The Pentose Cycle and Insulin Release in Isolated Mouse Pancreatic Islets during Starvation

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When islets from mice were incubated with 16.7 mM-glucose, previous starvation for 48 h decreased the rate of insulin release by approx. 50% and glucose utilization was decreased by approx. 35%. The maximally extractable activity of glucose 6-phosphate dehydrogenase was diminished by 28% after starvation. The formation of $^{14}\text{CO}_2$ from both $[1\text{-}^{14}\text{C}]\text{glucose}$ and $[6\text{-}^{14}\text{C}]\text{glucose}$ was unchanged by starvation. The rate of oxidation of $[1\text{-}^{14}\text{C}]\text{glucose}$ was, however, higher than the rate of oxidation of $[6\text{-}^{14}\text{C}]\text{glucose}$ in islets from both fed and starved mice. The fraction of glucose utilized that was oxidized (specific $^{14}\text{CO}_2$ yield) ranged from one-fifth to one-third and was higher in islets from starved mice with both $[1\text{-}^{14}\text{C}]\text{glucose}$ and $[6\text{-}^{14}\text{C}]\text{glucose}$ as substrate. The contribution of pentose-cycle oxidation to total glucose metabolism was small (3% in the fed state and 4% in the starved state). The absolute rates of glucose carbon metabolism via the pentose cycle and the turnover of NADPH in this pathway were identical in islets from fed and starved animals. After incubation at 16.7 mM-glucose for 30 min the contents of glucose 6-phosphate and 6-phosphogluconate were both unchanged by starvation. It is concluded that there is no correlation between the decreased sensitivity of the insulin secretory mechanism during starvation and the metabolism of glucose via the pentose cycle, the islet content of glucose 6-phosphate or 6-phosphogluconate.

The role of the pentose phosphate pathway in insulin secretion is still unsettled. Matschinsky *et al.* (1968) have measured the activities of most of the enzymes that are involved in the pentose phosphate pathway and they have also demonstrated a marked increase in the concentration of 6-phosphogluconate in islets from obese-hyperglycaemic mice when the extracellular concentration of glucose was raised. Montague & Taylor (1970) demonstrated a close connexion between the islet concentration of 6-phosphogluconate and the rate of insulin secretion under a variety of experimental conditions and suggested that the metabolism of glucose through the pentose phosphate pathway was closely related to the secretion of insulin.

The main functions of the pathway are the supply of pentose phosphates and reducing equivalents (NADPH) for biosynthetic purposes, or in, e.g., erythrocytes, for the regeneration of GSH from GSSG. GSH may again be needed for the maintenance of optimum erythrocyte membrane function. Hellman *et al.* (1974) have suggested that disulphide-bridge conformation of the cell membrane was associated with a resting state of the β -cells and that shifting the equilibrium away from the disulphide to the thiol conformation would enhance insulin release. NADPH may play an important role here by keeping glutathione in a reduced state,

because GSH is a potent reductant of protein disulphides. It is also known that NADPH may react with the insulin-granule membrane to facilitate insulin release (Watkins & Cooperstein, 1970). Heinze & Steinke (1971) have shown that the transition from glucose-insensitive islets in foetal and neonatal rats to fully glucose-sensitive islets in 5-day-old rats was associated with an increase in the rate of oxidation to $^{14}\text{CO}_2$ of $[1\text{-}^{14}\text{C}]\text{glucose}$ compared with the rate of oxidation of $[6\text{-}^{14}\text{C}]\text{glucose}$. On the other hand, Snyder *et al.* (1970) and Ashcroft *et al.* (1972) were unable to demonstrate any increase in the absolute rate of metabolism of glucose through the pentose phosphate pathway when islets were stimulated by high glucose concentrations.

Hedekov & Capito (1974) demonstrated that the maximally extractable activities of hexokinase, glucokinase and glucose 6-phosphatase in mouse pancreatic islets were not affected by starvation, whereas there was indirect evidence that the activity of 6-phosphogluconate dehydrogenase might be diminished during starvation where a decreased insulin response to glucose also could be demonstrated. To assess the possible significance of the pentose phosphate pathway for the decreased sensitivity of the insulin-secretory mechanism during starvation, we have investigated the effect of starvation on the maximally extractable activities of

glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase and the effect on the concentration of their substrates, glucose 6-phosphate and 6-phosphogluconate. Further, the effect of starvation on the flux of glucose carbon through the pentose phosphate pathway has been evaluated.

Experimental

Materials

Luciferase from *Achromobacter fischerii* and 3,5-diaminobenzoic acid were from Sigma Chemical Co., St. Louis, Mo., U.S.A. Other enzymes (including collagenase) and coenzymes were from Boehringer, Mannheim, Germany. Mouse insulin, ¹²⁵I-labelled pig insulin, anti-(pig insulin) and guinea-pig serum were gifts from Dr. J. Schlichtkrull, Novo Research Institute, Copenhagen, Denmark. Human serum albumin was from Behringswerke A.G., Marburg-Lahn, Germany. Sodium merthiolate and the sodium salt of calf thymus DNA were from BDH Chemicals Ltd., Poole, Dorset, U.K. [5-³H]Glucose (2 Ci/mmol), [1-¹⁴C]glucose (201 μCi/mmol) and [6-¹⁴C]glucose (3 mCi/mmol) were from The Radiochemical Centre, Amersham, Bucks., U.K. Instagel was from Packard Instrument Co., La Grange, Ill., U.S.A. Myristaldehyde was from Aldrich Chemical Co., Milwaukee, Wis., U.S.A. Other reagents used were of the purest grade obtainable.

Methods

Preparation of islets. Pancreas from male albino mice (Theillers original strain; Tuck and Son, Rayleigh, Essex, U.K.) was used. For each experiment 12–20 mice of the same age and weight (20–22 g) were selected and divided into two groups. One group was fed on a standard laboratory diet *ad libitum* for 24 h and one group was starved for 48 h (with free access to drinking water) before being killed by decapitation. Starvation was carried out in wire-bottomed metabolic cages to minimize coprophagy. Islets were separated by a collagenase method and harvested with a wire loop as described by Coll-Garcia & Gill (1969).

Islet homogenates. For preparation of a homogenate 200 islets were collected in 50 μl of ice-cold 100 mM-Tris-HCl-10 mM-MgCl₂-0.5 mM-EDTA. The islet suspension was sonicated at 4°C for 15 s on a Branson Sonifier B-12 (set at position 1, 45 W). The homogenate was dialysed against the same buffer and diluted with the appropriate assay buffer.

Insulin release. Batches of five islets were incubated (with shaking) in 0.3 ml of Krebs bicarbonate medium (Krebs & Henseleit, 1932) containing human serum albumin (2 mg/ml) and glucose (3 mg/ml). After 2 h of incubation the tubes were gently centrifuged and

a sample (0.1 ml) of the medium was removed, diluted with 0.04 M-sodium phosphate buffer, pH 7.4, containing human serum albumin (10 mg/ml) and stored at -20°C until assay by radioimmunoassay (Heding, 1966) with mouse insulin as standard.

Glucose utilization. This was determined as the formation of ³H₂O from [5-³H]glucose essentially as described by Ashcroft *et al.* (1972) and Hedekov & Capito (1974).

Oxidation of [1-¹⁴C]glucose and [6-¹⁴C]glucose. Batches of 15 islets were incubated in 10 μl of gassed (O₂+CO₂, 95:5) Krebs-Henseleit buffer containing [1-¹⁴C]glucose or [6-¹⁴C]glucose at concentrations given in the Tables. Incubations were carried out in small glass tubes (5 mm×30 mm) placed inside stoppered scintillation vials for 2 h at 37°C. Blanks without islets were taken through the whole procedure. After incubation, 0.3 ml of Hyamine was injected into the outer vessel and 15 μl of 0.2 M-HCl was injected into the small tube containing the islets to arrest metabolism and liberate CO₂ into the Hyamine. The whole assembly was left overnight to permit complete absorption of CO₂, and the radioactivity in Hyamine was measured by liquid-scintillation spectrometry after addition of 3 ml of Instagel.

The specific radioactivity of glucose in the medium was determined by liquid-scintillation spectrometry and enzymic assay of glucose (Slein, 1963). Glucose-oxidation rates were calculated as pmol of glucose oxidized/2 h per 10 islets from the formula:

$$\text{Glucose oxidized (pmol)} = \frac{{}^{14}\text{CO}_2 \text{ formed (d.p.m.)}}{\text{sp. radioactivity of } [{}^{14}\text{C}]\text{glucose (d.p.m./pmol)}}$$

Specific yields were calculated as pmol of glucose oxidized per pmol of glucose utilized. According to Katz & Wood (1963) the contribution of the pentose cycle pathway to total glucose metabolism can be calculated from the equation:

$$\frac{G1_{\text{CO}_2} - G6_{\text{CO}_2}}{1 - G6_{\text{CO}_2}} = \frac{3\text{PC}}{1 + 2\text{PC}}$$

where $G1_{\text{CO}_2}$ and $G6_{\text{CO}_2}$ are the specific yields of ¹⁴CO₂ from [1-¹⁴C]glucose and [6-¹⁴C]glucose respectively and PC is the fraction of utilized glucose that is metabolized by the pentose cycle pathway. Absolute rates of glucose metabolism can be calculated as PC×glucose utilization. The calculation is only concerned with the oxidative part of the pathway and the pentose cycle pathway is defined according to the overall equation:

Assay of 6-phosphogluconate dehydrogenase activity.

The activity was assayed at 22°C by the change in fluorescence on adding 40–60 µl of dialysed islet homogenate (prepared as described above) to tubes containing 2.45 ml of assay medium (100 mM-Tris-HCl, 10 mM-MgCl₂, 0.5 mM-EDTA and 0.02 mg of bovine plasma albumin/ml, adjusted to pH 7.8, containing 1 mM-6-phosphogluconate and 0.2 mM-NADP⁺). Fluorescence was recorded continuously by using a thermostatically controlled Aminco-Bowman spectrofluorimeter. The formation of NADPH was linear with time and proportional to the amount of homogenate added. Blank incubations without homogenate and blank samples without substrate as well as appropriate spectrophotometrically checked NADPH standards were included in each experiment. All determinations were either in duplicate or in triplicate. The DNA content of 10 µl of homogenate was determined in duplicate for each assay by the method of Kissane & Robins (1958) with calf thymus DNA as standard.

Assay of glucose 6-phosphate dehydrogenase activity.

The same procedure and assay medium described above was used, except that 6-phosphogluconate was replaced by 1 mM-glucose 6-phosphate and 6-phosphogluconate dehydrogenase (0.02 unit/ml) was added. The enzyme was dialysed against 100 mM-Tris-HCl buffer, pH 7.8, before addition to remove sulphate, as this might inhibit the glucose 6-phosphate dehydrogenase activity. In this assay it was assumed that oxidation of 1 mol of glucose 6-phosphate gave rise to 2 mol of NADPH. It was ascertained that further addition of 6-phosphogluconate dehydrogenase did not increase the rate of the reaction. The formation of NADPH was linear

with time and proportional to the amount of homogenate added.

Determination of 6-phosphogluconate. Some 20 islets were incubated for 30 min at 37°C in 25 µl of gassed (O₂+CO₂, 95:5) Krebs-Henseleit buffer containing 0.5 or 3.0 mg of glucose/ml. The reaction was stopped by the addition of 25 µl of 0.1 M-HCl. The islets were sonicated and 40 µl of the sonicate or standard 6-phosphogluconate solutions (made up in Krebs bicarbonate medium-HCl mixture similar in composition to the acidified medium) were analysed for 6-phosphogluconate by the addition of 27 µl of 0.2 M-Tris buffer, pH 8.0, 1.5 µl of 1 mM-NADPH⁺ and 1.5 µl of dialysed 6-phosphogluconate dehydrogenase (12 units/ml before dialysis). After incubation at room temperature for 60 min the content of NADPH was measured by means of bacterial luciferase as described by Ashcroft *et al.* (1973).

Determination of glucose 6-phosphate. Some 10 islets were incubated at 37°C in 28 µl of gassed (O₂+CO₂, 95:5) Krebs-Henseleit buffer containing 0.5 or 3.0 mg of glucose/ml. After 30 min the reaction was stopped by the addition of 0.1 M-HCl and the content of glucose 6-phosphate in the islets was measured as described in detail by Ashcroft *et al.* (1973).

Results

Table 1 shows that the rate of insulin release at 16.7 mM-glucose was diminished by approx. 50% and the rate of glucose utilization at the same glucose concentration was decreased by approx. 33% by starvation. The maximally extractable activity of

Table 1. *Effect of starvation on insulin release, glucose utilization and activities of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase*

For measurement of insulin release batches of five islets from fed and 48 h-starved mice were incubated for 2 h at 37°C in 0.3 ml of gassed Krebs-Henseleit buffer containing 16.7 mM-glucose and human serum albumin (2 mg/ml). The released insulin was determined by radioimmunoassay as described in the Experimental section. Glucose utilization was measured by incubating batches of 15 islets in 10 µl of Krebs-Henseleit buffer containing 16.7 mM-[5-³H]glucose (specific radioactivity 3–4 mCi/mmol). The glucose uptake was calculated from the amount of ³H₂O formed during the incubation. Glucose 6-phosphate dehydrogenase activity and 6-phosphogluconate dehydrogenase activity was measured at 22°C by the change in fluorescence on addition of dialysed homogenate to a reaction mixture as specified in the Experimental section. The enzyme activities are expressed as pmol of substrate converted/min per 100 ng of DNA. All results are given as means ± S.E.M. with the number of determinations in parentheses. Ten islets contain 156 ng of DNA (Hedekov & Capito, 1974).

	Fed	48 h Starved
Insulin release at 16.7 mM-glucose (µunits/ml per five islets per 2 h)	1581 ± 116 (27)	799 ± 107 (22)*
Glucose utilization at 16.7 mM-glucose (pmol/2 h per 10 islets)	1252 ± 63 (12)	821 ± 54 (14)*
Glucose 6-phosphate dehydrogenase activity (pmol/min per 100 ng of DNA)	13.2 ± 1.0 (8)	9.5 ± 0.8 (8)**
6-Phosphogluconate dehydrogenase activity (pmol/min per 100 ng of DNA)	10.1 ± 0.9 (9)	8.2 ± 0.9 (9)†

* $P < 0.001$ versus fed control.

** $P < 0.01$ versus fed control.

† Not significant versus fed control ($P \sim 0.1$).

Table 2. *Effect of starvation on the islet content of 6-phosphogluconate and glucose 6-phosphate*

Batches of 15 islets from fed or 48h-starved mice were incubated for $\frac{1}{2}$ h at 37°C in medium containing glucose at the indicated concentrations. The amount of 6-phosphogluconate and glucose 6-phosphate was determined as described in the Experimental section. The results are given as means $\pm$ S.E.M. with the number of batches of islets shown in parentheses. The values for glucose 6-phosphate (included in this Table for convenience) were taken from a series of parallel experiments published by Hedeksoy & Capito (1974).

Glucose concn. (mM)	Fed		48h Starved	
	2.5	16.7	2.5	16.7
6-Phosphogluconate (pmol/10 islets)	0.79 $\pm$ 0.08 (13)	0.87 $\pm$ 0.11 (13)	0.24 $\pm$ 0.05 (12)**	0.66 $\pm$ 0.08 (12)*
Glucose 6-phosphate (pmol/10 islets)	3.6 $\pm$ 0.4 (15)	11.3 $\pm$ 0.4 (30)*	2.8 $\pm$ 0.2 (19)**	10.5 $\pm$ 0.6 (30)*

* $P < 0.001$ versus low-glucose control.

** $P < 0.001$ versus fed control.

Table 3. *Effect of starvation on the rates of oxidation of [1- 14 C]glucose and [6- 14 C]glucose, the specific yields of 14 CO $_2$ from [1- 14 C]glucose and [6- 14 C]glucose and the absolute rates of glucose oxidation via the pentose cycle pathway*

Batches of 15 islets were incubated in 10 μ l of Krebs-Henseleit bicarbonate medium for 2h at 37°C with radioactive glucose, [1- 14 C]glucose and [6- 14 C]glucose, at the given concentrations. Rates of glucose oxidation were measured and specific yields and rates of metabolism via the pentose cycle were calculated as described in the text. Rates of glucose utilization ranged from 1490 to 1550 pmol/2h per ten islets in the fed state, and from 1040–1100 pmol/2h per ten islets in the starved state. The glucose concentration in the medium is given as 16.7 mM-glucose, but varied in different experiments from 16.6 mM to 18.1 mM. Glucose utilization was determined from a curve relating glucose utilization to medium glucose concentration from a series of parallel experiments. The results are given as means $\pm$ S.E.M. with the number of determinations in parentheses.

Glucose concn. (mM)	Fed			48h Starved		
	2.5	16.7		2.5	16.7	
Oxidation of [1- 14 C]glucose (pmol/2h per 10 islets)		373 $\pm$ 24	(27)*		352 $\pm$ 12	(22)**†
Oxidation of [6- 14 C]glucose (pmol/2h per 10 islets)		287 $\pm$ 23	(28)		273 $\pm$ 22	(23)†
Specific yields of 14 CO $_2$ from [1- 14 C]-glucose	0.499 (2)	0.242 $\pm$ 0.014	(27)‡	0.536 (2)	0.328 $\pm$ 0.010	(22)***‡‡
Specific yields of 14 CO $_2$ from [6- 14 C]-glucose	0.302 (2)	0.187 $\pm$ 0.014	(28)	0.282 (2)	0.257 $\pm$ 0.020	(23)****
Pentose cycle (%)	12.6 (2)	3.0 $\pm$ 0.4	(23)	15.2 (2)	4.0 $\pm$ 0.7	(20)†
Absolute rate of glucose metabolism via the pentose phosphate pathway (pmol/2h per 10 islets)	30 (2)	47 $\pm$ 6	(23)	24 (2)	43 $\pm$ 8	(20)†

† Not significant versus fed control ($P > 0.05$); * $P < 0.02$ versus [6- 14 C]glucose, ** $P < 0.005$ versus [6- 14 C]glucose, *** $P < 0.001$ versus fed control, **** $P < 0.01$ versus fed control, ‡ $P < 0.01$ versus [6- 14 C]glucose, ‡‡ $P < 0.005$ versus [6- 14 C]glucose.

glucose 6-phosphate dehydrogenase was diminished by approx. 30% after 48h starvation. 6-Phosphogluconate dehydrogenase activity was decreased by approx. 20%, but this fall in activity was not statistically significant ($P \sim 0.1$).

Table 2 shows that the content of 6-phosphogluconate in the islets after 30min of incubation at 16.7mM-glucose was not changed by starvation, whereas there was a decrease in 6-phosphogluconate content when islets from starved mice were incubated at 2.5mM-glucose. Incubation of the islets at 16.7mM-glucose compared with incubation at 2.5mM-glucose increased the content of 6-phosphogluconate in islets from starved mice but not in islets from fed mice. The content of glucose 6-phosphate in the islets after 30min of incubation at 16.7mM-

glucose was unchanged by starvation, whereas there was a 30% decrease in islet glucose 6-phosphate content after incubation at 2.5mM-glucose.

Rates of glucose oxidation and glucose utilization were linear over the period of incubation used in these experiments. Table 3 shows that at 16.7mM-glucose the rate of oxidation of both [1- 14 C]glucose and [6- 14 C]glucose was unchanged by starvation. The rate of oxidation of [1- 14 C]glucose was, however, higher than the rate of oxidation of [6- 14 C]glucose in islets from both fed and starved mice. The fraction of glucose utilized that was oxidized ranged from one-fifth to one-third and was definitely higher in islets from starved mice with both [1- 14 C]glucose and [6- 14 C]glucose as substrate. Since specific 14 CO $_2$ yields from [6- 14 C]glucose

equal that fraction of C_3 of pyruvate that is oxidized in the tricarboxylic acid cycle, the percentage contribution of this pathway to total glucose metabolism may be increased during starvation. The contribution of the pentose cycle to total glucose metabolism was small in both the fed and the starved state, but tended to be somewhat higher in the starved state although not statistically significant (3% versus 4% respectively). As the glucose utilization was decreased in the starved state the absolute rates of glucose carbon metabolized via the pentose phosphate pathway were identical in islets from fed and starved mice (47 and 43 pmol/2h per 10 islets respectively), which also means that the turnover of NADPH in this pathway is unchanged by starvation. In two experiments at 2.5mm-glucose the percentage contribution of the pentose cycle to total glucose metabolism was increased to 12.6 and 15.2% in islets from fed and starved mice respectively, but the absolute rates of glucose carbon metabolized via the pentose phosphate pathway was within the range of the values obtained at 16.7mm-glucose, in accordance with the results of Ashcroft *et al.* (1972).

Discussion

It has been concluded by some authors who have measured higher yields of $^{14}CO_2$ from $[1-^{14}C]$ glucose than from $[6-^{14}C]$ glucose by islet tissue that the pentose phosphate pathway plays a significant role in islet glucose metabolism (Field *et al.*, 1960; Jarrett & Keen, 1966; Heinze & Steinke, 1971). However, as stressed by Katz & Wood (1963), for estimation of the flux of glucose carbon through the pentose cycle measurements of specific yields, e.g. the fraction of utilized $[^{14}C]$ glucose oxidized to $^{14}CO_2$ from $[1-^{14}C]$ glucose and $[6-^{14}C]$ glucose, are needed and therefore the experiments must include measurements of total glucose utilization. Certain assumptions have to be fulfilled to use the model of Katz & Wood (1963) for estimation of pentose-cycle flux. Application of the method to islet tissue has been discussed in detail by Ashcroft *et al.* (1972) with the conclusion that the method is valid for in mouse islet tissue.

The main observation on pentose-cycle flux during starvation is that at 16.7mm-glucose the total utilization of glucose was decreased, but the fraction of glucose metabolized by the pentose phosphate pathway was increased and the absolute amount of glucose carbon metabolized via the pentose-cycle remained identical in islets from fed and starved mice respectively, which also means that the turnover of NADPH in this pathway is the same in the fed and in the starved state.

As the rate of insulin secretion at 17mm-glucose is decreased by approx. 50% by starvation there is no correlation between pentose-cycle flux, NADPH

turnover on the one hand and insulin release on the other, in agreement with the conclusions of Ashcroft *et al.* (1972) and Snyder *et al.* (1970).

The maximally extractable activity of the first enzyme in the pathway, glucose 6-phosphate dehydrogenase, was decreased by approx. 30% by starvation without an accompanying decrease in flux through the pathway. This may have its explanation in the fact that the oxidative enzymes of the pentose cycle are inhibited by some 98–99% in the intact islet [compare the flow in Table 3 with the activity of these enzymes as measured in islet homogenates (Table 1), taking into account that 100ng of DNA = approx. six islets]. The activity of these enzymes may be controlled by the cytosolic NADPH/NADP⁺ ratio (Ashcroft & Randle, 1970) and by de-inhibition by AMP or GSSG (Eggelston & Krebs, 1974).

We have given indirect evidence for a decrease in islet 6-phosphogluconate dehydrogenase activity after 48h starvation (Hedekov & Capito, 1974). Direct assay showed that the enzyme activity was decreased by 19% but this was not statistically significant ($P \sim 0.1$). The change in activity may be real but evidently small. More refined methods and a larger number of experiments may be needed to detect it.

Montague & Taylor (1970) found a close connexion between the concentration of 6-phosphogluconate in rat islets and the rate of insulin secretion. In our experiments there was no correlation between the concentration of 6-phosphogluconate and insulin release. At 16.7mm-glucose, starvation causes a decrease in insulin secretion rate, but there is no difference in the islet content of 6-phosphogluconate in the fed and starved state. When the extracellular glucose concentration is raised from 2.5 to 16.7mm there is a bigger increase in insulin-secretion rate in islets from fed mice than in islets from starved mice (Hedekov & Capito, 1974). With 6-phosphogluconate content exactly the converse is true; there is no rise in 6-phosphogluconate in the fed state but almost a three-fold increase in the starved state. This lack of correlation of 6-phosphogluconate concentration with insulin release agrees with the results of Idahl *et al.* (1971), who showed that the addition of adrenalectomy to or omission of Ca^{2+} from a high-glucose medium (both experimental conditions that inhibit insulin release) gave a significant elevation of the islet 6-phosphogluconate content.

At 16.7mm-glucose the metabolism of glucose carbon via the different pathways seems to be altered by starvation. Glycolysis is diminished, but the absolute rate of pentose-cycle flux is unchanged, and the same can be said about the tricarboxylate-cycle activity. The specific yield of $^{14}CO_2$ from $[6-^{14}C]$ glucose (which means that fraction of C_3 of pyruvate that is oxidized in the tricarboxylate cycle) was increased in islets from starved mice, but the absolute

rate of oxidation of $[6-^{14}\text{C}]$ glucose to $^{14}\text{CO}_2$ was unaltered during starvation.

In conclusion, there does not seem to exist a correlation between the decreased sensitivity of the insulin-secretory mechanism during starvation and the pentose-cycle flux, the tricarboxylic acid-cycle activity or the islet content of 6-phosphogluconate and glucose 6-phosphate, whereas the decreased insulin secretion seen during starvation is clearly associated with a decreased glycolysis. The decreased glycolysis may again effect either Ca^{2+} distribution in the islets and/or their ability to raise their content of cyclic AMP on stimulation, as discussed in more detail elsewhere (Hedekov & Capito, 1975).

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