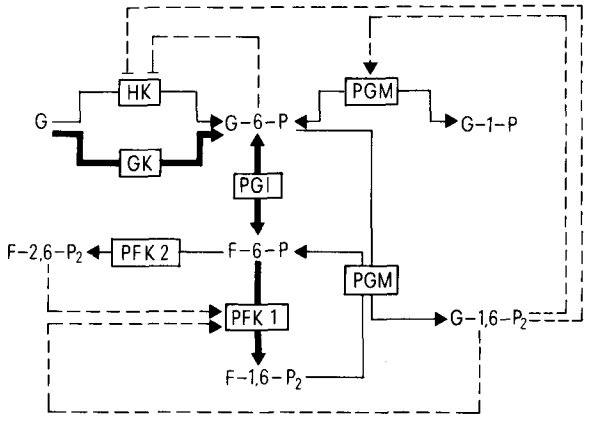

Figure 4. Schematic view of some early steps of glucose metabolism in pancreatic islets. The activation and inhibition of enzymes (GK: glucokinase; HK: hexokinase; PGI: phosphoglucose isomerase; PGM: phosphoglucomutase, PFK1: phosphofructokinase; PFK2: fructose-6-phosphate, 2-kinase) are indicated by dashed arrows and T-bars, respectively. The major metabolic pathway is indicated by heavy arrows and minor pathways by fine arrows.

cose 6-phosphate acts as a noncompetitive inhibitor of hexokinase and its concentration in intact islet cells is sufficiently elevated to exert a marked inhibitory effect upon glucose phosphorylation by the low-Km enzyme[21]. The high Km enzyme glucokinase may thus play an essential role in glucose phosphorylation within the physiological range of circulating glucose concentrations. It was even proposed that this enzyme should be considered as the key glucoreceptor system in islet cells[80]. Glucokinase may display limited anomeric specificity, with opposite preference for either α- or β-D-glucose, pending on the concentration of these anomers[80]. An adaptative decrease in the islet content in glucokinase was observed during prolonged starvation and may play a role in the decreased secretory response to glucose of islets removed from fasted animals[13,67]. Likewise, a low activity of glucokinase in tumoral or fetal insulin-producing cells could also account for the poor secretory responsiveness of such cells to glucose[1,24].

Glucose 6-phosphatase activity was also identified in islet homogenates, but a participation of this enzyme in the net balance between glucose phosphorylation and glucose 6-phosphate hydrolysis in intact islet cells was questioned by certain authors[5,26,108].

3.1.4 Glycogen synthesis and glycogenolysis

As already mentioned, islets removed from normoglycemic rats contained little or no detectable amount of glycogen (< 1 pmole glucose residue/islet). According to Hedeskov and Capito[26], mice islets exposed to [5-^{3}H]glucose (16.7 mM) incorporate the sugar into glycogen at a rate not exceeding 0.12 (fed mice) to 0.28 (48-h-starved mice) pmoles/60 min per islet. Over 20 h incubation and at a much higher concentration of glucose (83.3 mM), glycogen accumulated in rat islets to a maximal level of 75.8 ± 11.5 pmoles of glucose residue/islet[66]. Hellman and Idahl[30] recorded glycogen content averaging 10.2 ± 0.9 and 3.6 ± 0.4 pmoles of glucosyl units/μg dry wt in islets removed from obese-hyperglycemic mice and their lean litter mates, respectively. The glycogen content of islets rapidly decreases, when the islets are incubated in the absence of glucose or at low extracellular glucose concentration (3.3 mM)[30,66]. Glucagon, theophylline or dibutyryl-cyclic AMP, but not epinephrine, accelerate the rate of glycogenolysis in the islets[37]. It should be stressed that, even in the obese-hyperglycemic mice, the glycogen content would not be sufficient to maintain for more than a few min a significant rate of glycolysis. Thus, when such islets are exposed to extracellular glucose (10 mM), the rate of glucose utilization averaged 1.3 pmoles/min per islet[31]. Incidentally, acid amyloglucosidase activity is present in pancreatic islets, with apparent lysosomal localization, and administration of exogenous amyloglucosidase extracted from *Aspergillus niger* is reported to increase the level of circulating insulin[45,46].

3.1.5 Pentose cycle

Glucose 6-phosphate dehydrogenase which displays anomeric specificity for β-D-glucose 6-phosphate[65] and 6-phosphogluconate dehydrogenase activities are both present in islet cells[6,27,65]. The reaction velocity in islet homogenates (2–10 pmoles/min per islet) exceeds by 1–2 orders of magnitude the flow rate through the pentose phosphate pathway as measured in intact islet cells. This suggests a key role for the availability of $NADP^+$ in the regulation of metabolism in the pentose cycle. This is supported by the fact that a marked increase in the rate of circulation in the pentose cycle is observed in islets exposed to menadione[55]. Glucose causes a rapid and sustained increase in the 6-phosphogluconate content of isolated islets[27,38,83]. The flow rate through the pentose cycle is relatively low in the islets. At low glucose concentration (< 5.6 mM), it represents no more than about 10% of the rate of conversion of glucose 6-phosphate to triose phosphate[7,27,56,106,111]. When the glucose concentration is increased, such a ratio falls to 1–3%.

3.1.6 Conversion of glucose 6-phosphate to fructose 1,6-bisphosphate

Glycolysis represents the quantitatively major pathway for glucose metabolism in pancreatic islets[56]. Within the framework of the findings so far summarized, an increase in the rate of glycolysis subsequent to an increase in extracellular glucose concentration could be viewed as a mere mass action phenomenon ruled by an increase in the steady state values of glucose 6-phosphate and fructose 6-phosphate, the content of the latter metabolite usually representing close to one-third of the former metabolite. It should be noted, however, that in islets exposed to β-D-glucose, the conversion of glucose 6-phosphate to fructose 6-phosphate may play a role in the anomeric specificity of glucose metabolism in pancreatic islets, since phosphoglucose isomerase behaves as an α-stereospecific enzyme[60,65].

It is well established that, under suitable experimental conditions, the rate of glycolysis in intact islets may be rate-limited at a site distal to glucose phosphorylation. To cite only one example, iodoacetate impairs glycolysis apparently by inhibiting glyceraldehyde 3-phosphate dehydrogenase[102]. The question must be considered, therefore, whether under physiological conditions, the rate of glucose phosphorylation is the sole determinant of the rate of glycolysis. There are reasons to believe that such is not the case. For instance, in islets removed from starved animals, glucokinase activity is reduced by no more than one to two thirds. Yet, the rate of glycolysis is more severely affected[20]. Likewise, the influence of the anomers of D-mannose upon both lactic acid output and $^{14}CO_2$ production from D-[U-^{14}C]-mannose cannot be accounted[53,97] solely by the alleged preference of glucokinase for α-D-mannose[81].

The enzyme phosphofructokinase represents a likely candidate for regulation of glycolysis beyond the level of glucose phosphorylation. Upon optimal assay conditions, the maximal activity of this enzyme in islet homogenates exceeds that of the glucose-phosphorylating islet enzymes. However, at physiological concentrations of both the two substrates (fructose 6-phosphate and ATP) and a number of environmental factors influencing the activity of phosphofructokinase (H^+, K^+, NH_4^+, citrate, ADP, AMP), the reaction velocity in islet

homogenates may well be low enough not to differ vastly from that of glucose phosphorylation. Hence, it is conceivable that, in intact islets, the activity of phosphofructokinase indeed becomes rate-limiting, at least under certain conditions. The regulation of phosphofructokinase activity may well represent, therefore, an essential feature in the overall control of glycolysis in islet cells. In this perspective, hexose bisphosphates, especially glucose 1,6-bisphosphate and fructose 2,6-bisphosphate may play a critical role as activators of phosphofructokinase[59]. Exposure of intact islets to increasing concentrations of extracellular glucose indeed results in activation of phosphofructokinase[62], and this coincides with a rapid, dose-related and sustained increase in the islet content of both glucose 1,6-bisphosphate and fructose 2,6-bisphosphate[58, 74, 100, 104]. Incidentally, the concentration of these two hexose-phosphates in glucose-stimulated intact islet cells is sufficiently elevated to postulate that they participate, probably in concert, in the activation of phosphofructokinase.

3.1.7 Conversion of fructose 1,6-bisphosphate to pyruvate

The presence and activity of the enzymes involved in the stepwise conversion of fructose 1,6-bisphosphate to pyruvate were studied in pancreatic islets[11, 12, 92, 107]. Moreover, the islet content of such intermediates as fructose 1,6-bisphosphate measured together with triose phosphates, 3-phosphoglycerate, 2-phosphoglycerate and phospho-enol-pyruvate is increased in glucose-stimulated islets[3, 31, 36, 92, 107, 109]. Relatively little attention was so far paid, however, to regulatory processes in this lower segment of glycolysis.

The flow rate through the reaction catalyzed by glyceraldehyde-3-phosphate dehydrogenase obviously depends on the continuous regeneration of NAD^+ from NADH. At least two mechanisms may here play a role. First, the conversion of pyruvate to lactate, which is then released by the islet cells, may provide a first modality for the regeneration of NAD^+[93]. In this respect, it should be noted that the glucose-induced increment in lactate output accounts, in the islets, for 20–40% of the rate of glucose utilization[86, 93]. Second, the regeneration of NAD^+ may be linked to operation of the glycerol phosphate shuttle. The flow rate through such a shuttle remains to be assessed in intact islets. The fact that anoxia inhibits glycolysis in intact islets[31] could be related to the operation of glycerol phosphate shuttle. MacDonald found that in rat islet mitochondria, the activity of the flavin-linked glycerol-3-phosphate dehydrogenase was about 50 times higher than in liver or heart mitochondria and even 5–9 times higher than in skeletal muscle mitochondria[47]. This author also documented the activation of this enzyme by Ca^{2+} (10^{-8} to 10^{-5} M), which lowers the Km of the enzyme in respect to glycerol-3-phosphate without affecting the maximal velocity measured at saturating concentrations of the substrate[48]. By increasing Ca^{2+} activity, glucose may thus stimulate the glycerol phosphate shuttle. Incidentally, the cytosolic NAD-linked glycerol-3-phosphate dehydrogenase is also present in rat islets, with an activity close to that of the mitochondrial flavin-linked enzyme[47].

The activity of pyruvate kinase could be rate-limiting in the control of glycolysis in islets deprived of extracellular K^+, but a regulatory role for this enzyme at normal K^+ concentrations is not evident[92].

3.1.8 Oxidation of pyruvate

The production of $^{14}CO_2$ from D-[U-^{14}C] glucose accounts for approximately 20–30% of the rate of glucose utilization by the islets[4, 93], such a ratio being little affected by the ambient glucose concentration. Because of the modest contribution of the pentose pathway to $^{14}CO_2$ production, this ratio documents on the approximate rate of oxidation of glucose metabolites in the mitochondria.

At this point, it should be stressed, that the ^{14}C atoms of D-[U-^{14} C]glucose are also recovered, to a limited extent, in a number of other metabolites including amino acids and glycerolipids[10, 23].

3.1.9 Sparing action of endogenous nutrients

Glucose causes a dose-related but partial inhibition of $^{14}CO_2$ output from islets prelabelled with [U-^{14}C]palmitate, and also decreases NH_4^+ output from the islets[52, 54]. Glucose, however, increases the production of $^{14}CO_2$ from islets prelabelled with L-[U-^{14}C]glutamine[52]. Thus, glucose may exert a sparing action on both the oxidation of endogenous fatty acids and the oxidative deamination of glutamate, whilst facilitating the catabolism of certain amino acids. The latter effect could be due, in part at least, to the fact that glucose acts as a precursor of pyruvic acid, which may act as a partner in the transamination of endogenous amino acids. It should also be kept in mind that glucose may facilitate the oxidation of certain amino acids, e.g. L-asparagine[91]. In other words, the sparing effect of glucose upon the catabolism of certain endogenous nutrients may be compensated, to a limited extent, by its facilitating action upon the oxidation of selected amino acids. It is not surprizing, therefore, that the output of $^{14}CO_2$ derived from exogenous D-[U-^{14}C]glucose barely exceeds the increment in O_2 uptake evoked by this hexose[54]. Hence, it does not appear, from short-term experiments, that glucose, even when used in high concentrations, is able to cover in its entirety, the fuel requirement of the islet cells.

3.2 Metabolism of non-carbohydrate nutrients in islet cells

In considering the metabolism of non-carbohydrate nutrients in pancreatic islets, we will purposedly restrict the discussion to the major circulating nutrients which, at their physiological concentration, could play a significant role in the respiration of islet cells.

3.2.1 Fatty acids

The rate of oxidation of exogenous [U-^{14}C]palmitate (0.7–0.8 mM) is close to 1.5–3.0 pmoles/h per islet[9, 40]. This would account for a rate of O_2 uptake not exceeding 1.1 pmoles/min per islet. Glucose inhibits the oxida-

tion of exogenous palmitate[9]. It should be noted, however, that these data refer solely to the prompt oxidation of exogenous [U-^{14}C]palmitate and, hence, may not inform on the total rate of fatty acid oxidation in islet cells. The view that the latter process represents a significant fraction of the utilization of nutrients in islet cells is supported by a recent study dealing with an inhibitor of long chain fatty acid oxidation, methyl palmoxirate[73]. Methyl palmoxirate acts as an inhibitor of carnitine acyl transferase I[110]. At a concentration of 0.1 mM, the drug immediately suppresses the oxidation of exogenous fatty acids. With a time lapse which could reflect the oxidation of fatty acids already located in the mitochondria, methyl palmoxirate also inhibits the oxidation of endogenous fatty acids. The drug fails to affect, however, the oxidation of exogenous D-[U-^{14}C]glucose and the output of NH_4^+. Methyl palmoxirate also fails to affect the output of $^{14}CO_2$ from islets prelabelled with L-[U-^{14}C]glutamine and incubated for 30 min in the absence of exogenous nutrient. At the concentration of 0.1 mM, methyl palmoxirate inhibits insulin release evoked by D-glucose, D-glyceraldehyde, 2-ketoisocaproate, L-leucine, its nonmetabolized analog BCH or 3-phenylpyruvate. Methyl palmoxirate, however, does not affect insulin release whenever the oxidation of endogenous fatty acids is already suppressed, e.g. in the simultaneous presence of L-glutamine and either D-glucose or L-leucine[73]. These findings indeed suggest that the oxidation of fatty acids contributes to a significant extent to the overall respiratory rate of the pancreatic B-cell.

3.2.2 Amino acids

Considerable work remains to be performed in order to characterize the oxidation rate of different amino acids when they are offered to the islets at their physiological concentration and the simultaneous presence of the normal constellation of other circulating nutrients. In most cases, it can presently only be inferred from either metabolic data obtained at high concentrations of certain amino acids or secretory data obtained under more physiological conditions that circulating amino acids, indeed participate in the physiological regulation of islet metabolism and, hence, insulin release. For instance, although detailed information is available on the metabolism in pancreatic islets of L-leucine and its deamination product 2-ketoisocaproate, when used in the 5–20 mM range (see Malaisse[51] for review), we are aware of only one study, based mainly on secretory data, which suggests that at physiological concentrations of other nutrients, especially D-glucose and L-glutamine, branched chain amino acids, also used at their physiological concentration, indeed augment insulin release by the islets[96].

It was proposed that, among amino acids, L-glutamine represents a major candidate for participation in the fuel metabolism of islet cells[63, 101]. Thus, at a concentration slightly in excess of its normal plasma concentration, L-glutamine is rapidly taken up and duly oxidized in islet cells, whilst exerting a marked sparing action upon the oxidation of endogenous fatty acids. An initial step in the regulation of L-glutamine metabolism in the islet cells may consist in the rate of conversion of L-glutamate to 2-ketoglutarate as modulated by either the activity of the mitochondrial enzyme glutamate dehydrogenase or the availability of suitable 2-keto acids acting as transamination partners[69, 70].

4. The balance between ATP production and breakdown in islet cells

It is generally believed that the rate of ATP formation, as coupled to the oxidation of nutrients, matches its rate of breakdown in ATP-consuming reactions. There are no obvious reasons to believe that such would not be the case in the islet cells. Hence, if the B-cell is to be looked upon as a fuel-sensor organ, the regulation of ATP consumption should be considered with the same interest given to its rate of formation.

4.1 ATP turnover in islet cells

In two reports, an attempt was made to assess the fractional turnover rate of ATP by glucose-stimulated islets exposed to one or several mitochondrial poisons[8, 54]. The interpretation of the data was obscured by a lack of information on the effect of these drugs on ATP expenditure, glycolytic flux and creatine-phosphate concentration and on the possible existence of a compartmentalized stable pool of ATP characterized by a low fractional turnover rate. Because of such limitations, we feel that the measurement of O_2 uptake, although providing indirect information, may as well be used to estimate ATP turnover in islet cells. For instance in the basal state, the steady-state ATP content (ca. 4–6 pmoles/islet) and O_2 consumption rate (ca. 4–6 pmoles/min) would yield a fractional turnover rate of ATP of about 6/min.

4.2 Environmental regulation of nutrient catabolism

In an attempt to identify ATP-consuming processes in islet cells, Sener et al.[101] have examined the influence of several environmental factors upon both D-[U-^{14}C]glucose and L-[U-^{14}C]glutamine oxidation by isolated islets. There was a fair correlation between the two series of observations, which suggested that the handling of Ca^{2+}, the active transport of univalent cations and, in glucose-stimulated islets, the biosynthesis of proteins represented three major ATP-consuming processes in the islet cells. This list should not be considered as exhaustive.

4.3 Islet kinases and ATPases

ATP is converted to ADP in reaction catalyzed by several kinases or ATPases. ATP is also used as a substrate in other reactions, such as that catalyzed by adenylate cyclase. The characterization of these enzymes in islet homogenates or subcellular fractions may help to elucidate the site and regulation of ATP consumption in intact islet cells.

The participation of kinases in the phosphorylation of proteins and lipid molecules (e.g. in the phosphatidylinositol cycle) is evoked elsewhere in this monography.

Several ATPases were already identified in islet cells. For instance, the proton-driven ATPase present in secretory granules is discussed in detail in another report in this series[33]. An ouabain-sensitive $Na^+ + K^+$, Mg^{2+}-ATPase[41,44], a HCO_3^--sensitive Mg^{2+}-ATPase[103] and two Ca^{2+}-sensitive ATPases, located respectively in the plasma membrane and endoplasmic reticulum[15,18,39,42,85] were also identified in crude homogenates or subcellular fractions of pancreatic islets.

5. Conclusion

The present report provides information on the metabolism of nutrients in islet cells, with emphasis on the fate of glucose. When islets are deprived of exogenous nutrient, they maintain for a few hours a stable rate of respiration, which reflects the oxidation of endogenous nutrients, mainly amino acids and fatty acids. Glucose, which only exerts a modest sparing action on the metabolism of endogenous nutrients, is rapidly transported into the pancreatic B-cell and phosphorylated at the intervention of both a glucokinase and a hexokinase, the latter enzyme being inhibited in intact cells by such metabolites as glucose 6-phosphate. Glycolysis represents the major pathway of glucose metabolism and may be regulated not solely at the level of glucose phosphorylation but also by the activity of phosphofructokinase, which is activated by hexose-bisphosphates. The regeneration of NAD^+ from NADH, as coupled for instance to either the conversion of pyruvate to lactate or the operation of the glycerol phosphate shuttle, is required to maintain a sustained rate of glycolysis at the level of glyceraldehyde-3-phosphate dehydrogenase. The oxidation of glucose accounts for about 30% of its rate of utilization. At their physiological concentrations, several other circulating nutrients, e.g. L-glutamine, branched chain amino acids and fatty acids, may, in addition to glucose, contribute to the respiration of islet cells. The oxidation of these nutrients and the coinciding generation of ATP may be matched by the rate of ATP utilization in the islet cells, although further information is required to identify the nature and regulation of ATP-consuming processes in this fuel-sensor organ.

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Coupling factors in nutrient-induced insulin release

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Introduction

In the first report in this series, information was provided on the regulation of nutrient metabolism in islet cells, the view being emphasized that an acceleration of oxidative fluxes may represent an early causal event in the process of nutrient-stimulated insulin release[80]. The present report deals with the coupling of metabolic events to more distal events in the secretory sequence. We wish to make it immediately clear that this funda-