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Glycogen Accumulation in Rat Pancreatic Islets

In Vitro Experiments

Margaret Doherty and Willy J. Malaisse

Laboratory of Experimental Medicine, Brussels Free University, Belgium

Under conditions of sustained hyperglycemia, glycogen accumulates in pancreatic islets, but not so in acinar pancreatic cells. Advantage conceivably could be taken from such a situation in the perspective of the noninvasive imaging of the endocrine pancreas. The present experiments aim, therefore, at characterizing the time course for glycogen accumulation in pancreatic islets cultured at a high concentration (30 m*M*) of D-glucose in the presence of tracer amounts of either D-[U-^{14}C]glucose or 2-deoxy-2-[^{18}F]fluoro-D-glucose. The ^{14}C-labeled glycogen content of the cultured islets increased with time (150 min to 72 h), exceeded that found in acinar tumoral cells, and did not decrease over 60 min of incubation at 30 m*M* D-glucose in the absence of D-[U-^{14}C]glucose. Glycogenolysis was observed, however, when the concentration of D-glucose was decreased to 2.8 m*M* and, in such a case, was further enhanced by forskolin and theophylline. Such a glycogenolysis coincided with the generation of $^{14}CO_2$ from radioactive intracellular precursors and alteration of the B-cell secretory response to D-glucose. The radioactive glycogen content was higher in islets exposed to 2-deoxy-2-[^{18}F]fluoro-D-glucose than D-[U-^{14}C]glucose. Prior exposure of the islets to streptozotocin suppressed the accumulation of glycogen during their subsequent culture at high D-glucose concentration. These findings may help to define the experimental conditions optimal for the labeling and accumulation of islet glycogen in vivo.

Key Words: Glycogen; pancreatic islets; endocrine pancreas imaging.

Introduction

Under conditions of sustained hyperglycemia, e.g., in animal models of type-2 diabetes, glycogen accumulates in pancreatic islets, but not so in acinar pancreatic cells (*1–5)*. Advantage conceivably could be taken from such a situation in the perspective of the noninvasive imaging of the endocrine pancreas.

In such a perspective, the present work was aimed at answering the following questions. First, what is the time course for glycogen accumulation in islets exposed to D-[U-^{14}C]glucose and how does this compare with tumoral acinar cells? Second, is it possible to label the glycogen pool in islets cultured for 1–3 d at high D-glucose concentrations and exposed to D-[U-^{14}C]glucose only for a few hours at the end of this culture period? Third, under the same experimental conditions, is it possible to maintain the pool of ^{14}C-labeled glycogen when the islets are placed, after the labeling period, in a medium still containing the high concentration of the hexose but in the absence of D-[U-^{14}C]glucose? Fourth, and for the opposite purpose, is it possible to rapidly deplete the islets of their ^{14}C-labeled glycogen content by exposing them to a low concentration of D-glucose and/or to agents raising the cell content in cyclic adenosine monophosphate? Fifth, could 2-deoxy-2-[^{18}F]fluoro-D-glucose be used to label islet glycogen? Finally, does a prior exposure of the islets to streptozotocin abolish the accumulation of glycogen during the subsequent culture at high D-glucose concentration?

Results

Glycogen Accumulation in Cultured Islets

Figure 1 illustrates the time course for the accumulation of radioactive glycogen in rat islets cultured in the presence of 30 m*M* D-glucose mixed with a tracer amount of D-[U-^{14}C]glucose. The radioactive glycogen content of the islets increased from 3.37 ± 0.59 to 5.76 ± 0.65 pmol of D-[U-^{14}C]glucose equivalent/islet ($n = 12$ in both cases; $p < 0.02$) as the incubation time with D-[U-^{14}C]glucose (30 m*M*) was increased from 150 to 300 min (Table 1, experiment 1). A comparable time course for the deposition of radioactive glycogen was observed when the islets were exposed to D-[U-^{14}C]glucose after a prior incubation of 150–240 min in the sole presence of unlabeled D-glucose (30 m*M*). This is illustrated in Fig. 2 (left), in which the amount of ^{14}C-labeled glycogen is expressed relative to the corresponding value for the intracellular pool of radioactive amino acids.

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Author to whom all correspondence and reprint requests should be addressed: Dr. Willy J. Malaisse, Laboratory of Experimental Medicine, Brussels Free University, 808 Route de Lennik, B-1070 Brussels, Belgium. E-mail: malaisse@med.ulb.ac.be

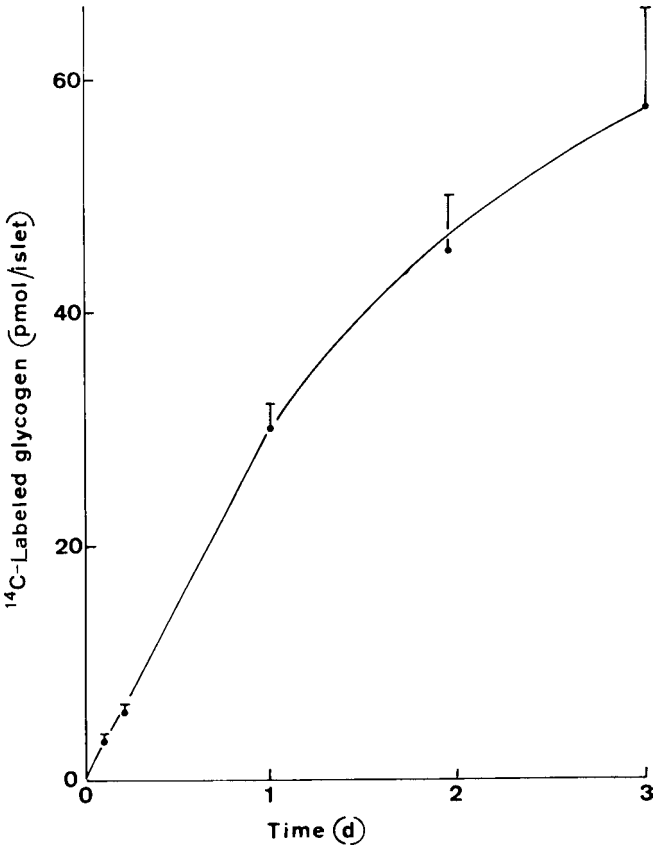

Fig. 1. Time course for the accumulation of ^{14}C-labeled glycogen in islets exposed to 30.0 mmol/L D-glucose in the presence of D-[U-^{14}C]glucose. Mean values (±SEM) refer to 10–69 individual observations.

When the rat islets were cultured for 72 h in the presence of 30.0 m*M* D-glucose (mixed with a tracer amount of D-[U-^{14}C]glucose), their final content in ^{14}C-labeled glycogen averaged 57.2 ± 8.7 pmol/islet (n = 69), when expressed as glucose residues with the same specific radioactivity as D-[U-^{14}C]glucose in the culture medium (Table 1, experiment 2). The corresponding values for the islet content in ^{14}C-labeled acidic metabolites and amino acids, expressed in the same manner, were significantly lower ($p < 0.001$), not exceeding, respectively, 23.0 ± 1.0 and 16.5 ± 1.0 pmol/islet (n = 69 in both cases).

In fair agreement with the time course illustrated in Fig. 1, the generation of radioactive glycogen by islets incubated for 180 min at 30.0 m*M* D-glucose in the presence of a tracer amount of D-[U-^{14}C]glucose was lower ($p < 0.001$) when the islets had been first cultured for 48 h at the same high concentration of D-glucose (6.61 ± 0.28 pmol of D-glucose equivalent/islet; n = 24) than in islets first cultured for 48 h in the presence of only 5.6 m*M* D-glucose (11.50 ± 0.57 pmol of D-glucose equivalent/islet; n = 24). This contrasted with the fact that, in these experiments, the preincubation at low vs high concentration of D-glucose failed to affect significantly the radioactive content of the perchloric acid (PCA)-precipitable material, acidic metabolite pool, and amino acid pool of the islets, the readings made in islets first cultured at 30.0 m*M* D-glucose averaging, respectively, 97.0 ± 10.5% (n = 24), 89.5 ± 9.7% (n = 12), and 105.3 ± 3.7% (n = 23) of the mean corresponding values (100.0 ± 7.4%, n = 24; 100.0 ± 12.0%, n = 11; and 100.0 ± 3.0%, n = 24) found in islets first cultured at 5.6 m*M* D-glucose. In the case of ^{14}C-labeled glycogen, the value found after the prior culture at 30.0 m*M* D-glucose only represented 57.5 ± 2.4% (n = 24) of that measured after culture at 5.6 m*M* D-glucose (100.0 ± 5.0%; n = 24).

Experiments in AR42J Cells

Control experiments conducted in tumoral acinar cells of the AR42J line indicated that after 72 h of culture in the presence of 30.0 m*M* D-glucose (mixed with a tracer amount of D-[U-^{14}C]glucose), the radioactive glycogen content of the cells did not exceed 1.35 ± 0.20 pmol/2000 cells (n = 48), compared with 57.2 ± 8.7 pmol/islet (see above). Such a mean value was lower ($p < 0.02$ or less) than the cell content in ^{14}C-labeled acidic metabolites (3.84 ± 0.37 pmol/2000 cells) or amino acids (2.03 ± 0.17 pmol/2000 cells), in sharp contrast to the situation found in pancreatic islets under otherwise comparable experimental conditions. All mean values just mentioned are derived from 48 individual measurements, are expressed as D-glucose equivalent by reference to the specific radioactivity of D-[U-^{14}C]glucose in the culture medium, and refer to 2000 cells for the purpose of comparison with islets, each of which contains approximately the same number of cells.

Transient Exposure to D-[U-^{14}C]Glucose of Islets Cultured at High Concentration of Hexose

To simulate the experimental conditions used in some of the in vivo experiments to be presented in a separate report, the islets were cultured for 2 d at 30.0 m*M* D-glucose in the absence or presence of D-[U-^{14}C]glucose. In the former case, they were then incubated for 120 min at 30.0 m*M* D-glucose in the presence of the ^{14}C-labeled hexose. In both cases, some groups of islets were eventually further incubated for 60 min, still at 30.0 m*M* D-glucose but now in the absence of D-[U-^{14}C]glucose. In these experiments, it was first observed that in islets first incubated in the absence of D-[U-^{14}C]glucose, the content in ^{14}C-labeled acidic metabolites increased in proportion to the length of the incubation with D-[U-^{14}C]glucose at a mean rate of 7.8 ± 0.7 pmol/(islet·h) (n = 30) (Fig. 2, middle). Such a progressive increase was much less pronounced, however, in the case of ^{14}C-labeled amino acids. As shown in Fig. 2 (right), after 120 min of incubation in the presence of D-[U-^{14}C]glucose, the islet content in labeled glycogen averaged 15.3 ± 1.9% (n = 39) of the corresponding mean value found within the same experiment(s) after 48 hof culture in the presence of the radioactive hexose, i.e., 45.3 ± 4.8 pmol/islet (n = 25).

When the islets cultured for 2 d in the presence of 30.0 m*M* D-glucose (together with D-[U-^{14}C]glucose) were fur-

Table 1
Accumulation of ^{14}C-Labeled Acidic Metabolites, Amino Acids, and Glycogen in Islets Cultured in Presence of 30.0 m*M* D-glucose With or Without D-[U-^{14}C]Glucose

Experiment no.	D-[U-^{14}C]Glucose (min)	No D-[U-^{14}C]Glucose (min)	Acidic metabolites (pmol/islet)[a]	Amino acids (pmol/islet)[a]	Glycogen (pmol/islet)[a]
1	0–150	—	10.0 ± 1.2 (12)	4.6 ± 0.8 (12)	3.4 ± 0.6 (12)
	0–300	—	9.7 ± 1.1 (12)	5.4 ± 0.4 (12)	5.8 ± 0.7 (12)
	151–300	0–150	13.6 ± 2.3 (12)	7.6 ± 1.2 (12)	5.5 ± 0.5 (12)
	241–300	0–240	9.7 ± 0.8 (12)	4.9 ± 0.5 (12)	2.8 ± 0.4 (12)
2	0–4320	—	23.0 ± 1.0 (69)	16.5 ± 1.0 (69)	57.2 ± 8.7 (69)
3	0–2820	—	20.1 ± 3.8 (16)	10.1 ± 3.0 (25)	45.3 ± 4.8 (25)
	0–2820	2821–2880	11.5 ± 2.4 (18)	3.9 ± 2.2 (18)	39.7 ± 3.3 (18)
	2701–2820	0–2700	15.6 ± 1.5 (30)	5.4 ± 1.0 (29)	6.3 ± 1.0 (39)
	2701–2820	0–2700 and 2821–2880	9.2 ± 2.8 (18)	2.4 ± 0.8 (18)	11.9 ± 2.1 (16)

[a]Results are expressed as picomoles of D-[U-^{14}C]glucose equivalent/islet by reference to the specific radioactivity of the labeled hexose in the culture medium.

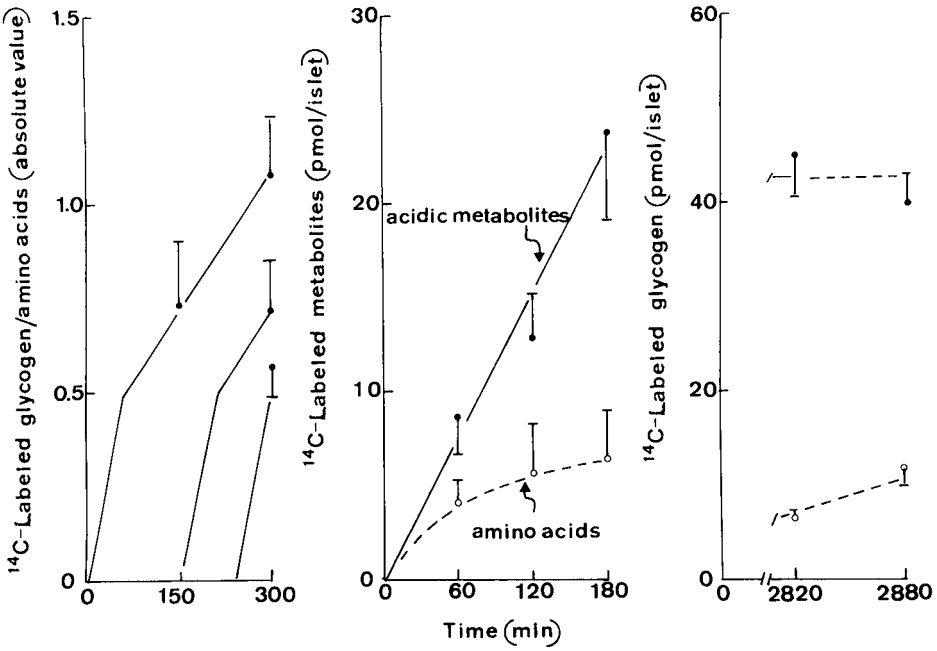

Fig. 2. (**Left**) Time course for the accumulation of ^{14}C-labeled glycogen (relative to the paired value for the islet content in radioactive amino acids) in islets incubated for 150–300 min in the presence of 30.0 mmol/L of D-glucose and exposed to D-[U-^{14}C]glucose either throughout incubation or after 150–240 min of incubation in its absence. Mean values (±SEM) refer to 12 individual observations in all cases. (**Middle**) Time course for the accumulation of ^{14}C-labeled acidic metabolites and amino acids in islets incubated in the presence of 30.0 mmol/L of D-glucose and exposed for 60–180 min to D-[U-^{14}C]glucose after culture for 2 d at the high hexose concentration in the absence of labeled tracer. Mean values (±SEM) refer to seven individual observations in all cases. (**Right**) Time course for the changes in the ^{14}C-labeled glycogen content of islets first cultured for 47 h at 30.0 mmol/L D-glucose and exposed to D-[U-^{14}C]glucose either for the same period (●) or only for the last 120 min of culture (○) and then incubated for 60 min at 30.0 mmol/L of D-glucose in the nominal absence of the labeled hexose. Mean values (±SEM) refer to 16–25 individual observations.

ther incubated for 60 min at the same hexose concentration but in the absence of D-[U-^{14}C]glucose, their glycogen content was barely decreased ($p > 0.25$), averaging 87.7 ± 7.3% ($n = 18$) of the mean corresponding value found with the same experiments at the end of the culture period in the presence of the radioactive hexose (Fig. 2, right). The islet content in ^{14}C-labeled acidic metabolites and amino acids was more severely decreased ($p < 0.05$), however, down to an overall mean value of 47.8 ± 12.2% ($n = 36$).

A comparable fall in the islet content of radioactive acidic metabolites and amino acids was observed when the islets were incubated for 60 min in the presence of 30.0 m*M* D-glucose but in the absence of D-[U-^{14}C]glucose after only 120 min of exposure to the radioactive hexose at the end of the 2-day culture period (Table 1, experiment 3). Thus, the ^{14}C-labeled acidic metabolites and amino acids only represented 52.0 ± 11.8% ($n = 36$; $p < 0.02$) of the mean corresponding value found within the same experiments imme-

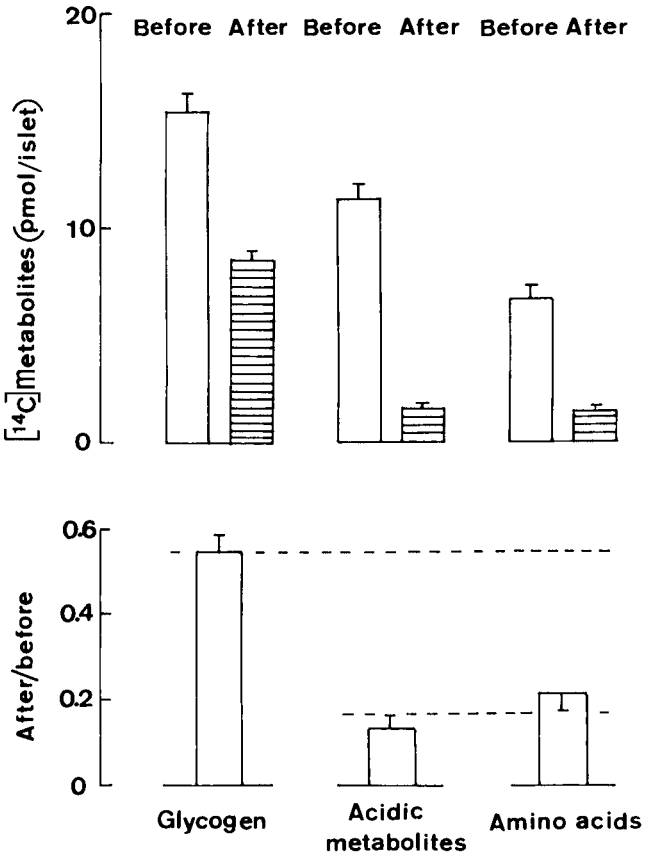

Fig. 3. Islet content in ^{14}C-labeled glycogen, acidic metabolites, and amino acids before and after a last day of culture at 30.0 m*M* D-glucose in the nominal absence of D-[U-^{14}C]glucose. The islets were first cultured for 1 d at the same high concentration of D-glucose and exposed to D-[U-^{14}C]glucose during the last 120 min of this first culture period. Mean absolute values (±SEM) refer to 57–60 individual measurements (**top**). Hence, the ratios after/before the last day of culture (**bottom**) corresponds to degrees of freedom of 115–118.

diately after exposure to D-[U-^{14}C]glucose. However, in these experiments, the islet content in radioactive glycogen failed to decrease during the last 60 min of incubation in the nominal absence of the labeled hexose and, on the contrary, was somewhat higher ($p < 0.025$) after than before such an incubation.

As shown in Fig. 3, a somewhat comparable situation was found when the period of culture in the presence of 30.0 m*M* D-glucose but nominal absence of D-[U-^{14}C]glucose amounted to 24 h after a first day of culture at the same high concentration of D-glucose including exposure of the islets to D-[U-^{14}C]glucose for the last 120 min. Thus, the islet content in radioactive glycogen decreased, after the last day of culture, to 8.44 ± 0.45 pmol of D-glucose equivalent ($n = 60$), representing 54.6 ± 4.0% of the mean value (15.46 ± 0.79 pmol/islet; $n = 60$) recorded immediately after the 120-min exposure to D-[U-^{14}C]glucose. The islet content in radioactive acidic metabolites and amino acids decreased to a much larger relative extent ($p < 0.001$), averaging at the end of the experiments only 16.6 ± 2.1% (233 df) of the measurements made immediately after the labeling period.

Glycogenolysis

In the next set of experiments, the possible stimulation of glycogenolysis by forskolin and theophylline was investigated. The islets were cultured for 48 h in the presence of D-glucose (30 m*M*) mixed with a tracer amount of D-[U-^{14}C]glucose and then examined for their content in ^{14}C-labeled glycogen either before or after a further incubation for 15 min conducted in the absence or presence of forskolin and theophylline.

Relative to the mean final content of the islets in ^{14}C-labeled glycogen after 48 of culture, that recovered in islets further incubated for 15 min in the nominal absence of D-[U-^{14}C]glucose but in the presence of 30.0 m*M* unlabeled D-glucose was slightly, albeit not significantly ($p < 0.1$), increased to a mean value of 140.8 ± 15.3% ($n = 32$). This coincided with the fact that, after the last 15-min incubation, the radioactive content of the media in neutral metabolites averaged, when expressed relative to the specific radioactivity of D-[U-^{14}C]glucose in the initial culture medium, 3.39 ± 0.31 m*M* ($n = 32$). The latter value would correspond to a contamination of the islet pellet, after removal of most of the culture medium, by 45.3 ± 4.2 μL of such a medium. The presence of forskolin (10 μ*M*) and theophylline (1.4 m*M*) in the final incubation medium failed to decrease significantly the amount of ^{14}C-labeled glycogen recovered in the islets, with a mean ratio between experimental/control values of 87.8 ± 20.6% (30 df).

A different situation prevailed when the final incubation of 15 min in the nominal absence of D-[U-^{14}C]glucose was conducted in the presence of only 2.8 m*M* D-glucose. In this case, the glycogen content of the islets, expressed by reference of the specific radioactivity of D-[U-^{14}C]glucose in the culture medium, decreased ($p < 0.001$) from a mean value of 47.2 ± 3.5 pmol/islet ($n = 8$) before the final incubation to 28.6 ± 1.7 pmol/islet ($n = 8$) thereafter. The presence of forskolin (10 μ*M*) and theophylline (1.4 m*M*) in the final incubation medium now caused a further decrease ($p < 0.03$) of the glycogen content to 24.1 ± 0.9 pmol/islet ($n = 9$). In these experiments, the apparent contamination of the islet pellet, after removal of most of the culture medium, did not exceed 22.8 ± 2.4 μL of such a medium ($n = 18$).

When groups of 30 islets each were cultured for 24 h in the presence of 30.0 m*M* D-glucose mixed with a tracer amount of D-[U-^{14}C]glucose, washed thrice, and eventually incubated for 60 min at 37°C in a medium (40 μL) containing 2.8 m*M* unlabeled D-glucose, the production of $^{14}CO_2$ during the final incubation failed to be significantly affected by the presence of cytochalasin B (0.2 m*M*) in the medium (data not shown). However, it was decreased ($p < 0.001$) from a control value of 9.44 ± 0.97 pmol of D-glucose equivalent (with the same specific radioactivity as that of the culture medium) per islet ($n = 20$) to 4.32 ± 0.98

pmol/islet in the presence of 1.25 m*M* KCN, 2.5 μ*M* antimycin A, and 2.5 μ*M* rotenone. The difference between these two sets of data (5.13 ± 1.34 pmol/islet; 37 df) documents the oxidation during the final incubation of ^{14}C-labeled metabolites derived from intracellular precursors, e.g., radioactive glycogen.

Even after only 180 min of incubation at 20.0 m*M* D-glucose, the accumulation of glycogen may be sufficient to affect, during a subsequent short incubation of 20 min, the secretory response of the islets to the hexose. Thus, under these conditions, the output of insulin during the last incubation of 20 min was paradoxically not lower ($p > 0.8$) when the islets were incubated in the absence of any exogenous nutrient (66.1 ± 5.9 μU/islet; $n = 20$) than in islets maintained at 20.0 m*M* D-glucose (63.3 ± 5.4 μU/islet; $n = 20$). Moreover, when both theophylline (1.4 m*M*) and cytochalasin B (20.0 μ*M*) were added to the incubation medium containing no exogenous nutrient, the output of insulin was further increased ($p < 0.02$) to 85.4 ± 4.9 μU/islet ($n = 40$), although theophylline and cytochalasin B normally fail to affect insulin release from glucose-deprived islets *(6,7)*.

Experiments with 2-Deoxy-2-[^{18}F]fluoro-D-Glucose

A further set of experiments was aimed at comparing the labeling of islet glycogen by D-[U-^{14}C]glucose and by 2-deoxy-2-[^{18}F]fluoro-D-glucose. Groups of 20 islets each were first cultured for 2 d in the presence of 30.0 m*M* D-glucose and then incubated for 60 min, at the same high concentration of the hexose, in the presence of either D-[U-^{14}C]glucose or 2-deoxy-D-[^{18}F]fluoro-D-glucose. In the former case, the radioactive glycogen content of the islets averaged 5.1 ± 0.9 pmol/islet ($n = 48$), when expressed by reference to the specific radioactivity of D-[U-^{14}C]glucose in the final incubation medium. In the islets exposed for 60 min to the tracer amount of 2-deoxy-2-[^{18}F]fluoro-D-glucose in the presence of 30.0 m*M* unlabeled D-glucose, the amount of labeled glycogen recovered in the islets, when expressed by reference to the calculated specific radioactivity of the hexose, averaged 11.2 ± 2.5 pmol/islet ($n = 44$), a value significantly higher ($p < 0.02$) than that found, within the same experiments, with D-[U-^{14}C]glucose. Likewise, the islet content in radioactive acidic metabolites, expressed in the same manner as indicated above, was higher ($p < 0.005$) in the islets exposed to 2-deoxy-2-[^{18}F]fluoro-D-glucose (33.9 ± 2.4 pmol/islet; $n = 46$) than in those incubated with D-[U-^{14}C]glucose (9.9 ± 1.9 pmol/islet; $n = 48$).

This finding coincided with the fact that, when tested at a 10.0 m*M* concentration, unlabeled 2-deoxy-2-fluoro-D-glucose decreased ($p < 0.03$ or less) over 24 h of culture both the conversion of D-[U-^{14}C]glucose (30.0 m*M*) to ^{14}C-labeled acidic metabolites from a control value of 2.22 ± 0.07 to 1.70 ± 0.03 nmol of D-[U-^{14}C]glucose equivalent/islet and the final content of the islets in such acidic metabolites from 58.6 ± 1.3 to 48.1 ± 4.1 pmol/islet. Nevertheless, under the same conditions, 2-deoxy-2-fluoro-D-glucose increased ($p < 0.005$) the islet content in ^{14}C-labeled glycogen from a control value of 30.1 ± 2.1 to 41.4 ± 2.5 pmol/islet ($n = 10$ in all cases). However, in these experiments, the release of ^{14}C-labeled amino acids in the culture medium (0.27 ± 0.02 vs 0.26 ± 0.02 n*M*/islet) and their final content in the islets (47.3 ± 3.8 vs 41.6 ± 3.6 pmol/islet) were not significantly decreased by 2-deoxy-2-fluoro-D-glucose. This finding is consistent with the knowledge that the generation of such radioactive amino acids from D-[U-^{14}C]glucose refers to a pool rapidly reaching its close-to-equilibrium value *(8)*.

Effects of Streptozotocin on Hormonal and Metabolic Variables

To facilitate the interpretation of data collected in vivo, the effect of streptozotocin on glycogen accumulation and other variables was examined in the last set of experiments. Groups of 30 islets each were first incubated for 60 min at 37°C in 1.0 mL of a HEPES- and bicarbonate-buffered medium *(4)* containing 8.3 m*M* D-glucose in either the absence or presence of 3.8 m*M* streptozotocin *(9)*. The islets were then cultured for 72 h at 30.0 m*M* D-glucose (mixed, when required, with a tracer amount of D-[U-^{14}C]glucose).

The release of insulin in the culture medium was decreased ($p < 0.001$) from a control value of 4.13 ± 0.20 to 0.50 ± 0.17 mU/islet ($n = 24$ in both cases) in the islets first exposed to streptozotocin. The insulin content of the islets, after the culture period, was significantly higher ($p < 0.001$), however, in the islets first exposed to streptozotocin (1.00 ± 0.05 mU/islet; $n = 24$) than in the control islets (0.71 ± 0.04 mU/islet; $n = 24$). Thus, the total amount of insulin released during the 72-h culture period and recovered in the islets thereafter was about three times higher ($p < 0.01$) in the control islets (4.84 ± 0.21 mU/islet) than in those first exposed to streptozotocin (1.51 ± 0.17 mU/islet), indicating severe impairment of proinsulin biosynthesis in the latter islets.

During the culture period, the release of ^{14}C-labeled acidic metabolites and amino acids exceeded, in the control islets, by 25.64 ± 1.47 and 1.41 ± 0.07 nmol of D-glucose equivalent/islet (df = 46 and $p < 0.001$ in both cases), respectively, that found in the islets first exposed to streptozotocin (i.e., ≤3.07 ± 0.04 and ≤0.39 ± 0.01 nmol/islet; $n = 24$ in both cases). In other words, the release of ^{14}C-labeled acidic metabolites and amino acids represented, in the streptozotocin islets, at most 10.7 ± 5.1 and 21.6 ± 5.0% (46 df in both cases) of the corresponding control value.

The prior exposure of the islets to streptozotocin decreased ($p < 0.001$) their final content in ^{14}C-labeled glycogen from 72.8 ± 1.5 to 9.4 ± 1.3 pmol of D-glucose equivalent/islet ($n = 24$ in both cases). Likewise, the islet content in radioactive trichloroacetic acid (TCA)-precipitable material was decreased ($p < 0.001$) from 118.6 ± 11.2 to 28.0 ± 2.7 pmol of D-glucose equivalent/islet ($n = 24$ in both cases). Last, the islet content in ^{14}C-labeled acidic metabolites and amino

acids averaged, respectively, 72.6 ± 3.0 and 154.2 ± 2.0 pmol of D-glucose equivalent/islet in the control islets, as distinct ($p < 0.001$) from only 15.9 ± 2.6 and 22.5 ± 2.1 in the streptozotocin islets ($n = 24$ in all cases).

Discussion

The present study provides four essential pieces of information in the perspective of using the accumulation of radioactive glycogen in pancreatic islets as a possible tool for the preferential labeling of the endocrine moiety of the pancreas after iv injection of ^{11}C-labeled D-glucose or 2-deoxy-2-[^{18}F]fluoro-D-glucose in either normal human subjects or animals rendered hyperglycemic by a constant infusion of D-glucose.

First, it confirms that pancreatic islets accumulate sizable amounts of glycogen when cultured at a high concentration of D-glucose. A different situation may well prevail in pancreatic acinar cells. Because of the short lifetime of dispersed nonimmortalized pancreatic acinar cells, the stable rat pancreatic acinar cell line AR42J was used as the culture system for investigating glycogen accumulation in the exocrine pancreas. Such tumoral cells differ, however, from normal acinar cells in several respects (*10*). With this reservation in mind, the accumulation of glycogen, relative to the generation of ^{14}C-labeled acidic metabolites and amino acids from D-[U-^{14}C]glucose, was much lower in the AR42J cells than in islets examined under the same experimental conditions.

Second, a sizable labeling of islet glycogen was achieved after only 1–5 h of culture in the presence of D-[U-^{14}C]glucose. Such a labeling was less pronounced in islets first cultured for 2 d at high (30.0 m*M*), as distinct from low (5.6 m*M*), D-glucose concentration. The labeling of islet glycogen was not decreased when the islets were eventually incubated for 60 min at the high concentration of D-glucose but in the nominal absence of D-[U-^{14}C]glucose. After the final incubation conducted in the absence of D-[U-^{14}C]glucose, the islet content in radioactive acidic metabolites and amino acids was markedly decreased, however. This suggests that advantage could be taken from a comparable situation in vivo in order to decrease the contribution of acidic metabolites and amino acids to the total radioactive content of the pancreatic gland. The labeled islet glycogen pool was decreased, however, within 15 min of incubation at a low D-glucose concentration (2.8 m*M*). Under the latter experimental conditions, the amount of ^{14}C-labeled glycogen was further decreased by forskolin and theophylline. Such was not the case when the islets were incubated for 15 min at the same high concentration of D-glucose (30.0 m*M*) as that used during the prior culture period. These findings are consistent with the regulation of both glycogen synthase and glycogen phosphorylase activities in pancreatic islets (*11*). The glycogenolysis observed during incubation at the low concentration of D-glucose (2.8 m*M*) was associated with a production of $^{14}CO_2$ resistant to cytochalasin B and in excess of that found in islets exposed to metabolic poisons. It apparently coincided with stimulation of insulin release, as previously already documented in glycogen-enriched islets (*2*,*3*).

Third, our work documents that 2-deoxy-2-[^{18}F]fluoro-D-glucose could be used, instead of D-[U-^{14}C]glucose, to label islet glycogen. This finding does not conflict with the concept that 2-deoxy-2-fluoro-D-glucose 6-phosphate is not further metabolized in the glycolytic pathway and acts as a substrate in the reaction catalyzed by glucose-6-phosphatase. Indeed, if the presence of the F atom in position 2 prevents the conversion of 2-deoxy-2-fluoro-D-glucose 6-phosphate to its fructose 6-phosphate ester in the reaction catalyzed by phosphoglucoisomerase, because of the impossibility of generating the intermediate enediol on the enzyme, this does not apply to the sequence of reactions catalyzed by phosphoglucomutase, UDP-glucose pyrophosphorylase, and glycogen synthase (*12*). In fact, the accumulation of radioactive glycogen was higher in islets exposed to 2-deoxy-2-[^{18}F]fluoro-D-glucose rather than D-[U-^{14}C]glucose. Such a difference could well be attributable to the just mentioned inability of 2-deoxy-2-fluoro-D-glucose 6-phosphate to be metabolized in the glycolytic pathway. It indeed coincided with a higher content of the islets in labeled acidic metabolites after exposure to 2-deoxy-2-[^{18}F]fluoro-D-glucose, as distinct from D-[U-^{14}C]glucose. It obviously represents a favorable attribute in the perspective of using the accumulation of radioactive glycogen in the islets as a tool to label preferentially the endocrine moiety of the pancreas.

Fourth, the experiments conducted in islets first exposed to streptozotocin indicate that this β-cytotoxic agent prevents the accumulation of radioactive glycogen after subsequent culture of the islets at a high concentration of D-glucose. Hence, streptozotocin-induced diabetes would indeed appear as a suitable model to compare in vivo the accumulation of glycogen in islet cells of control and diabetic animals, even when all B-cells are not destroyed by streptozotocin.

In conclusion, the present work warrants further investigations on the use of radioactive glycogen accumulation in islet cells as a tool for the preferential labeling of the endocrine moiety of the pancreatic gland. Parallel work conducted in vivo and to be reported elsewhere further supports such a proposal.

Materials and Methods

D-[U-^{14}C]glucose was purchased from NEN (Boston, MA), and 2-deoxy-2-[^{18}F]fluoro-D-glucose was prepared as described elsewhere (*13,14*) and provided by P. Damhaut (PET/Biomedical Cyclotron Unit, Erasmus Hospital, Brussels, Belgium).

Pancreatic tumoral cells of the AR42J line (*10*), kindly given by Dr. M. Svodoba (Biological Chemistry and Nutri-

tion Laboratory, Brussels University, Brussels, Belgium), were grown in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY) supplemented with 5.6 m*M* D-glucose, 4.0 m*M* L-glutamine, 100 U/mL of penicillin, 100 µg/mL of streptomycin, and 10% (v/v) fetal bovine serum in a 5% CO_2 humidified incubator at 37°C. The increase in the number of cells (N) from its initial value (N_0) over 1–3 d of culture (t) yielded, according to the equation $N = N_0.e^{gt}$ (*15*), a mean multiplication factor (g) of 0.258 ± 0.009 d^{-1} ($n = 9$).

Pancreatic islets were isolated by the collagenase technique (*16*) from fed female Wistar rats (Iffa Credo, L'Arbresle, France). The islets were cultured in RPMI-1640 (Gibco) supplemented with 10% (v/v) heat-inactivated fetal calf serum, 2 m*M* L-glutamine, 100 U/mL of penicillin, and 100 µg/mL of streptomycin, and containing 30 m*M* D-glucose (except if otherwise mentioned) mixed, when required, with a tracer amount of D-[U-^{14}C]glucose.

After culture, the islets were washed once with a non-radioactive culture medium and then sonicated (three times for each 10 s on ice) in a solution (250 µL) of perchloric acid (5% [v/v]) containing 25 mg/mL of carrier glycogen. After centrifugating for 5 min at 4°C and 1800*g*, an aliquot (200 µL) of the PCA extract was mixed with 600 µL of ethanol. The glycogen pellet obtained after centrifugating for 10 min at 4°C and 2000*g* was resuspended in 100 µL of H_2O and again mixed with 400 µL of ethanol. This washing procedure was repeated four times before measuring the radioactive content of glycogen by liquid scintillation (or using an automatic gamma counter in the case of experiments conducted in the presence of 2-deoxy-2-[^{18}F]fluoro-D-glucose).

An aliquot (600 µL) of the supernatant solution obtained after the first centrifugation for glycogen deposition was mixed with 150 µL of KOH (1.0 *M*) and left on ice for 10 min. After centrifugating for 10 min at 4°C and 2000*g*, an aliquot (500 µL) of the neutralized PCA extract was passed successively through a AG 1-X8 (Bio-Rad, Hercules, CA) anion-exchange resin and Dowex 50 WX8 (Fluka Chemie AG, Buchs, Switzerland) cation-exchange resin columns to separate ^{14}C-labeled acidic metabolites and amino acids (*17,18*).

The methods used to measure insulin release and content (*16*), and the production of ^{14}C-labeled CO_2 (*19*), acidic metabolites (*17*), and amino acids (*18*) by the islets were all described in the cited references.

All results are expressed as the mean values (±SEM) together with either the number of individual observations (n) or degrees of freedom (df). The statistical significance of differences between mean values was assessed by use of student's t-test.

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