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Labeling of Pancreatic Glycogen by D-[U-^{14}C]Glucose in Hyperglycemic Rats

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Under conditions of sustained hyperglycemia, glycogen accumulates in pancreatic islets, but not so in acinar pancreatic cells. We investigated whether advantage could be taken of such a situation in the perspective of the noninvasive imaging of the endocrine pancreas. Control rats or animals injected with streptozotocin (STZ) were infused with solutions of D-glucose mixed with a tracer amount of D-[U-^{14}C]glucose, and the radioactive glycogen content of both liver and pancreas was then measured. After 48 h of infusion, the radioactive glycogen content of the pancreas was 30 times lower in STZ rats than in control animals, coinciding with a 50 times lower insulin content. In the control rats, a sizable labeling of pancreatic glycogen was also recorded when D-[U-^{14}C]glucose was infused for only the last 4 h of unlabeled D-glucose infusion; such a labeling was not decreased when the animals were further infused for 1 h with only the unlabeled hexose. Moreover, a pronounced difference in the pancreatic gland and blood radioactive content of control rats was still observed when the hyperglycemic animals were killed only 40 min after the iv injection of D-[U-^{14}C]glucose. In STZ rats transplanted with islets and later infused with D-[U-^{14}C]glucose, the total radioactive content and radioactive glycogen content were both much higher in the transplanted islets than in the pancreatic gland. These results allow one to define the conditions under which the administration of either 2-deoxy-2-[^{18}F]fluoro-D-glucose or ^{11}C-labeled D-glucose could conceivably be used to favor the selective labeling of the endocrine, as distinct from exocrine, pancreas.

Key Words: Glycogen; pancreas; hyperglycemia; D-[U-^{14}C]glucose.

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Introduction

The labeling of pancreatic glycogen in protracted provoked hyperglycemia was recently proposed as a possible tool for imaging of the endocrine moiety of the pancreatic gland by a noninvasive procedure *(1)*. Such a proposal was inspired by the knowledge that under conditions of sustained hyperglycemia, e.g., in animal models of type 2 diabetes, glycogen accumulates in pancreatic islet B-cells *(2, 3)*, but not so in acinar pancreatic cells *(4)*. In a prior report, the optimal conditions for the labeling of glycogen in isolated pancreatic islets was already investigated *(5)*. The major aim of the present study was to extend these investigations to experiments conducted in vivo in either control or streptozotocin (STZ)-induced diabetic rats infused with a hypertonic solution of D-glucose mixed with a tracer amount of D-[U-^{14}C]glucose.

Results

Long-Term Infusion of D-[U-^{14}C]Glucose

In the first set of experiments, D-[U-^{14}C]glucose was infused for 48 h together with unlabeled D-glucose (1.67 *M* in the control rats and 0.25 *M* in the STZ rats). As shown in Table 1, the STZ rats lost, over the period of 5 d following the administration of STZ, 18.5 ± 4.3 g, whereas the control animals gained 5.3 ± 2.5 g over the same period ($p < 0.005$). When compared with the control animals, the STZ rats were severely hyperglycemic and hypoinsulinemic. The insulin content of the pancreas averaged 930 ± 42 and 12 ± 4 mU ($n = 4$ in both cases; $p < 0.001$) in control and STZ rats, respectively. Shortly after halting the infusion of D-glucose, the control rats displayed a lower plasma D-glucose concentration but higher plasma insulin concentration than before the infusion of the hexose, in fair agreement with a prior observation *(3)*. Thus, in the control rats, the paired (d 5–d 3) difference in plasma D-glucose concentration, plasma insulin concentration, and insulinogenic index averaged, respectively, –2.00 ± 0.45 m*M* ($n = 4$; $p < 0.025$), +32.7 ± 6.1 μU/mL ($n = 4$; $p < 0.02$), and +9.29 ± 2.16 U/mol ($n = 4$; $p < 0.025$). When expressed relative to the specific radioactivity of the infused D-glucose, the radioactive gly-

Table 1
Metabolic and Hormonal Data in Glucose-Infused Rats

Rats	Control	STZ	*p*
Body wt (g)			
Day of surgery (d 0)	258.3 ± 6.6 (4)	236.5 ± 9.8 (4)	>0.1
Onset of infusion (d 3)	262.3 ± 7.9 (4)	226.3 ± 9.3 (4)	<0.05
At sacrifice (d 5)	263.5 ± 7.0 (4)	218.0 ± 8.4 (4)	<0.01
Paired change (d 3–d 0)	+4.0 ± 4.1 (4)	−10.3 ± 5.2 (4)	<0.1
Paired change (d 5–d 3)	+1.3 ± 3.1 (4)	−8.3 ± 1.5 (4)	<0.05
Paired change (d 5–d 0)	+5.3 ± 2.5 (4)	−18.5 ± 4.3 (4)	<0.005
Plasma D-glucose (mmol/L)			
Day 3	6.98 ± 0.24 (4)	33.93 ± 2.04 (4)	<0.001
Day 5	4.98 ± 0.34 (4)	46.83 ± 2.10 (3)	<0.001
Plasma insulin (μU/mL)			
Day 3	41.3 ± 7.3 (54)	6.9 ± 4.5 (4)	<0.01
Day 5	74.0 ± 4.8 (4)	12.1 ± 9.6 (3)	<0.001
Insulinogenic index (U/mol)			
Day 3	5.89 ± 0.97 (4)	0.23 ± 0.15 (4)	<0.005
Day 5	15.18 ± 1.80 (4)	0.27 ± 0.21 (3)	<0.001
Pancreas			
Weight (mg)	1053 ± 55 (4)	664 ± 51 (4)	<0.005
Insulin content (μU/mg)	893.9 ± 78.4 (4)	19.1 ± 6.4 (4)	<0.001
Liver			
Weight (g)	10.35 ± 0.20 (4)	11.33 ± 0.67 (4)	>0.2
Glycogen			
Liver (nmol/mg)[a]	149.6 ± 18.0 (4)	4.3 ± 1.3 (4)	<0.001
Pancreas (pmol/mg)[a]	1729 ± 371 (4)	84 ± 20 (4)	<0.005
(nmol/pancreas)[a]	1760 ± 279 (4)	54 ± 11 (4)	<0.001

[a]Results are expressed by reference to the specific radioactivity of infused D-glucose.

cogen content of both the liver and the pancreas was much lower in STZ rats than in control animals. The pancreatic content in ^{14}C-labeled glycogen in the control and STZ rats was virtually proportional to the insulin content of the pancreatic gland (Fig. 1). As shown in Table 2, even when expressed relative to the radioactive content of the infusate, the radioactive glycogen content of the liver and pancreas remained significantly lower ($p < 0.025$ or less) in STZ rats than in control animals. This contrasted with the fact that the radioactive content of the neutralized perchloric acid (PCA) and ethanol extract prepared from the liver and pancreas was not lower in STZ rats than in control animals. The paired ratio between radioactive glycogen and ^{14}C-labeled material recovered in the neutralized PCA and ethanol extracts indeed averaged, in control and STZ rats, respectively, 136.3 ± 14.7 and 21.0 ± 4.9% in the liver ($p < 0.001$) and 7.2 ± 1.3 and 1.8 ± 0.7% in the pancreas ($p < 0.01$).

When the radioactive material present in the neutralized PCA and ethanol extracts was analyzed by ion-exchange chromatography, the recovery averaged 94.8 ± 0.8 and 95.4 ± 3.8% ($n = 8$ in both cases) in the liver and pancreas, respectively. The mean amount of ^{14}C-labeled acidic metabolites was somewhat lower, albeit not significantly, in the liver or pancreas of STZ rats, as compared with control rats. Likewise, the amount of radioactive amino acids was not significantly different in STZ and control rats, whether in the liver or pancreas. In sharp contrast to these results, the amount of ^{14}C-labeled neutral metabolites (e.g., radioactive D-glucose or sorbitol) was about 50% higher in the liver ($p < 0.05$) and nine times higher in the pancreas ($p < 0.005$) of STZ rats, as distinct from control rats. When expressed by reference to the specific radioactivity of infused D-[U-^{14}C]glucose, this neutral material averaged in the pancreas of control and STZ rats, 2.53 ± 0.17 and 3.54 ± 0.60 nmol/mg, respectively ($n = 4$ in both cases; $p < 0.15$). These findings strongly suggest that, at least in the pancreas, the specific radioactivity of D-glucose, relative to that of infused D-[U-^{14}C]glucose, was only slightly lower in STZ rats than in control animals. It reinforces, therefore, the view that the amounts of pancreatic ^{14}C-labeled glycogen, as given in Table 1, are close to the true values, being possibly slightly underestimated in the STZ rats, when compared with control animals.

Short-Term Infusion of D-[U-^{14}C]Glucose

In the next series of experiments, normal rats were infused for 2 d with the hypertonic solution of D-glucose (1.67 *M*), but D-[U-^{14}C]glucose was infused only for 240 min, and the animals were killed either immediately or 1 h after the administration of the radioactive hexose. In all cases, the in-

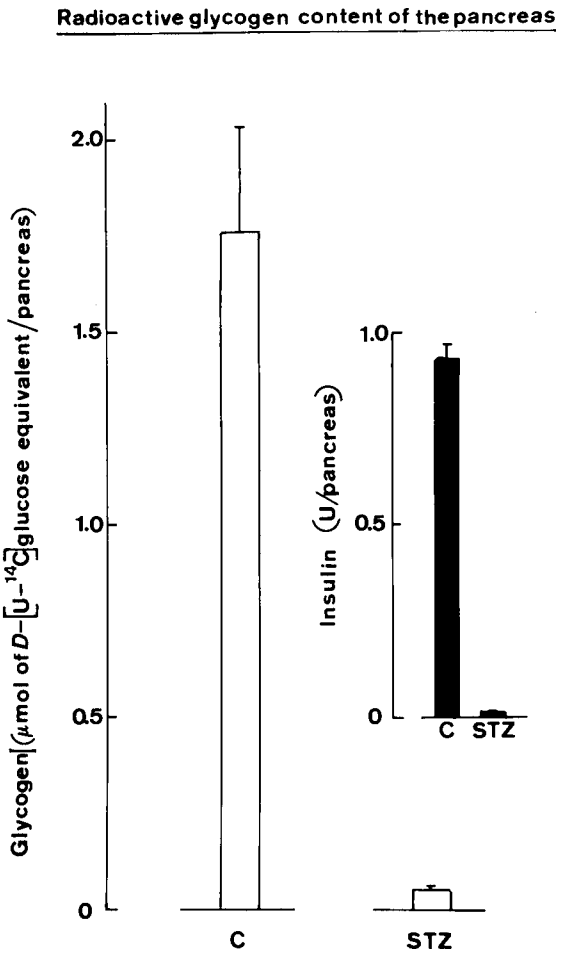

Fig. 1. Pancreatic content in ^{14}C-labeled glycogen and insulin (inset) in control (C) and STZ rats infused for 48 h with a hypertonic solution of D-glucose mixed with a tracer amount of D-[U-^{14}C]glucose. Mean values (±SEM) refer to four individual observations in all cases.

fusion of unlabeled D-glucose (1.67 *M*) was continued up to the time of sacrifice. In this second series of experiments, the body wt of the rats failed to change significantly over the period of 5 d between surgery and sacrifice (Table 3). The plasma D-glucose and insulin concentrations were much higher ($p < 0.02$ or less) after than before D-glucose infusion. When all available measurements made before and either during or shortly after the infusion of D-glucose were pooled, the insulinogenic index also increased ($p < 0.001$) from a basal value of 5.25 ± 0.93 U/mol ($n = 10$) to 27.69 ± 6.71 U/mol ($n = 14$).

The insulin content of the pancreas (1147.8 ± 164.6 μU/mg; $n = 10$) was comparable ($p > 0.3$) with that recorded in the first series of experiments.

The glycogen content of the pancreas (as measured by a nonradioisotopic procedure) was much lower than that of the liver (Table 3). In both cases, it largely exceeded that of ^{14}C-labeled glycogen, if the assumption were made that the specific radioactivity of circulating D-glucose at the end of the experiments would be identical to that of the infused hexose. The five values for pancreatic glycogen content exceeding 10.0 nmol/mg (25.7 ± 5.0 nmol/μg) were found in rats with a mean plasma D-glucose concentration of 18.14 ± 3.95 m*M*, while the five values for pancreatic glycogen content below 10.0 nmol/mg (6.4 ± 0.7 nmol/μg) were recorded in rats in which the plasma D-glucose concentration did not exceed 6.58 ± 0.46 m*M*; the differences between these two sets of animals were significant in the case of both pancreatic glycogen content ($p < 0.01$) and plasma D-glucose concentration ($p < 0.02$).

When D-[U-^{14}C]glucose was infused only during the last 4 h of administration of unlabeled D-glucose (1.67 mol/L of solution infused for 48 h) to normal rats, the radioactive glycogen content of the liver and pancreas, relative to the concentration or specific radioactivity of D-[U-^{14}C]glucose in the solution infused during the last 4 h of the experiments, was one order of magnitude lower than in the first set of experiments, i.e., when D-[U-^{14}C]glucose was infused for 48 h (Table 4). The relative contribution of different metabolites to the overall radioactive content of the liver and pancreas was not identical, however, in the two series of experiments. For instance, the paired ratio between ^{14}C-labeled glycogen and the radioactive material present in the neutralized PCA and ethanol extracts was decreased ($p <$ 0.05 or less) to mean values of 13.0 ± 3.6 and 0.9 ± 0.2% in the liver and pancreas, respectively, as compared with corresponding percentages of 136.3 ± 14.7 and 7.2 ± 1.3% ($n = 4$ in all cases) in the normal rats infused with D-[U-^{14}C]glucose throughout the experiments (Fig. 2).

When the rats were perfused for 60 min with only unlabeled D-glucose (1.67 mol/L) after the administration of D-[U-^{14}C]glucose, the content of both the liver and pancreas in ^{14}C-labeled glycogen was not lower than in the rats that received D-[U-^{14}C]glucose for 4 h up to the time of sacrifice. In both the liver and pancreas, the paired ratio between ^{14}C-labeled glycogen and the radioactive material present in the neutralized PCA and ethanol extracts was even higher ($p < 0.005$ or less) in the former than latter animals (Fig. 2).

Prolonged Infusion of Unlabeled D-Glucose After Transient Administration of D-[U-^{14}C]Glucose

The results summarized in Fig. 2 document that the infusion of unlabeled D-glucose for 60 min after the administration of D-[U-^{14}C]glucose favors, in both the liver and pancreas, the contribution of radioactive glycogen relative to that of other ^{14}C-labeled metabolites. It was investigated, therefore, whether by prolonging the infusion of unlabeled D-glucose to 24 h after the administration of D-[U-^{14}C]glucose the fractional contribution of radioactive glycogen to the total ^{14}C content of the liver and pancreas could be further enhanced in normal and STZ rats. For such a purpose, either control or STZ rats were infused for 3 d with D-glucose (1.67 *M* in con-trol rats and 0.25 *M* in STZ rats). The infusate con-tained D-[U-^{14}C]glucose from h 45 to h 48 of infusion inclusive.

Table 2
Radioactive Content of Tissue Homogenates and Extracts in Glucose-Infused Rats

	Liver			Pancreas		
	Control rats	STZ rats	*p*	Control rats	STZ rats	*p*
Total homogenate	357.0 ± 11.6 (4)[a]	133.9 ± 16.3 (4)	<0.001	141.2 ± 19.3 (4)	55.1 ± 7.6 (4)	<0.01
PCA-soluble material	164.3 ± 13.4 (4)	91.0 ± 17.1 (4)	<0.02	24.3 ± 4.8 (4)	26.9 ± 3.7 (4)	>0.6
Glycogen	89.6 ± 10.8 (4)	17.2 ± 5.3 (4)	<0.001	1.04 ± 0.22 (4)	0.33 ± 0.08 (4)	<0.025
Neutralized PCA and ethanol extract	65.5 ± 3.1 (4)	76.3 ± 9.7 (4)	>0.3	14.37 ± 1.05 (4)	21.87 ± 3.11 (4)	<0.1
Neutral metabolites	32.83 ± 0.96 (4)	50.33 ± 6.12 (4)	<0.05	1.52 ± 0.10 (4)	14.01 ± 2.37 (4)	<0.005
Acidic metabolites	23.65 ± 0.78 (4)	16.88 ± 2.68 (4)	<0.1	9.73 ± 0.70 (4)	5.90 ± 1.61 (4)	<0.1
Amino acids	5.64 ± 2.00 (4)	4.31 ± 0.34 (4)	>0.5	1.57 ± 0.12 (4)	1.87 ± 0.49 (4)	>0.5

[a]All results are expressed as nanoliters of infusate equivalent per milligram of wet wt.

Table 3
Metabolic and Hormonal Data in Glucose-Infused Rats
Examined Before or After 60-min Period of Unlabeled D-Glucose Infusion

Rats	Before	After	*p*
Body wt (g)			
Day of surgery (d 0)	290.8 ± 10.3 (4)	238.0 ± 12.3 (6)	<0.02
At sacrifice (d 5)	292.0 ± 8.4 (4)	236.8 ± 11.5 (6)	<0.01
Paired change (d 5–0)	+1.3 ± 2.3 (4)	−1.2 ± 3.3 (6)	>0.5
Plasma D-glucose (mmol/L)			
Day 3	6.56 ± 0.19 (4)	7.91 ± 0.67 (6)	>0.1
Day 5	13.17 ± 3.34 (4)	16.02 ± 3.86 (6)	>0.6
Plasma insulin (μU/mL)			
Day 3	41.9 ± 10.8 (4)	44.9 ± 11.5 (6)	>0.8
Day 5	388.0 ± 157.6 (4)	654.7 ± 124.2 (6)	>0.2
Insulinogenic index (U/mol)			
Day 3	5.80 ± 1.36 (4)	4.91 ± 1.32 (6)	>0.6
Day 5	18.00 ± 5.82 (4)	49.14 ± 10.56 (6)	<0.1
Pancreas			
Weight (mg)	893 ± 75 (4)	864 ± 77 (6)	>0.8
Insulin content (μU/mg)	667.3 ± 162.2 (4)	1468.2 ± 139.5 (6)	<0.01
Glycogen content (nmol/mg)	6.58 ± 0.85 (4)	22.39 ± 5.36 (6)	<0.05
Liver			
Weight (g)	11.08 ± 1.08 (4)	11.90 ± 0.52 (6)	>0.4
Glycogen content (nmol/mg)	298.3 ± 83.8 (4)	334.5 ± 43.9 (6)	>0.6

Table 4
Radioactive Content of Tissue Homogenates and Extracts
in Glucose-Infused Rats Examined Before or After 60-min Period of Unlabeled D-Glucose Infusion

	Liver			Pancreas		
	Before	After	*p*	Before	After	*p*
Total homogenate	83.6 ± 7.0 (4)[a]	78.8 ± 12.5 (6)	>0.7	23.7 ± 4.5 (4)	19.2 ± 1.7 (6)	>0.3
PCA-soluble material	39.8 ± 7.2 (4)	25.0 ± 4.5 (6)	>0.1	11.1 ± 0.6 (4)	7.3 ± 0.7 (6)	<0.01
Glycogen	6.34 ± 2.31 (4)	11.26 ± 2.69 (6)	>0.2	0.11 ± 0.02 (4)	0.16 ± 0.05 (6)	>0.4
Neutralized PCA and ethanol extract	40.8 ± 5.3 (4)	15.7 ± 2.3 (6)	<0.005	10.8 ± 1.2 (4)	4.7 ± 2.0 (6)	<0.1
Neutral metabolites	12.93 ± 2.39 (4)	6.94 ± 1.37 (6)	<0.05	1.71 ± 0.57 (4)	1.16 ± 0.20 (6)	>0.3
Acidic metabolites	10.38 ± 2.07 (4)	6.75 ± 0.95 (6)	>0.1	6.53 ± 0.51 (4)	3.27 ± 0.27 (6)	<0.001
Amino acids	0.87 ± 0.06 (4)	0.68 ± 0.12 (6)	>0.2	1.02 ± 0.10 (4)	0.75 ± 0.15 (6)	>0.2

[a]All results are expressed as nanoliters of infusate equivalent per milligram of wet wt.

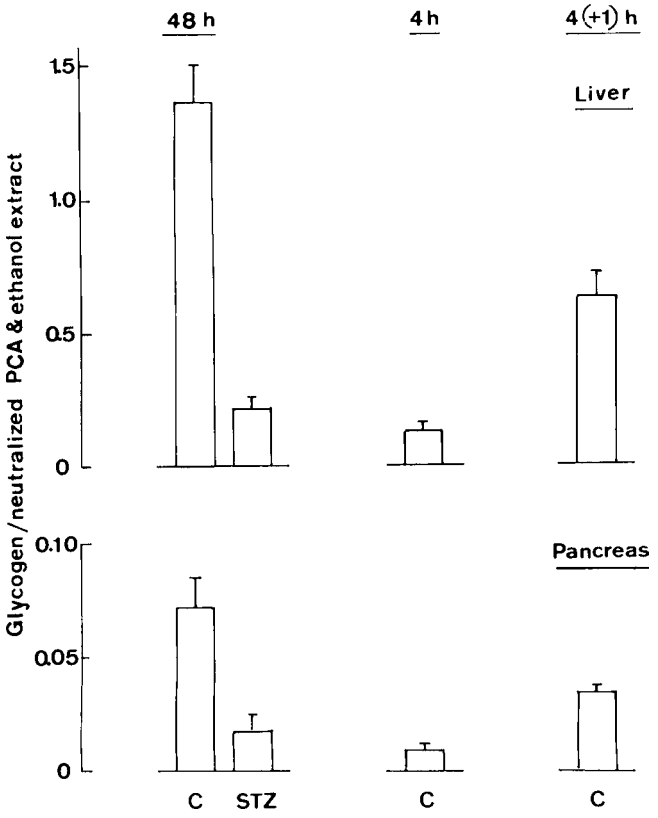

Fig. 2. Paired ratio between ^{14}C-labeled glycogen and radioactive material present in the neutralized PCA and ethanol extracts in the liver (**top**) or pancreas (**bottom**) of either control (C) or STZ rats infused with a hypertonic solution of D-glucose for 48 to 49 h; (**left**) D-[U-^{14}C]glucose is also present in the infusate throughout the infusion period, (**middle**) only for the last 4 h of a 48-h infusion period, or (**right**) from h 45 to h 48 inclusive during a 49-h infusion period. Mean values (±SEM) refer to four to six individual observations.

Whereas over the period of 3 to 4 d following surgery the control rats gained 1.1 ± 0.5 g/d, the STZ rats lost 7.1 ± 0.6 g/d (4 d) after the administration of STZ and subsequent surgery (n = 3 in both cases; $p < 0.001$). The wet wt of the liver and pancreas was not significantly different, however, in control and STZ rats (Table 5).

The plasma D-glucose concentration was much higher ($p < 0.001$) in STZ rats than in control animals before the infusion of D-glucose. In the former rats, it increased ($p < 0.05$) by 6.02 ± 1.08 mM (n = 3) during infusion of the hexose. In the control rats, however, the changes in plasma D-glucose concentration caused by the infusion of D-glucose were quite variable, including a minor decrease (−1.88 mM), virtually no change (+0.60 mM), and a dramatic increase (+14.57 mM) in such a concentration. Nevertheless, in all control rats, the infusion of D-glucose markedly augmented the plasma insulin concentration, resulting in a mean paired rise of the insulinogenic index (i.e., the paired ratio between plasma insulin and D-glucose concentrations) of 23.8 ± 5.9 U/mol (n = 3; $p < 0.01$). Such was not the case in STZ rats, in which the insulinogenic index averaged no more than 1.26 ± 0.17 and 1.31 ± 0.16 U/mol before and after the infusion of D-glucose, respectively (n = 3 in both cases). The insulin content of the pancreas averaged 1.69 ± 0.45 mU/mg in control rats, as compared ($p < 0.001$)with only 32.9 ± 6.3 μU/mg in STZ rats (n = 3 in both cases).

The radioactive content of the liver, when expressed as nanoliters/milligram by reference to the radioactive content of the infusate, was higher ($p < 0.005$) in control animals (45.7 ± 14.2 nL/mg; n = 3) than in STZ rats (3.9 ± 0.8 nL/mg; n = 3). In the control rats, there was a highly significant correlation between such a radioactive liver content (nanoliters/milligram) and the plasma D-glucose concentration at sacrifice (r = 1.0000). The hepatic radioactive PCA-soluble material, expressed in the same manner, was also higher ($p < 0.005$) in control animals (9.4 ± 1.8 nL/mg) than in STZ rats (1.8 ± 0.3 nL/mg). In the control rats, the ^{14}C-labeled glycogen content of the liver, expressed by reference to the specific radioactivity of infused D-glucose, was inversely related to the plasma glucose concentration at sacrifice (r = 0.7980). In fact, the ^{14}C-labeled glycogen content of the liver (expressed as nanomoles/milligram of wet wt by reference to the specific radioactivity of infused D-glucose) when multiplied by the paired plasma D-glucose concentration at sacrifice yielded closely comparable values in all control rats (45.5 ± 4.9 nmol·mM/mg; n = 3). Even the lowest value for such a hepatic radioactive glycogen content found in the control rats (0.8 nmol/mg) largely exceeded that found in the STZ rats (17 ± 7 pmol/mg; n = 3). Likewise, the total glycogen content of the liver was significantly higher ($p < 0.01$) in control animals (344 ± 61 nmol/mg) than in STZ rats (52 ± 8 nmol/mg). The liver content in ^{14}C-labeled neutral molecules recovered in the neutralized PCA and ethanol extracts, expressed as D-glucose equivalent with the same specific radioactivity as that of the infused hexose, averaged 0.96 ± 0.25 nmol/mg in control rats, as distinct ($p < 0.01$) from only 0.17 ± 0.03 nmol/mg in STZ rats (n = 3 in both cases). Such a difference is consistent with the knowledge that gluconeogenesis contributes, in the STZ rats, to the generation of D-glucose in the hepatocytes. This is also supported by the fact that the mean ^{14}C-labeled glycogen/total glycogen ratio was about 34 times higher ($p < 0.02$) in the liver of control animals than in the STZ rats, in which it did not exceed 0.34 ± 0.19‰ (n = 3).

Even when expressed relative to the radioactive content of the infusate, that of the pancreas remained much higher ($p < 0.001$) in the control animals (19.89 ± 3.79 nL/mg; n = 3) than in the STZ rats (1.38 ± 0.19 nL/mg; n = 3). However, the mean radioactive glycogen content of the pancreas was now barely higher ($p < 0.09$) in the control animals (16.9 ± 3.6 pmol/mg; n = 3) than in the STZ rats (8.6 ± 0.8 pmol/mg; n = 3). Moreover, in the control rats, the radioactive glycogen content accounted for only 0.60 ± 0.15‰ (n = 3) of the total pancreatic radioactivity.

Table 5
Metabolic and Hormonal Data in Rats Infused with D-[U-^{14}C]Glucose for 240 min (h 45–48) During 3-d Infusion of Unlabeled D-Glucose

Rats	Control	STZ	*p*
Body wt (g)			
Day zero	207.3 ± 6.2 (3)	241.0 ± 5.2 (3)	<0.02
Day 3 (or 4)	211.0 ± 6.9 (3)	212.7 ± 7.5 (3)	>0.8
Day 6 (or 7)	202.7 ± 4.7 (3)	212.7 ± 10.9 (3)	>0.4
Plasma D-glucose (m*M*)			
Before infusion	7.25 ± 0.82 (3)	32.90 ± 2.83 (3)	<0.001
After infusion	11.68 ± 8.41 (3)	38.92 ± 3.87 (3)	<0.05
Paired change	+4.43 ± 5.12 (3)	+6.02 ± 1.08 (3)	>0.7
Plasma insulin (μU/mL)			
Before infusion	64.3 ± 44.3 (3)	43.0 ± 8.4 (3)	>0.6
After infusion	517.6 ± 311.8 (3)	51.4 ± 7.4 (3)	>0.2
Paired change	+453.3 ± 329.9 (3)	+8.4 ± 6.4 (3)	>0.2
Liver			
Wet wt (g)	10.81 ± 0.91 (3)	9.22 ± 0.41 (3)	>0.1
(% of body wt)	5.30 ± 0.53 (3)	4.34 ± 0.07 (3)	>0.1
Pancreas			
Wet wt (mg)	715 ± 53 (3)	942 ± 94 (3)	>0.1
(‰ of body wt)	3.51 ± 0.20 (3)	4.40 ± 0.23 (3)	<0.05
Insulin content (μU/mg)	1687 ± 446 (3)	33 ± 6 (3)	<0.001

These findings clearly indicate that a prolonged delay (24 h) between the end of D-[U-^{14}C]glucose infusion and that of unlabeled D-glucose infusion was not favorable for enhancing the relative contribution of ^{14}C-labeled glycogen to the total radioactive content of the pancreatic gland. Nevertheless, the dramatic difference in such a total radioactive content between control and STZ rats should not be ignored, for at least two reasons. First, a high total pancreatic radioactive content (13.38 nL/mg, when expressed relative to the radioactive content of the infusate) was also observed in the sole control rat that became severely hyperglycemic as a result of the infusion of D-glucose. Second, the neutral radioactive molecules recovered in the neutralized PCA and ethanol pancreatic extracts when expressed as D-glucose equivalent with the same specific radioactivity as that of the infusate were only twofold higher ($p < 0.02$) in the control animals (128.9 ± 18.0 pmol/mg; $n = 3$) than in the STZ rats (51.7 ± 10.0 pmol/mg; $n = 3$). Thus, even if it is ignored that such a difference may reflect, to some extent at least, the presence of ^{14}C-labeled D-glucose in the islet B-cells of the control rats, it suggests that the specific radioactivity of D-glucose was, at the end of the experiments and relative to that of the infused hexose, not vastly lower in the STZ rats than in the control animals. Such a view is further supported by the fact that the paired ratio between ^{14}C-labeled and total glycogen content of the pancreatic gland was indeed comparable ($p > 0.15$) in control and STZ rats, with mean respective values of 0.87 ± 0.25 and 0.49 ± 0.01‰ ($n = 3$ in both cases). Moreover, even when the aforementioned mean values for the neutral radioactive molecules recovered in the neutralized PCA and ethanol pancreatic extracts were expressed relative to the mean corresponding plasma D-glucose concentrations at the time of sacrifice, they yielded a control:STZ ratio (8.31) that re-mained one order of magnitude lower than the control:STZ ratio for the total radioactive content of the pancreatic gland (also expressed by reference to the specific radioactivity of the infusate, namely 81.77, i.e., 31.71 ± 6.32/0.38 ± 0.05).

Intravenous Injection of D-[U-^{14}C]Glucose 180 min Before Sacrifice

To simulate the experimental conditions that could be used for imaging of the endocrine pancreas after injection of 2-deoxy-2-[^{18}F]fluoro-D-glucose *(6)*, control rats were infused with a hypertonic solution of D-glucose (1.67 *M*) for 240 min and injected intravenously with D-[U-^{14}C]glucose 180 min before sacrifice (i.e., at min 60 of the D-glucose infusion). For purposes of comparison, STZ rats were also injected with D-[U-^{14}C]glucose 180 min before sacrifice.

Over the 4 d following surgery, the body wt of the control rats was not significantly affected (+0.3 ± 1.9 g), whereas the STZ rats lost 12.8 ± 3.2 g over a same period of 4 d after the administration of STZ ($n = 4$ in both cases; $p < 0.02$). The liver wet wt, when expressed in absolute terms, was not significantly different in control and STZ rats. However, relative to paired body wt, it was significantly lower ($p < 0.01$) in STZ rats than in control animals (Table 6).

Table 6
Metabolic and Hormonal Data
in Control and STZ Rats Examined 180 min After Injection of D-[U-^{14}C]Glucose

Rats	Control	STZ	*p*
Body wt (g)			
Before (d 0)	234.0 ± 3.9 (4)	252.5 ± 5.3 (4)	<0.05
After (d 4)	234.3 ± 5.7 (4)	239.8 ± 7.6 (4)	>0.5
Paired change	+0.3 ± 1.9 (4)	−12.8 ± 3.2 (4)	<0.02
Plasma D-glucose (m*M*)			
Before infusion	8.18 ± 0.42 (4)	41.70 ± 2.06 (4)	<0.001
After infusion	16.44 ± 1.81 (4)	24.72 ± 1.08 (4)	<0.01
Paired change	+8.26 ± 2.02 (4)	−16.93 ± 2.67 (4)	<0.001
Plasma insulin (μU/mL)			
Before infusion	25.0 ± 11.7 (4)	3.5 ± 1.3 (4)	>0.1
After infusion	361.5 ± 73.4 (4)	7.4 ± 0.8 (4)	<0.005
Paired change	+336.5 ± 92.0 (4)	+4.0 ± 1.0 (4)	<0.2
Liver			
Wet wt (g)	9.47 ± 0.32 (4)	8.81 ± 0.38 (4)	>0.2
(% of body wt)	4.04 ± 0.09 (4)	3.67 ± 0.05 (4)	<0.01
Pancreas			
Wet wt (g)	831 ± 46 (4)	996 ± 40 (4)	<0.05
(‰ of body wt)	3.54 ± 0.24 (4)	4.15 ± 0.06 (4)	<0.1

Such was not the case for the paired ratio between pancreas and body wts.

As a result of the infusion of D-glucose, the plasma D-glucose and insulin concentrations increased in the control rats by 8.26 ± 2.02 m*M* and 336.5 ± 92.0 μU/mL, respectively ($n = 4$ in both cases; $p < 0.05$ or less), which coincided with an increase ($p < 0.01$) in the insulinogenic index from 3.07 ± 1.71 to 22.38 ± 4.51 U/mol ($n = 4$ in both cases). At sacrifice, the plasma D-glucose concentration averaged in STZ rats 24.72 ± 1.08 m*M*, as distinct ($p < 0.01$) from 16.44 ± 1.81 m*M* ($n = 4$ in both cases) in control rats, despite the fact that it decreased ($p < 0.01$) over the 3 h of observation in the former animals. In the STZ rats, all individual measurements of plasma insulin remained below 10.0 μU/mL. In these animals, the fall in plasma D-glucose concentrations during the 180 min of the experiments coincided with an increase ($p < 0.01$) in the insulinogenic index from 0.09 ± 0.04 to 0.31 ± 0.04 U/mol ($n = 4$ in both cases).

Fifteen minutes after the injection of D-[U-^{14}C]glucose, its apparent distribution space, as judged from the plasma radioactivity, was not significantly different in control and STZ rats, with an overall mean value of 0.39 ± 0.07 mL/g of body wt ($n = 8$). The *K* value for D-[U-^{14}C]glucose assimilation *(7)* averaged, as judged from the decrease in blood radioactivity between the min 15 and min 180 after injection of the radioactive hexose, 1.13 ± 0.08 10^{-2}/min ($n = 4$) in STZ rats, as distinct ($p < 0.02$) from 1.76 ± 0.16 10^{-2}/min ($n = 4$) in control animals.

The paired blood:plasma radioactive ratio was comparable in control and STZ rats 15 min after the injection of D-[U-^{14}C]glucose, with an overall mean value of 68.5 ± 6.8% ($n = 8$). At the time of killing, however, such a paired ratio was higher ($p < 0.005$) in STZ rats (91.7 ± 3.2%; $n = 4$) than in control animals (61.1 ± 4.2%; $n = 4$). Such a difference was borne out by the finding that the paired erythrocyte:plasma radioactive ratio was also significantly higher ($p < 0.001$) in STZ rats (71.9 ± 4.8%; $n = 4$) than in control animals (31.5 ± 2.4%; $n = 4$).

As illustrated in Fig. 3, the radioactive content of the liver (disintegrations per minute/milligram of wet wt), when expressed relative to the paired blood radioactivity (disintegrations/microliters), was about 20 times higher in control animals than in STZ rats. In the pancreas, such a paired organ:blood ratio yielded mean values of 4.31 ± 0.34 and 1.00 ± 0.18 μL/mg ($n = 4$ in both cases; $p < 0.001$) in control and STZ rats, respectively. Taking into account the paired ratio between plasma radioactivity and plasma D-glucose concentration at the time of sacrifice, the radioactive content of the pancreatic gland averaged 42.92 ± 4.77 and 23.11 ± 2.71 nmol of D-glucose equivalent per milligram of wet wt in control and diabetic rats, respectively ($n = 4$ in both cases; $p < 0.02$).

The essential information provided by these experiments is that 180 min after a single injection of D-[U-^{14}C]glucose to control rats rendered hyperglycemic through the infusion of a hypertonic solution of unlabeled D-glucose, the pancreas:blood radioactive ratio was largely in excess ($p < 0.001$)

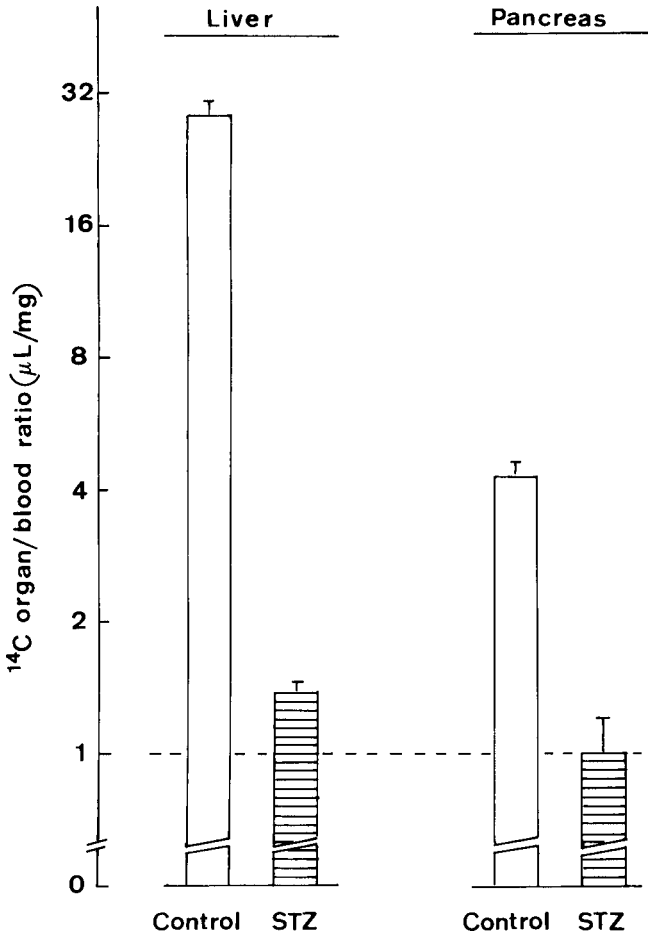

Fig. 3. Paired ratio between liver or pancreas and blood radioactivity in control animals (□) and STZ rats (▤) examined 180 min after the iv injection of D-[U-^{14}C]glucose. Mean values (±SEM) refer to four individual observations in all cases and are ranged on a logarithmic scale. The horizontal dotted line corresponds to a ^{14}C organ/blood ratio equal to unity.

of unity, thus theoretically allowing visualization of the pancreas, if comparable results were to be obtained with a radiolabeled D-glucose analog suitable for positron emission tomography. Such was no longer the case, however, in the STZ rats.

Since the plasma D-glucose concentration was higher ($p < 0.01$ or less) in the STZ rats than in the control animals at both the onset and end of the infusion of D-glucose, these experiments are not truly relevant, however, to the possible participation of the endocrine pancreas to the total radioactive content of the pancreatic gland. For instance, it could be argued that the mean plasma D-glucose concentration (as calculated from the measurements made at the onset and end of the experiments) represented in STZ rats about 2.7 times that in control animals, which coincides with a mean paired pancreas:plasma radioactive ratio 2.8 times higher in the latter than former case. Nevertheless, it should be underlined that when only animals with a comparable final plasma D-glucose concentration were considered in the control (21.2 m*M*) and STZ (21.7 m*M*) rats, the paired pancreas:plasma or pancreas:blood radioactive ratio remained 2.4–3.1 times higher in the former than latter case.

Intravenous Injection of D-[U-^{14}C]Glucose 40 min Before Sacrifice

To simulate more closely the experimental conditions that could be used in vivo for imaging of the endocrine pancreas with ^{11}C-labeled D-glucose, control and STZ rats were infused with a hypertonic solution of unlabeled D-glucose (1.67 *M*, infused at a rate of 2.8 mL/60 min per rat) for only 100 min, with a tracer amount of D-[U-^{14}C]glucose injected intravenously during this infusion 40 min before sacrifice. This delay of 40 min corresponds to twice the half-life of ^{11}C.

In these experiments, the control rats were examined 4 d after surgery. The STZ rats were treated with insulin (6 U/rat injected subcutaneously twice daily) between d 4 and d 6 inclusive after the administration of STZ and subsequent surgery. They received a last injection of insulin at 8 AM on d 7 after the administration of STZ and were then infused with the hypertonic solution of D-glucose. As indicated in Table 7, the daily change in body wt was not significantly different in the control animals and the insulin-treated STZ rats. Likewise, the plasma D-glucose concentration was not significantly different in control and STZ rats, whether immediately before or after the infusion of D-glucose. It increased ($p < 0.02$) by 6.28 ± 1.95 m*M* ($n = 8$; pooled data in control and STZ rats) during the infusion of D-glucose. In the control rats, the increase in glycemia resulted in a significant ($p < 0.05$) increase in plasma insulin concentration (Table 7) and a rise ($p < 0.001$) in the insulinogenic index from 2.50 ± 0.17 to 8.47 ± 1.00 U/mol ($n =$ 4 in both cases). In the insulin-treated STZ rats, the plasma insulin concentration was about 200 times higher (4.09 ± 0.82 mU/mL) than in the control rats before the infusion of D-glucose and failed to be affected significantly by such an infusion. Relative to paired body wt, that of the liver, but not that of the pancreas, was significantly higher ($p < 0.05$) in the insulin-treated STZ rats than in the control animals.

As judged from the plasma radioactivity, the apparent distribution space of D-[U-^{14}C]glucose was not different in control and STZ rats, with an overall mean value of 2.63 ± 0.41 mL/g of body wt ($n = 8$). The paired blood:plasma radioactive ratio also failed to differ significantly in control and STZ rats, with an overall mean value of 73.8 ± 3.2% ($n = 8$; $p < 0.001$ relative to unity). Likewise, the paired liver:blood, pancreas:blood and parotid:blood radioactive ratios were not significantly different in control and STZ rats, with respective overall mean values of 6.73 ± 0.75, 1.99 ± 0.23, and 1.58 ± 0.14 ($n = 8$ in all cases). The pancreas and parotid gland radioactivity (disintegrations per minute/milligram of wet wt) averaged, respectively, 29.4 ± 2.6 and 23.4 ± 1.7% of the paired liver value.

Table 7
Metabolic, Hormonal and Radioactive Data in Control and Insulin-Treated STZ Rats Examined 40 min After Injection of D-[U-^{14}C]Glucose During Infusion of Unlabeled D-Glucose

Rats	Control	STZ	*p*
Body wt (g)			
Before (d 0)	206.3 ± 2.3 (4)	225.0 ± 6.5 (4)	<0.05
After (d 4–7)	209.8 ± 3.9 (4)	235.3 ± 15.5 (4)	>0.1
Paired change (g/d)	+0.9 ± 1.5 (4)	+1.5 ± 1.3 (4)	>0.7
Plasma D-glucose (m*M*)			
Before infusion	7.71 ± 0.42 (4)	8.69 ± 2.80 (4)	>0.7
After infusion	14.78 ± 4.20 (4)	14.16 ± 3.50 (4)	>0.9
Paired change	+7.08 ± 3.90 (4)	+5.47 ± 1.27 (4)	>0.7
Plasma insulin (μU/mL)			
Before infusion	19.4 ± 1.8 (4)	4086 ± 818.2 (4)	<0.005
After infusion	122.0 ± 26.4 (4)	4066 ± 734.2 (4)	<0.005
Paired change	+102.6 ± 26.3 (4)	−29.9 ± 252.5 (4)	>0.6
D-[U-^{14}C]glucose apparent distribution space (mL/g body wt)			
By reference to plasma radioactivity	2.85 ± 0.71 (4)	2.43 ± 0.53 (4)	>0.6
Blood/plasma radioactive ratio (%)	72.0 ± 6.3 (4)	75.7 ± 1.9 (4)	>0.5
Liver			
Wet wt (mg)	9202 ± 253 (4)	15,125 ± 2403 (4)	<0.05
(% of body wt)	4.39 ± 0.09 (4)	6.23 ± 0.72 (4)	<0.05
Liver/blood radioactive ratio (μL/mg)	6.61 ± 1.06 (4)	6.84 ± 1.22 (4)	>0.8
Pancreas			
Wet wt (mg)	761 ± 16 (4)	950 ± 69 (4)	<0.05
(‰ of body wt)	3.63 ± 0.11 (4)	4.03 ± 0.39 (4)	>0.3
Pancreas/blood radioactive ratio (μL/mg)	1.78 ± 0.27 (4)	2.19 ± 0.41 (4)	>0.4
Parotid			
Parotid/blood radioactive ratio (μL/mg)	1.64 ± 0.19 (4)	1.51 ± 0.22 (4)	>0.6

These experiments thus clearly document that even only 40 min after D-[U-^{14}C]glucose injection to hyperglycemic rats, the radioactive content of the pancreatic gland (disintegrations per minute/milligram of wet wt) represents about twice that of blood (disintegrations per minute/microliter). This represents a favorable situation for imaging of the pancreas by positron emission tomography after iv injection of ^{11}C-labeled D-glucose. However, under these experimental conditions, no difference could be detected between control animals and insulin-treated STZ rats, except for the extremely high plasma insulin concentration found in the latter animals. Hence, the following question should be raised. Is the fact that the radioactive content of the pancreas was no longer different in control rats and insulin-treated STZ rats, under the present experimental conditions, attributable to either hyperinsulinism in the STZ rats or an insufficient accumulation of ^{14}C-labeled glycogen in the islets of control rats, as a result of the short period of exposure (40 min) to circulating D-[U-^{14}C]glucose?

Long-Term Infusion of D-[U-^{14}C]Glucose in Control and Insulin-Treated STZ Rats

The experiments so far presented aimed mainly at investigating the optimal conditions for the preferential labeling of glycogen, as distinct from other metabolites, in the pancreas of control rats. The next set of experiments aimed at assessing whether the obvious difference in the radioactive labeling of pancreatic glycogen in control vs STZ rats, as observed in the first series of experiments described herein, was indeed attributable to the virtual absence of insulin-producing cells in the pancreas of STZ rats, rather than to the metabolic consequences of hypoinsulinemia. For such a purpose, the first set of experiments was repeated, except that the STZ rats were now intensively treated with insulin in order to restore normoglycemia before the onset of D-glucose infusion. Such an infusion was then conducted using the same concentration of D-glucose (1.67 *M*) and same rate of infusion (2.8 mL/60 min per rat) in both control and STZ animals.

Starting with an initial body wt of 250.0 ± 4.8 g ($n = 4$), the STZ rats lost 41.8 ± 8.3 g over the period of 3 d (d 0–3) following the injection of the β-cytotoxic agent and the surgical procedure (Table 8). On d 3, their plasma D-glucose and insulin concentrations averaged 43.75 ± 2.39 m*M* and 51.0 ± 14.7 μU/mL, respectively ($n = 4$ in both cases). At that time, the insulinogenic index did not exceed 0.90 ± 0.52 U/mol ($n = 4$), as distinct ($p < 0.02$) from 6.70 ± 0.91 U/mol ($n = 4$) in the control rats. One rat died after the

Table 8
Metabolic and Hormonal Data in Control and Insulin-Treated STZ Rats

Rats	Control	STZ	*p*
Body wt (g)			
Day 0	251.5 ± 5.0 (4)	250.0 ± 4.8 (4)	>0.8
ΔDay 3–0	+7.8 ± 1.3 (4)	–41.8 ± 8.3 (4)	<0.005
Day 3	259.3 ± 5.3 (4)	208.2 ± 3.8 (4)	<0.001
ΔDay 5 (or 6)–3	–10.0 ± 2.1 (4)	+22.7 ± 17.8 (3)	
Day 5 (or 6)	249.3 ± 6.9 (4)	233.7 ± 14.7 (3)	
Plasma D-glucose (m*M*)			
Day 3	7.24 ± 0.16 (4)	43.75 ± 2.39 (4)	<0.001
Day 5 (or 6)	16.65 ± 1.88 (4)	12.72 ± 7.50 (3)	
Plasma insulin (μU/mL)			
Day 3	49.6 ± 5.5 (4)	51.0 ± 14.7 (4)	>0.9
Day 5 (or 6)	769.2 ± 278.2 (4)	666.9 ± 303.0 (3)	
Liver weight (g)	13.20 ± 0.58 (4)		
(% of body wt)	5.31 ± 0.31 (4)		
Pancreatic insulin content (μU/mg)	260 ± 36 (4)		
Islet insulin content (μU/islet)	559 ± 99 (15)		
Glycogen content (μmol/g wet wt)			
Liver	376.31 ± 47.81 (4)		
Pancreas	14.42 ± 8.70 (4)		

second injection of insulin. The other three rats gained 22.7 ± 17.8 g over the period of 3 d during which they were treated with insulin (d 3–5 inclusive). On d 6, their plasma D-glucose averaged 12.72 ± 7.50 m*M*, which included one hypoglycemic animal (3.12 m*M*), one normoglycemic animal (7.54 m*M*), and one hyperglycemic animal (27.50 m*M*). At the same time, the plasma insulin concentration was raised to 0.67 ± 0.30 mU/mL, with an inverse correlation ($r = -0.8904$) between plasma D-glucose and insulin concentrations. Unexpectedly, these animals died within either the first 24 h (two rats) or 48 h (one rat) of D-glucose infusion, despite the fact that the insulin treatment (6 U/rat twice daily) was continued during such an infusion.

Starting with an initial body wt of 251.5 ± 5.0 g ($n = 4$), the control rats gained 7.8 ± 1.3 g ($n = 4$; $p < 0.01$) over the period of 3 d following surgery. They then lost 10.0 ± 2.1 g ($n = 4$; $p < 0.02$) during the infusion of glucose. The liver wet wt averaged 5.31 ± 0.31% ($n = 4$) of paired body wt at sacrifice.

The plasma D-glucose concentration increased ($p < 0.005$) from an initial value of 7.24 ± 0.16 to 16.65 ± 1.88 m*M* after infusion of D-glucose. Likewise, the plasma insulin concentration rose ($p < 0.05$) from 49.6 ± 5.5 μU/mL to 0.77 ± 0.28 mU/mL, and the insulinogenic index from 6.70 ± 0.91 to 39.87 ± 15.96 U/mol ($n = 4$ in all cases; $p < 0.06$).

The pancreatic insulin content, as measured in the PCA-precipitable material, averaged 260 ± 36 μU/mg ($n = 4$) and the islet insulin content 559 ± 99 μU/islet ($n = 15$).

The sum of the radioactive neutral molecules, acidic metabolites, and amino acids recovered after chromatography of the plasma averaged 75.9 ± 1.1% ($n = 4$) of the paired total plasma radioactivity. Relative to such a sum, the contribution of neutral molecules represented 49.8 ± 2.8%, that of acidic metabolites 39.9 ± 2.9%, and that of amino acids 10.2 ± 0.7% ($n = 4$ in all cases). The absolute value for the neutral molecules when expressed as D-glucose equivalent with the same specific radioactivity as infused D-[U-^{14}C]glucose yielded a mean value (35.77 ± 2.13 m*M*; $n = 4$) well in excess ($p < 0.001$) of the measured plasma D-glucose concentration (16.65 ± 1.88 m*M*). It should be kept in mind, however, that several ^{14}C-labeled molecules other than D-glucose may be present in this neutral material (e.g., sorbitol and cholesterol).

The blood radioactive content (counts per minute/microliter) when expressed by reference to the specific radioactivity of infused D-[U-^{14}C]glucose (cycles per minute/nanomoles) yielded a mean value of 67.3 ± 4.9 nmol/μL ($n = 4$), representing 70.7 ± 3.5% of the paired plasma value.

The radioactive content of the pancreas (counts per minute/microliter) expressed relative to the paired blood value (counts per minute/nanomoles) averaged 1.39 ± 0.36 μL/mg ($n = 4$), a value not significantly different from unity ($p > 0.25$). The liver radioactive content (counts per minute/milligram) represented 9.24 ± 0.77 times the paired pancreatic value ($n = 4$; $p < 0.001$). The radioactive content of the parotid gland, however, averaged when expressed relative to the paired blood value no more than 2.46 ± 0.29 μL/mg, with a paired parotid:pancreas ratio of 1.76 ± 0.41, not significantly different from unity ($p > 0.1$).

As in previous experiments, the radioactive content of the PCA-soluble material averaged 60.3 ± 6.4% ($n = 8$) of

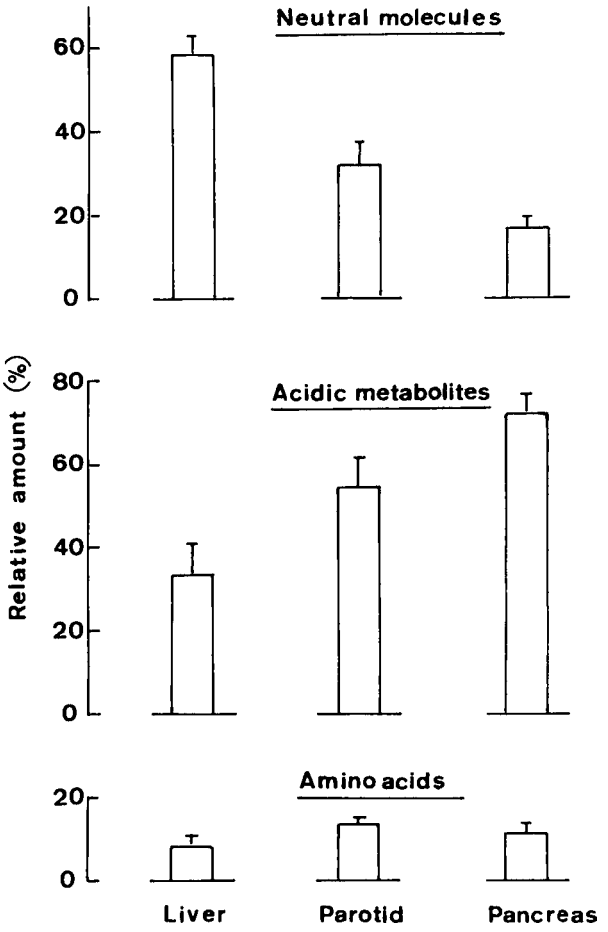

Fig. 4. Relative contribution of ^{14}C-labeled neutral molecules, acidic metabolites, and amino acids in the neutralized PCA and ethanol extracts from livers, parotid glands, and pancreases obtained from normal rats infused for 48 h with a hypertonic solution of D-glucose mixed with a tracer amount of D-[U-^{14}C]glucose. Mean values (±SEM) refer to four individual observations in all cases.

the paired total homogenate radioactivity (pooled data collected in liver and parotid gland extracts).

The radioactive glycogen content of the pancreas represented per unit wet wt, 1.28 ± 0.41% of the paired liver value (n = 4)—i.e., 227 ± 75 nmol/mg when expressed as D-glucose equivalent with the same specific radioactivity as that of infused D-[U-^{14}C]glucose, in fair agreement with the results of the first series of experiments presented herein. The radioactive glycogen content of the parotid gland was somewhat higher, albeit not significantly, than that of the pancreas, averaging 5.10 ± 1.02 nmol/mg.

The liver content in ^{14}C-labeled neutral molecules recovered in the PCA and ethanol extracts averaged 50.9 ± 9.3 nmol/mg (n = 4) when expressed as D-glucose equivalent with the same specific radioactivity as infused D-[U-^{14}C] glucose. In the pancreas, the same measurement only averaged 6.4 ± 1.2% (n = 4) of that found in the liver. In the parotid gland, however, the results were 2.74 ± 0.58 times higher than in the pancreas (n = 4; $p < 0.02$).

The sum of the radioactivity found in the glycogen pellet and in the neutralized PCA and ethanol extract represented 84.8 ± 3.9% (n = 12) of the paired value found in the supernatant of the PCA extract (pooled data for liver, pancreas, and parotid gland).

As illustrated in Fig. 4, the relative contribution of neutral molecules, acidic metabolites, and amino acids to the total amount of radioactive material recovered after chromatography of the PCA and ethanol extracts was far from identical in the three organs under consideration. In the liver, the major fraction corresponded to neutral molecules, with their percentage higher ($p < 0.02$ or less) than in the parotid and pancreas. The opposite situation prevailed in the case of ^{14}C-labeled acidic metabolites, with the following hierarchy: pancreas ≥ parotid ≥ liver. The relative contribution of radioactive amino acids was not significantly different, however, in the liver, pancreas, and parotid. Relative to the radioactive content of the neutralized PCA and ethanol extracts, the sum of the neutral, acidic, and amino acid molecules recovered after chromatography averaged 94.5 ± 2.2% in the liver and 89.3 ± 5.8% in the pancreas, but only 66.7 ± 6.5% in the parotid.

By taking into account the measurements of DNA in groups of 40 islets each (70.7 ± 9.1 ng of DNA/islet; n = 16) and groups of 40 pieces of edematous acinar tissue each (405.7 ± 45.3 ng of DNA/piece; n = 16) prepared by the collagenase method *(8)* from a portion of the pancreas in each of the glucose-infused animals, as well as the mean volume of both rat islet cells (604 μm^3, i.e., 70% of B-cells with a volume of 776 fL/cell and 30% of non-B islet cells with a volume of 201 fL/cell; see ref. *9)* and rat pancreatic acinar cells (1254 μm^3; see ref. *10)*, we calculated that the radioactive content measured in both the islets and acinar pieces was, relative to cellular volume, about 30 times higher ($p < 0.001$) in the isolated islets than in the pancreatic acinar tissue. This ratio happens to be in fair agreement with the data listed in Table 2, when comparing the radioactive content of the total homogenate in the liver of control animals vs the pancreas of STZ rats and correcting for the difference in D-glucose concentration of the infusate in these two types of rats; indeed, this yields in terms of D-glucose content a value about 40 times higher in the liver of the control rats than in the pancreas of the STZ rats.

The essential novel information provided by these experiments consists of the fact that even after 48 h of infusion of D-glucose (1.67 M) mixed with a tracer amount of D-[U-^{14}C]glucose to normal rats, the radioactive content of the pancreatic gland (counts per minute/milligram) was not significantly higher than that of blood, at variance with the situation found in the liver.

Long-Term Infusion of D-[U-^{14}C]Glucose in STZ Rats Transplanted with Islets

In view of the limitations encountered when infusing D-glucose to the insulin-treated STZ rats, a last series of

experiments was conducted in four STZ rats that were transplanted with islets under the left kidney capsule 6 to 7 d after the administration of the β-cytotoxic agent. Fourteen to 16 d after islet transplantation (i.e., 5.0 ± 1.2 d after insertion of a catheter in the right heart) these rats were infused for 24 h with either a 0.28 *M* (one rat) or a 1.67 *M* (three rats) solution of D-glucose that contained a tracer amount of D-[U-^{14}C]glucose and was administered at the usual rate of 2.8 mL/60 min per rat.

Note that this procedure could be completed in only 4 of 12 transplanted rats. The other animals all lost weight after the administration of STZ. Seven to 8 d thereafter, just prior to islet transplantation, their plasma D-glucose and insulin concentration averaged 27.53 ± 0.58 m*M* and 13.2 ± 6.9 μU/mL ($n = 3$ in both cases). One rat died following anesthesia and during surgery for islet transplantation. Two animals died before surgery for insertion of the catheter, and this was attributable in one case to infection of the abdominal wound. After islet transplantation and up to the time of surgery for insertion of the catheter in the right heart, the surviving animals gained 3.8 ± 0.2 g/d ($n = 3$; $p < 0.005$). At that time, their plasma D-glucose and insulin concentrations were, respectively, 8.60 ± 0.68 m*M* lower and 8.6 ± 11.6 μU/mL higher than the paired value recorded just before islet transplantation ($n = 2$ in both cases). Two rats died on the occasion of the second surgical intervention (insertion of the catheter), and three other rats died during the infusion of D-glucose (0.28 *M* in two cases and 1.67 *M* in the third case).

The initial body wt of the rats averaged 238.8 ± 5.1 g ($n = 4$ in this and all following cases, unless mentioned otherwise) at the time of administration of STZ (day 0). Six to 7 d later, they were transplanted with 2925 ± 75 islets under the left kidney capsule. Seven to 10 d thereafter, they underwent surgery to insert a cardiac catheter. Over the first 6–7 d after the administration of STZ, the rats failed to gain weight (–6.3 ± 3.8 g). After islet transplantation, however, their body wt increased by 2.1 ± 0.4 g/d ($p < 0.02$) between d 6 to 7 and 13–17. The infusion of D-glucose mixed with a tracer amount of D-[U-^{14}C]glucose was initiated on d 20–22. Except in one rat that underwent a second surgical procedure because the first catheter had been pulled out, the animals continued to gain weight (+1.1 ± 0.2 g/d; $n = 3$; $p < 0.05$) over the period of 4–7 d separating surgery and the onset of D-glucose infusion. Over the 24-h infusion period, the animals lost 15.8 ± 3.1 g ($p < 0.02$).

At sacrifice, the wet wt of the liver and pancreas averaged, respectively, 9.93 ± 0.40 g and 691.3 ± 65.6 mg, representing 4.25 ± 0.23 and 0.30 ± 0.03% of paired body wt.

In the three rats later infused with a 1.67 *M* solution of D-glucose, the plasma D-glucose and insulin concentrations averaged on d 13, just before surgery for insertion of the catheter in the right heart, 20.1 ± 5.7 m*M* and 16.9 ± 7.8 μU/mL ($n = 3$). At that time, only one rat displayed a normal plasma D-glucose concentration (8.74 m*M*), whereas the other two rats remained severely hyperglycemic (25.79 ± 1.45 m*M*). A modest but further deterioration of glucose homeostasis was observed 7–9 d later just before initiating the infusion of D-glucose. The plasma D-glucose concentration was indeed 29.4 ± 4.4% ($n = 3$; $p < 0.025$) higher than the paired preceding value. The infusion of D-glucose further raised the plasma D-glucose concentration by 15.19 ± 1.12 m*M* ($n = 3$; $p < 0.01$), the value recorded at sacrifice amounting to 29.40 m*M* in the initially normoglycemic animal and 46.55 ± 3.01 m*M* in the other two animals. In the latter two animals, the plasma insulin concentration averaged no more than 2.5 ± 0.6 and 3.6 ± 3.6 μU/mL at the onset and end of D-glucose infusion. In the initially normoglycemic animal, however, the plasma insulin concentration rose from 25.5 μU/mL on d 13 to 51.6 μU/mL before D-glucose infusion and 251.4 μU/mL thereafter. In the sole rat eventually infused with a 0.28 *M* solution of D-glucose, the plasma D-glucose concentration decreased from 27.36 mM before islet transplantation to 9.70 mM before the infusion of the hexose. After such an infusion, the plasma D-glucose concentration did not exceed 9.50 mM. The plasma insulin concentration at sacrifice however, amounted to 74.4 μU/mL.

The insulin content of the pancreas did not exceed 60.7 ± 38.0 μU/mg of wet wt. The insulin content of the transplanted islets amounted to 24.8 ± 9.0 μU/islet (or about 4960 μU/mg of wet wt). It was 38 times higher than that found in the contralateral kidney capsule sample.

The blood radioactive content when expressed relative to the specific radioactivity of infused D-[U-^{14}C]glucose averaged 32.68 ± 7.03 m*M*, representing 63.4 ± 6.4% of the paired plasma value. The major fraction of the plasma radioactivity (64.6 ± 8.4%) corresponded to neutral molecules, and ^{14}C-labeled acidic metabolites and amino acids represented no more than 26.7 ± 7.1 and 8.7 ± 1.3%, respectively, of the total radioactive content recovered in these three fractions. When the plasma D-glucose concentration was estimated by dividing the radioactivity of neutral molecules (disintegrations per minute/microliter of plasma) by the specific radioactivity of infused D-[U-^{14}C]glucose, it averaged 29.56 ± 6.26 m*M*, as compared with a true value of 33.00 ± 8.90 m*M*. The paired ratio between these two variables was not significantly different from unity (99.1 ± 15.1%). These findings thus suggest that at the time of sacrifice, the specific radioactivity of circulating ^{14}C-labeled D-glucose was close to that of the infused D-[U-^{14}C]glucose.

The radioactive content of the pancreas (disintegrations per minute/milligram of wet wt) when expressed relative to the paired blood value (disintegrations per minute/microliter) did not exceed 1.19 ± 0.27 μL/mg. The radioactive content of the liver, however, represented 3.54 ± 0.53 times the paired pancreas value. Likewise, assuming a wet wt of 5.0 μg/transplanted islet, the radioactive content of the

islets, expressed relative to the paired blood value, averaged, after exclusion of one extremely low value (0.62 µL/mg), 5.71 ± 0.54 µL/mg (n = 3), a value about five times higher ($p < 0.001$) than that recorded in the pancreas.

The glycogen content of the liver averaged 136.4 ± 25.1 nmol of D-glucose equivalent/mg of wet wt. Such a value was not significantly different from that found in the transplanted islets (i.e., 104.6 ± 47.9 nmol/mg of wet wt). The glycogen content of the pancreas was one order of magnitude lower than that found in the liver or transplanted islets, with a paired ratio averaging 10.3 ± 2.8% (n = 8).

Likewise, the radioactive glycogen content of the liver, which averaged 5.73 ± 3.04 nmol/mg of wet wt when expressed as D-glucose equivalent with the same specific radioactivity as infused D-[U-^{14}C]glucose, was close to that found in the transplanted islets (i.e., 3.20 ± 0.60 nmol/mg) (n = 4). The latter mean value represented about 70 times that found in the pancreas of the animals infused with the 1.67 M solution of D-glucose (0.046 ± 0.017 nmol/mg; n = 3).

Comparable results were obtained when the ^{14}C-labeled glycogen content of the transplanted islets and pancreatic gland was expressed relative to the DNA content of the samples. Thus, in such a case, the radioactive glycogen content (expressed as D-glucose equivalent by reference to the specific radioactivity of infused D-[U-^{14}C]glucose) averaged 2.69 ± 0.34 nmol/µg of DNA in the transplanted islets, as distinct ($p < 0.001$) from only 27.1 ± 8.1 pmol/µg of DNA in the pancreas.

It should be stressed that in the sole rat that was close to normoglycemic before and after infusion of D-glucose (0.28 M), both the total glycogen content (117.3 nmol/mg of wet wt) and radioactive glycogen content (2.53 nmol/mg of wet wt) of the transplanted islets were comparable with those found in the frankly hyperglycemic animals (see above). In this animal, the paired pancreas:liver ratio in glycogen content (i.e., 9.7%) remained as low as that found in the severely hyperglycemic rats.

In the three rats infused with the 1.67 M solution of D-glucose, the liver content in ^{14}C-labeled neutral molecules recovered in the PCA and ethanol extract averaged 50.30 ± 5.12 nmol/mg of wet wt, when expressed as D-glucose equivalent with the same specific radioactivity as that of infused D-[U-^{14}C]glucose. The latter value was not significantly different ($p > 0.25$) from the mean plasma D-glucose concentration at the time of sacrifice (i.e., 40.83 ± 5.97 mM). The paired pancreas:liver ratio for these radioactive neutral molecules averaged 14.2 ± 1.5%. Such a percentage was virtually identical to that found for the paired ratio between pancreatic gland and transplanted islets (i.e., 14.4 ± 4.6%). These findings are consistent with the equilibration between extracellular and cytosolic D-glucose concentrations in hepatocytes and islet B-cells, at variance with the situation prevailing in acinar cells. A comparable situation was documented in the rat infused with the 0.28 M solution of D-glucose, with the paired pancreas:liver and pancreas:transplanted islets ratios in ^{14}C-labeled neutral molecules averaging 8.5 ± 1.3%.

Discussion

The time course and concentration dependency for glucose-induced glycogen accumulation in pancreatic islets were recently investigated in rat islets cultured in vitro in the presence of D-[U-^{14}C]glucose *(1,5)*.

The present experiments, conducted in vivo, extend these observations, with emphasis on the following points. First, they indicate that, in situations of sustained hyperglycemia, the labeling of pancreatic glycogen by infused D-[U-^{14}C]glucose is indeed negligible in STZ rats, as compared with control animals. Second, they document that in vivo as in vitro, it is possible, in normal rats, to label pancreatic glycogen over a few hours of D-[U-^{14}C]glucose infusion and that such a labeling is not rapidly reversed after cessation of the latter infusion provided that hyperglycemia is maintained by the infusion of unlabeled D-glucose throughout the experiment. The in vivo experiments reveal, however, that, under the present experimental conditions, ^{14}C-labeled glycogen only represents a minor fraction of the total radioactive content of the pancreas. In this respect, the use of 2-deoxy-2-[^{18}F]fluoro-D-glucose, rather than D-[U-^{14}C]glucose, as the radioactive tracer may represent a major advantage. It may indeed be expected to prevent the labeling of amino acids and, hence, proteins (recovered in the PCA-precipitable material). For instance, if, in addition to radioactive glycogen, only ^{14}C-labeled acidic metabolites and neutral molecules are taken into consideration, the total value found in the pancreas of control and STZ rats would amount, according to the data listed in Table 2, to 21.38 ± 0.70 and 3.94 ± 0.29 µmol/pancreas, respectively (n = 4 in both cases). Thus, the value recorded in control animals would remain six to seven times higher ($p < 0.001$) than that found in STZ rats.

In the pancreas of normal rats, the paired ratio between ^{14}C-labeled glycogen and the radioactive molecules recovered in the neutralized PCA-soluble extract, although much lower after 4 h, rather than 48 h, of D-[U-^{14}C]glucose infusion, increased when the rats were further infused for 60 min with only unlabeled D-glucose (Fig. 2). This suggests that the most suitable timing for imaging of the endocrine pancreas would be a few hours after the iv injections of 2-deoxy-2-[^{18}F]fluoro-D-glucose, i.e., at a time when the contribution of extracellular radioactivity would become negligible, relative to the total content of the pancreatic gland *(6,11)*.

The experiments conducted in hyperglycemic control rats injected intravenously with D-[U-^{14}C]glucose 180 min before sacrifice indeed indicated that the paired ratio between pancreas and blood radioactive content averaged 4.31 ± 0.34 µL/mg, largely in excess of unity, whereas such

a ratio did not exceed 1.00 ± 0.18 µL/mg in STZ rats under otherwise the same experimental conditions.

In hyperglycemic control rats examined only 40 min after the iv administration of D-[U-^{14}C]glucose, the pancreas:blood radioactive ratio also remained higher than unity but did not exceed 1.78 ± 0.27 µL/mg. This would suggest that imaging of the pancreas could be achieved after injection of ^{11}C-labeled D-glucose. However, under these conditions, no difference could be detected between control and insulin-treated STZ rats.

Unexpectedly, when insulin-treated STZ rats were infused with the hypertonic solution of D-glucose (1.7 *M*) otherwise used in control rats, they died within the first 24 or 48 h. Nevertheless, an array of findings, including the comparison between the radioactive content of islets and exocrine tissue obtained from control rats infused with D-glucose (mixed with a tracer amount of D-[U-^{14}C]glucose), indicate that the difference in the radioactive content of the pancreatic gland in control vs STZ rats is mainly attributable to the virtual absence of insulin-producing cells in the latter animals.

Moreover, further experiments conducted in STZ rats that were transplanted with islets under the kidney capsule and later infused for 24 h with a hypertonic solution of D-glucose (1.67 *M*) containing a tracer amount of D-[U-^{14}C] glucose and delivered at the usual rate of 2.8 mL/h indicated that despite hyperglycemia (mean plasma D-glucose concentration before and after infusion: 25.64 ± 7.04 and 40.83 ± 5.97 m*M*; $n = 3$ in both cases) and despite the fact that D-glucose was infused for only 24 h, the specific radioactivity of circulating glucose (as judged from the paired ratio between the plasma content in radioactive neutral molecules and plasma D-glucose concentration at the time of sacrifice) was not significantly different from that of infused D-[U-^{14}C]glucose, the former value averaging 93.0 ± 18.3% of the paired latter one.

In these experiments, the radioactive content of the transplanted islets and their content in radioactive glycogen were, respectively, about 5 and 70 times higher than that found in the pancreatic gland; all results were expressed relative to either wet wt or DNA content.

In conclusion, the present results allow one to define the experimental conditions under which the iv administration of 2-deoxy-2-[^{18}F]fluoro-D-glucose could conceivably be used to favor the selective imaging of the endocrine, as distinct from exocrine, pancreas. Further work is presently under progress in human subjects to assess the validity of such a proposal.

Materials and Methods

D-[U-^{14}C]glucose was purchased from NEN (Boston, MA).

The experiments were conducted in fed female Wistar rats (Iffa Credo, L'Arbresle, France) given free access to food (KM-04-k12; Pavan Service, Oud Turnhout, Belgium) and tap water up to the time of killing by decapitation. Some rats were injected intravenously with STZ (Sigma, St. Louis, MO), at a dose of 0.25 µmol/g of body wt, a few days before the experiments. When treated with insulin, these STZ rats received twice daily an sc injection of 6 U of insulin (Insulatard, Novo Nordisk, Denmark).

For the purpose of iv infusion, a catheter was introduced in the right heart by a method described elsewhere *(12)*. Unless otherwise mentioned, the animals were infused with a hypertonic solution of D-glucose (1.67 *M* in control rats and 0.25 *M* in STZ rats) administered at a rate of 2.8 mL/h.

In one series of experiments, STZ rats were transplanted under the left kidney capsule with groups of 2700–3000 islets obtained from normal rats of the same strain.

Blood samples were collected in heparinized tubes from the severed tip of the tail during D-glucose infusion and at the time of killing by stunning and decapitation. The plasma was separated and stored at –20°C. Pieces of liver, pancreas, and, on occasion, parotid gland were removed and weighed. The total weight of the liver and pancreas was also recorded. The plasma concentrations of D-glucose *(13)* and insulin *(14)*, the glycogen content of liver and pancreas *(15)*, and the pancreatic insulin content *(14)* were measured by methods described in the cited references. The incorporation of D-[U-^{14}C]glucose into PCA-precipitable material, glycogen, as well as neutral molecules, acidic metabolites, and amino acids was determined by a method described elsewhere *(5)*.

The design of the present experiments was approved by the Commission d'Ethique du Bien-Etre Animal of our faculty.

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

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