Further Studies on the Metabolism of D-Glucose Anomers in Pancreatic Islets

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SUMMARY

The α and β anomers of commercially available D-(5-^{3}H)glucose were separated by miniaturized Hudson-Dale procedures based on precipitation with acetic acid. Reflectometric measurements of the reactivity with matrix-bound glucose oxidase showed that the preparations were about 90 per cent pure with respect to anomeric composition. Nonradioactive anomers separated by the same procedures were analyzed by optic polarimetry and gas chromatography. The preparations were about 90 per cent pure with respect to anomeric composition and produced no peaks but D-glucose on trimethylsilylation and chromatography. Microdissected pancreatic islets of noninbred ob/ob-mice exhibited a linear production of ^3H_2O for three to nine minutes when incubated with 6 mM α-D-(5-^{3}H)glucose, β-D-(5-^{3}H)glucose, or D-(5-^{3}H)glucose in anomeric equilibrium; the three glucose preparations did not differ in their rate of conversion to ^3H_2O. The rate of ^3H_2O production increased with glucose concentration (3-21 mM) during incubations for three minutes and, again, there was no evidence for the metabolic activity's being dependent on the anomeric composition of the labeled sugar. When microdissected islets were perifused without glucose and suddenly exposed to 5-6 mM α-D-glucose or β-D-glucose, the concentration of glucose-6-phosphate rose within five minutes and did not differ significantly between experiments with α-D-glucose and β-D-glucose. In the same perifusion experiments, only α-D-glucose caused a pronounced stimulation of insulin secretion, the difference from β-D-glucose being significant. The results indicate that the recognition of glucose as an insulin secretagogue does not only involve metabolism by glucose-6-phosphate. The possible roles of the sorbitol pathway and of hypothetical regulatory sites for the glucose molecule ("receptors") are briefly discussed. DIABETES 25:450-58, May, 1976.

Studies in vivo[1] as well as in vitro[2-5] have indicated that α-D-glucose is a more potent stimulus to insulin secretion than is β-D-glucose. We observed no corresponding difference between the glucose anomers in their capacity to rapidly raise the islet content of glucose-6-phosphate, induce counter-transport of 3-*O*-methyl-D-glucose, or dilute ^3H_2O arising through metabolism of equilibrated D-(5-^{3}H)glucose in microdissected islets.[4] It was therefore concluded that the discriminatory behavior of the insulin-releasing D-glucose recognition system probably cannot be reduced to that of the glucose-transporting carrier or the glucose-phosphorylating enzymes. In view of the central position of this problem in current islet research, we have tried to place our conclusion on a more secure basis by measuring the formation of ^3H_2O from (5-^{3}H)-labeled D-glucose anomers directly. This attempt necessitated a microscale separation of radioactively labeled anomers; the results obtained with miniaturizations of classic acetic-acid-precipitation methods[6] are reported here. In addition, anomer-induced changes of glucose-6-phosphate and insulin release were studied in a microperifusion system especially designed for parallel measurements of metabolic substrates and secretion in the same perifusion experiments.[7]

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MATERIALS AND METHODS

Animals and isolation of islets. Adult, noninbred ob/ob-mice were taken from a local colony (Umeå-ob/ob-mice) and fasted overnight. Fresh islets containing more than 90 per cent β-cells were isolated by freehand microdissection under a stereomicroscope[8] with the excised pancreas immersed in a salt-balanced buffer of the following composition (mM): Na^+ 130, K^+ 5.9, Ca^{2+} 2.6, Mg^{2+} 1.2, Cl^- 139.9, SO_4^{2-} 1.2, $H_2PO_4^-$ 1.2, *N*-hydroxyethyl-

piperazine-N'-2-ethane sulfonic acid 20.0, as well as Na^+ in the form of NaOH to adjust pH to 7.4. The buffer was equilibrated with ambient air and was also used as basal incubation medium.

Measurements of insulin release, glucose-6-phosphate, and ^3H_2O production. The same type of buffer as used in microdissection was also used as basal medium in the subsequent incubations. To study insulin release and islet content of glucose-6-phosphate, islets were subjected to nonrecirculating perifusion. The previously described apparatus[7] was modified to allow solutions of glucose anomers to be injected through a side arm into the stream of basal medium containing 1 mg. of serum albumin/ml. and passing groups of three islets at a rate of 17-18 μl./min. Commercially available glucose anomers were dissolved at 0-2° C. and kept at this temperature in the reservoir feeding glucose to the side arm. The volumes of glucose anomers injected were small enough for the temperature to equilibrate with that of the basal medium (37° C.) before glucose reached the islets in their chambers, about 45 seconds after injection. The proportion of the flow rates in the side arm and the main medium inlet was 1:13. In each experiment the medium was equally distributed to six perifusion chambers containing islets from the same animal. The effluent from two of these was continuously sampled and radioimmunoassayed for insulin with crystalline mouse insulin as reference. The effluent was also analyzed for total glucose with a coupled hexokinase/glucose-6-phosphate dehydrogenase assay after equilibration of the anomers at room temperature. Before as well as five and 30 minutes after the perifusion with glucose was started, two chambers were removed from the apparatus and plunged into melting isopentane. The islets were freeze-dried (−40° C., 0.1 Pa) overnight while still remaining in the disconnected perifusion chambers, and they were then brushed free of dry medium and weighed on a quartz-fiber balance as previously described.[7] The dry islets were analyzed for glucose-6-phosphate essentially as described by Matschinsky.[9]

Production of ^3H_2O from (5-^{3}H)-labeled glucose was measured as an index of the combined glycolytic and pentose phosphate shunt fluxes.[10] Microdissected islets were subjected to preliminary incubation at 37° C. in albumin-free basal medium for 30 minutes. Aliquots of 15 μl. of ice-cold basal medium (0-2° C.) were distributed to small tubes containing 45-315 nmol of freeze-dried radioactive anomer (1.7 Ci./mol) prepared as described below and giving the final concentrations stated in figures 3 and 4. The labeled medium was warmed to 37° C. by holding the thin tube in a water bath for 30 seconds. Two or three islets were then added to each tube, and the tubes were inserted into larger glass vials containing 0.5 ml. distilled water; the outer glass vials had been prewarmed to 37° C. In control experiments employing a thermistor it was determined that the radioactive incubation medium reached 37° C. within 30 seconds, i.e. before the islets were added. This control of temperature equilibration was essential, since the islets were to be incubated for unusually short periods of time, namely three to nine minutes. At the end of the incubations, metabolism was stopped by adding 5 μl. of 0.27 M HCl. The ^3H_2O produced was allowed to equilibrate overnight with the water in the enclosing, outer glass vial and was then determined by liquid-scintillation counting. Samples of radioactive medium were used as external standards in the counting procedure. The islets were freeze-dried (−40° C., 0.1 Pa) overnight and weighed on a quartz-fiber balance.

Microscale preparation of D-glucose anomers. The principles of Hudson and Dale[6] were adapted to the microscale as follows: To prepare α-D-(5-^{3}H)glucose, 250 mg. of equilibrated D-(5-^{3}H)glucose was dissolved in 125 μl. of distilled water and warmed without boiling on an oil bath until the solution was clear. The solution was removed from the oil bath, allowed to cool for a few minutes, mixed with 500 μl. of melting concentrated acetic acid, seeded with a few small crystals of nonradioactive α-D-glucose, and allowed to crystallize at room temperature overnight. To prepare β-D-(5-^{3}H)glucose, 250 mg. of equilibrated D-(5-^{3}H)glucose was dissolved in only 25 μl. of distilled water in an oil bath (about 110-120° C.). While still in the oil bath, the solution was mixed with 300 μl. of hot acetic acid (about 100° C.). After careful stirring, a few crystals of nonradioactive β-D-glucose were added, and the mixture removed from the oil bath. Crystallization of β-D-glucose occurred within minutes and was much faster than that of α-D-glucose. Both preparations were filtered with suction on Munktell paper OOH until near dryness. The crystals were washed with 95 per cent ethanol followed by absolute ethanol and suction to dryness. They were scraped off and dried in an oven (75° C.) for 10-15 minutes and were subsequently kept at room temperature. In pilot and control experiments, preparations of nonradioactive α-D-glucose were made from pure β-D-glucose and β-D-glucose from α-D-glucose according to the same protocols except

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that weights and volumes were twice as large.

To analyze the concentrations of radioactive glucose anomers in the preparations obtained, aqueous solutions were assayed with a reflectometric β-D-glucose oxidase method. The solutions to be assayed were made directly from the stocks of purified material as well as from samples that had first been dissolved in ice-cold water and freeze-dried overnight. This was done to check whether freeze-drying of small volumes of anomer solution could be safely used to subdivide the radioactive preparations into lots of convenient size for each day of subsequent physiological experimentation.

Assay of D-glucose anomers. Nonradioactive α-D-glucose and β-D-glucose were assayed by optic polarimetry at 589 nm (Na lamp) in 10-cm. cuvettes (Perkin-Elmer 141). Crystals of sugars were dissolved in ice-cold basal medium (20 mg./ml.) and the optic activity determined with the cuvette housing kept cold by circulating ice-cold water. Because of the low temperature, no anomerization was detectable during the measuring procedure. The samples were then allowed to equilibrate at room temperature and the optical activity at equilibrium determined.

To achieve sufficient analytical sensitivity and avoid radioactive contamination of the polarimeter, the preparations of α-D-(5-^{3}H)glucose and β-D-(5-^{3}H)glucose were analyzed with the aid of β-D-glucose oxidase, peroxidase, and a chromogenic oxygen acceptor attached to plastic strips (Dextrostix, Ames Company, Stoke Poges, Slough, Bucks., England). The darkening of the strips on reaction with β-D-glucose was measured in a light-reflectance meter (Eyetone, Ames Company). This equipment is commercially available for routine determinations of blood sugar in clinics, but its use for the present analytic purposes necessitated a careful standardization and evaluation of the assay. Although the meter scale is designed to give direct readouts of glucose concentrations in plasma, this was ignored and the meter readings were treated as arbitrary numbers in the construction of standard curves based on aqueous solutions of sugars. Figure 1 shows the standard curves obtained for β-D-glucose measured in the presence of two different concentrations of α-D-glucose. The assay was apparently specific for β-D-glucose, since the standard curve was the same whether β-D-glucose was mixed with 0-75 or 100-180 mg. of α-D-glucose per 100 ml. However, the standard curve was not strictly linear but slightly upwards-concave, a fact that had to be taken into account by using several standard points when measuring unknowns. The random error of the assay was estimated as the standard deviation of replicate determinations at each point in figure 1. In absolute terms the error was roughly proportional to the β-D-glucose concentration throughout the concentration range tested (20-200 mg./100 ml.); on an average, the error of a single determination was $\pm$ 7.9 per cent.

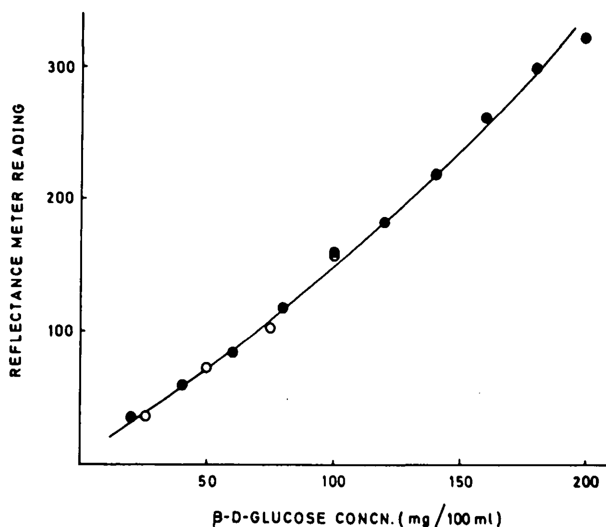

FIG. 1. **Specificity of reflectometric D-glucose assay for the β anomer. Meter readings (arbitrary scale units) are plotted against the concentration of β-D-glucose in solutions containing a total glucose concentration (α plus β) of 100 mg./100 ml. (O) or 200 mg./100 ml. (●). Each point is the mean of two or three replicates.**

Samples of sugar to be assayed were dissolved in ice-cold water, placed in a thin cuvette, and warmed for 10 seconds in a water bath of room temperature (21-22° C.). A β-D-glucose oxidase reagent strip was then immersed and left in the solution for 30 seconds. After the strip had been rinsed for two seconds under flowing distilled water and blotted, it was placed in the reflectance meter and the meter was read exactly 50 seconds after the strip had been immersed in the glucose solution. Care was taken to obtain exact timing for all steps.

Gas chromatography. After equilibration of D-glucose anomer preparations in distilled water, trimethyl silyl derivatives were prepared and subjected to gas-liquid chromatography. Carefully weighed samples of unknowns were silylated and run in the same experiments as external standards of commercially available D-glucose not subjected to the acetic acid precipitation procedures. In each run, arabinose served as internal standard, allowing a quantitative estimation of the amount of sugar in the D-glucose peaks.

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Chemicals. α-D-glucose and β-D-glucose were from Sigma Chemical Co., St. Louis, Mo., and equilibrated D-(5-^{3}H)glucose from the Radiochemical Centre, Amersham, England. The commercial D-(5-^{3}H)glucose had a specific activity of 1 Ci./mmol. and was diluted with nonradioactive glucose to give 1.7 Ci./mol. ^{125}I-insulin (150-160 mCi./mg.) was from Farbwerke Hoechst A.G., Frankfurt/Main, Germany, and guinea-pig anti-insulin antisera were obtained in the laboratory. Enzymes and cofactors for glucose-6-phosphate determinations were bought from C.F. Boehringer und Soehne GmbH, Mannheim, Germany.

RESULTS

Microscale separation of D-glucose anomers. The efficiency of the microadapted Hudson-Dale procedures for preparing D-glucose anomers was first examined by polarimetric measurements on nonradioactive sugar. In these experiments, 500 mg. of pure anomer was used as starting material for making the other optically active form. Following the protocols described in the Methods section, we obtained a product containing 91 per cent α-D-glucose from a starting material of pure β-D-glucose and a product containing about 85 per cent β-D-glucose when starting with pure α-D-glucose (table 1). When trimethyl silyl derivatives of the products were run on the gas chromatograph, no peaks were detected except those corresponding to D-glucose (figure 2). The purity of the products was also evident from the fact that more than 99 per cent of their weight was accounted for by D-glucose on comparisons with similarly chromatographed standards of commercially available D-glucose.

The purification methods were then scaled down by a factor of 2 and applied to batches of D-(5-^{3}H)glucose containing the α and β anomers in equilibrium. The reflectometric analyses of the batches of radioactive anomers thus prepared are summarized in table 2. In good agreement with the polarimetric analyses of nonradioactive anomer preparations, the microadapted Hudson-Dale procedures yielded α-D-(5-^{3}H)glucose and β-D-(5-^{3}H)glucose preparations containing about 90 per cent of the respective anomer. As in the polarimetric studies, the preparation of β-D-glucose

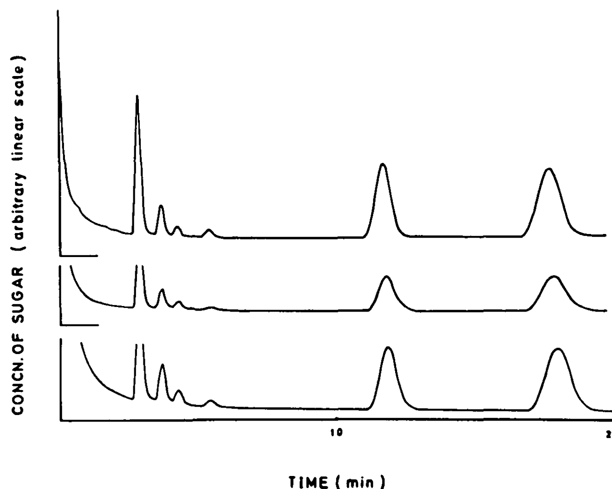

FIG. 2. Gas-liquid chromatographic evaluation of Hudson-Dale procedures for preparing anomers of D-glucose. From top to bottom the chromatograms refer to 100 μg. of Sigma α-D-glucose, 100 μg. of α-D-glucose prepared from Sigma β-D-glucose by acetic acid precipitation, and 100 μg. of β-D-glucose prepared from Sigma α-D-glucose by acetic acid precipitation. The first peaks are internal standards, while the peaks appearing between 10 and 20 minutes are the anomers of D-glucose. The occurrence of both anomeric forms in each run is due to the sugars' having been dissolved in water before the silylation procedure. Note the absence of any contaminating peak due to the acetic acid precipitation procedures. The same results were obtained in three chromatographic separations run with two different batches of the anomer preparations.

TABLE 1

Polarimetric evaluation of micro-adapted Hudson-Dale procedures for preparing anomers of D-glucose

Expt. no.	Starting material	Optic activity (degrees)		Calculated properties of product		
		Before equilibration	After equilibration	Total glucose concn. (mg./ml.)	% α	% β
I	β-D-glucose	2.10	1.07	20.3	90.8	9.2
II	β-D-glucose	2.09	1.06	20.1	91.4	8.6
III	α-D-glucose	0.60	1.00	19.0	13.8	86.2
IV	α-D-glucose	0.63	0.95	18.0	17.4	82.6

Samples of the products formed were weighed to give a concentration of approximately 20 mg./ml. The concentration and composition of the samples were calculated on the basis that the specific rotation is + 112 for α-D-glucose, + 18.7 for β-D-glucose, and + 52.7 for the anomers in equilibrium.[40]

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TABLE 2

Reflectometric analyses of radioactive anomer preparations obtained by applying the micro-adapted Hudson-Dale procedures to equilibrated D-(5-^{3}H) glucose

Product analyzed	Freeze-dried	β-D-glucose assayed		Calculated properties of product		
		Before equilibration (mg./100 ml.)	After equilibration (mg./100 ml.)	Total glucose concn. (mg./100 ml.)	% α	% β
α-D-(5-^{3}H)glucose	No	12.0	137.0	214.1	94.4	5.6
α-D-(5-^{3}H)glucose	Yes	17.0	112.5	175.8	90.3	9.7
β-D-(5-^{3}H)glucose	No	63.0	46.0	71.9	12.4	87.6
β-D-(5-^{3}H)glucose	Yes	65.5	45.0	70.3	6.8	93.2

Samples of the radioactive preparations of D-glucose anomers were dissolved in water and assayed by the reflectometric β-D-glucose oxidase method as described in the text. The composition of the samples was calculated on the assumption that equilibrated D-glucose contains 36 per cent α anomer and 64 per cent β anomer. The standard curve used to convert meter readings to concentrations of β-D-glucose is shown in figure 1. Each sample was assayed in two to five replicates, the mean values of which are shown in the table.

appeared to be slightly less pure than that of α-D-glucose. The freeze-drying procedure did not seem to induce any significant anomerization or decreased purity of the preparations. When samples of the radioactive preparations were carefully weighed, equilibrated at room temperature, and assayed for total glucose by a coupled hexokinase/glucose-6-phosphate dehydrogenase method, the recoveries were 103 per cent for the α-D-(5-^{3}H)glucose preparation and 89 per cent for the β-D-(5-^{3}H)glucose preparation.

Production of ^3H_2O from (5-^{3}H)-labeled D-glucose anomers. Initial studies showed that the β-D-(5-^{3}H)glucose batch, as well as the commercially available batch of equilibrated D-(5-^{3}H) glucose, yielded significantly higher blank values (about 1,000 c.p.m./incubation vial) for ^3H_2O production in the absence of islets than did the preparation of α-D-(5-^{3}H)glucose (about 100 c.p.m./vial). In those pilot studies, the anomer preparations were used without prior freeze-drying, and it seemed reasonable to suspect the occurrence of free ^3H_2O in the preparations of labeled glucose. In all experiments reported below, the radioactive anomers as well as the equilibrated sugar were therefore freeze-dried before use. This procedure reduced the blank values of the β-D-(5-^{3}H)glucose and of the equilibrated D-(5-^{3}H)glucose to the same level as that of the α-D-(5-^{3}H)glucose; it was requisite for the assay to detect metabolic production of ^3H_2O during only a few minutes of incubation with islets.

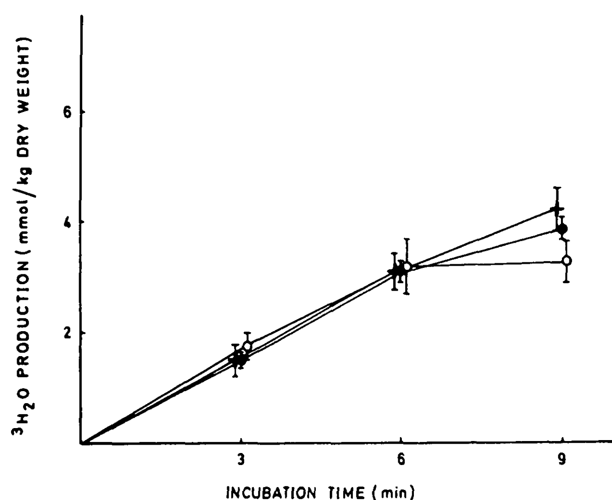

FIG. 3. Time course of ^3H_2O production in pancreatic islets incubated with 6 mM α-D-(5-^{3}H)glucose (●), 6 mM β-D-(5-^{3}H)glucose (O), or 6 mM equilibrated D-(5-^{3}H)glucose (+). Each point is the mean of six to fifteen experiments. Results are expressed in terms of glucose with the same specific radioactivity as in the medium.

Figure 3 shows that the production of ^3H_2O with time was apparently linear for three to six minutes whether the islets were exposed to 6 mM of the individual anomers or of the equilibrated glucose. The three glucose preparations did not differ significantly with respect to the rate of ^3H_2O production. The rate of anomerization in the incubation medium at 37° C. was studied by polarimetry. The time required for reaching half-ways to equilibrium was four minutes and 45 seconds in an experiment starting with α-D-glucose and four minutes and 30 seconds in an experiment starting with β-D-glucose. Subsequent measurements of metabolic flux were therefore based on incubations for only three minutes, about the shortest time allowing a reasonable accuracy in the determinations of ^3H_2O production. The effect of glucose concentration on the production of ^3H_2O during three minutes is shown in figure 4. With all the

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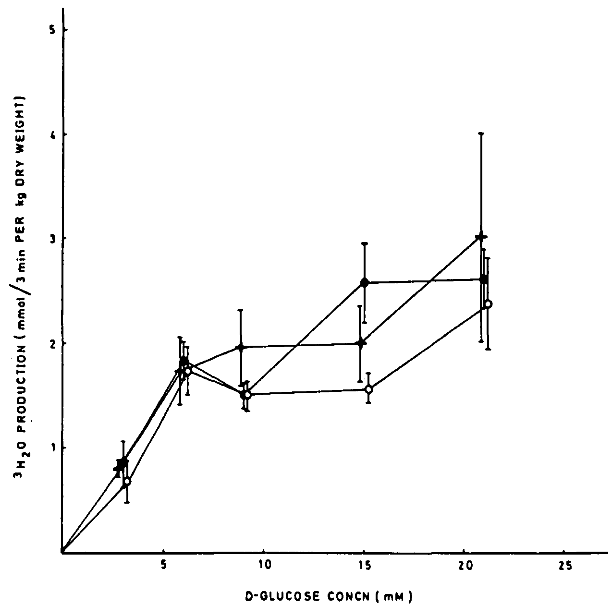

FIG. 4. Relationship between production of ^3H_2O during incubation for three minutes and the concentration of α-D-(5-^{3}H)glucose (●), β-D-(5-^{3}H)glucose (O), or equilibrated D-(5-^{3}H)glucose (+). Mean values ± S.E.M. are given for 7-17 different experiments. Results are expressed in terms of glucose with the same specific radioactivity as in the medium.

three glucose preparations the rate of ^3H_2O production increased with glucose concentration. The shape of the dose-response curves should be regarded as approximate in view of the small amounts of ^3H_2O produced and measured. Nonetheless, it seems safe to conclude that there was no striking and consistent difference in metabolic activity between the different preparations of (5-^{3}H)-labeled glucose.

Effects of perifusion with glucose anomers on islet glucose-6-phosphate and insulin release. After an hour without glucose, the islets were continuously perifused with glucose anomers for 30 minutes. The effluent collected two to four minutes after introducing the anomers contained 5.6 ± 0.2 mM glucose in six experiments with α-D-glucose, and 5.4 ± 0.2 mM glucose in six experiments with β-D-glucose (mean values ± S.E.M.). In the same experiments, the glucose concentration after 20-25 minutes of perifusion with anomers was 5.8 ± 0.2 mM in the case of α-D-glucose and 5.7 ± 0.2 mM in the case of β-D-glucose.

When the islets were exposed to either anomer, their glucose-6-phosphate content rose significantly within five minutes (table 3). After 30 minutes of perifusion with glucose, there was a slight further increase of the glucose-6-phosphate. At no time point

TABLE 3

Glucose-6-phosphate in islets perifused with D-glucose anomers

Time-point studied	Islet content of glucose-6-phosphate (μmol/kg. dry weight)	
	Expts. with α-D-glucose	Expts. with β-D-glucose
2 min. before perifusion with glucose	78.1 ± 4.2	71.7 ± 7.6
5 min. after commencing perifusion with glucose	120.5 ± 4.6	126.7 ± 12.7
30 min. after commencing perifusion with glucose	137.0 ± 6.5	149.2 ± 16.8

Mean values ± S.E.M. of six different experiments. Each experiment comprised duplicate determinations of glucose-6-phosphate at all time points, but the N-value used when calculating S.E.M. is the number of experiments. The parallel measurements of insulin release are shown in figure 4.

studied did the glucose-6-phosphate concentration differ significantly between experiments with α-D-glucose and β-D-glucose.

Figure 5 shows that the rate of insulin release rose rapidly on exposure of the islets to α-D-glucose and stayed at a stimulated level throughout the perifusion with this anomer. The stimulated secretory response was about seven times greater than the basal rate observed in the absence of glucose. In contrast,

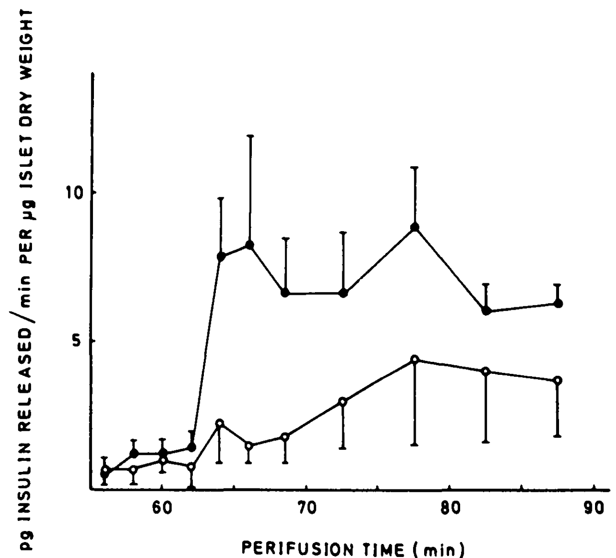

FIG. 5. Insulin release from islets perifused with D-glucose anomers. After perifusion with glucose-free medium for 60 minutes, 5-6 mM α-D-glucose (●) or β-D-glucose (O) was included in the medium and perifusion with the sugar continued for 30 minutes. The points denote the average rate of insulin release over each sampling period. Mean values ± S.E.M. are given for five (α) and six (β) different experiments, in each of which the islet glucose-6-phosphate was also measured (cf. table 3). The exact glucose concentrations during perifusions are stated in the text.

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β-D-glucose caused a very small and doubtful enhancement of insulin release during the first minutes. The release rate seemed to rise during the last half of the perifusion period, but this tendency is questionable in view of the associated random errors.

The probability that α-D-glucose and β-D-glucose had the same effects on insulin release was estimated by the Wilcoxon rank sum method. In each experiment, the amount of insulin released during minutes 61-63 was subtracted from that released during minutes 63-65 to give a measure of the initial secretory response. The initial response to α-D-glucose was significantly different from that to β-D-glucose ($P < 0.05$, two-sided). Likewise, the amounts of insulin secreted during minutes 63-70 differed significantly between the two anomer groups ($P < 0.05$, two-sided). To test directly whether a certain increase of glucose-6-phosphate was associated with the same enhancement of insulin release, irrespective of whether α-D-glucose or β-D-glucose was used as the stimulating sugar, the initial secretory response was divided by the initial rise of glucose-6-phosphate in each experiment (cf. table 3). The mean ratios thus computed were 147 pg. of insulin/min. per pmol glucose-6-phosphate for the α-D-glucose group and 26 pg./min. per pmol for the β-D-glucose group. These ratios were significantly different with $P < 0.02$ (two-sided Wilcoxon test).

DISCUSSION

The present experiments on the metabolic activity of D-glucose anomers differ in design from those previously reported in two salient respects. We have now measured the ^3H_2O production from labeled anomers directly instead of studying the ability of nonradioactive anomers to dilute the ^3H_2O arising through metabolism of equilibrated D-(5-^{3}H)glucose.[4] Moreover, glucose-6-phosphate and insulin release have been measured in the same experiments, each of which was designed to allow groups of islets from one animal to be perifused with the same anomer-containing medium in a strictly parallel fashion; this technic should eliminate the possibility that any between-experiment source of error could distort the comparison of the two parameters. The results confirm and extend previous studies indicating that α-D-glucose apparently is not a better substrate for glycolysis via glucose-6-phosphate[4] but is nonetheless a better stimulus of insulin release.[1-5] In our previous paper on the subject,[4] α-D-glucose even appeared to be less effective than β-D-glucose in raising the islet glucose-6-phosphate concentration during three minutes of static incubation. Such a significant difference was not obtained in the present study, but the mean values obtained after five minutes of perifusion with α-D-glucose were again slightly lower than those obtained with β-D-glucose.

The demonstration of correlations between glucose metabolism in islets and glucose-stimulated insulin release[10-15] has encouraged the "substrate-site hypothesis,"[16] which proposes that the recognition of glucose as an insulin secretagogue depends on the sugar's being metabolized in the β-cells.[17,18] More precisely, studies on the metabolism and insulin-releasing action of trioses,[19,20] as well as isotope tracer investigations of metabolic flux,[10] suggested that the signal to secretion is produced in the glycolytic pathway, perhaps at or below the triose phosphate step. The apparent discrepancies between the metabolic and insulin-releasing actions of the D-glucose anomers is not a compelling reason for abandoning the substrate-site hypothesis in its general form, or even the idea that glycolysis is significant for glucose recognition. However, the anomer data seem to require that auxiliary mechanisms be considered as well. The general concept of a dual or bimodal system for glucose recognition has been discussed by several authors,[16,19,21-23] but there is no consensus as to which molecular mechanisms may take part in such a system. At least the following candidates for a glucoreceptor mechanism have been discussed in various contexts: glycolysis giving rise to some specific signal substance,[20,24-26] glucose metabolism providing ATP ("fuel function" of glucose),[21,27] pentose phosphate pathway,[28-30] sorbitol pathway,[31] unspecified regulatory sites for the glucose molecule,[16,19] unspecified glucose sites in the β-cell plasma membrane,[22,32] and regulatory glucose sites coupled to adenylate cyclase.[21,23,33,34]

Within the framework of a mixed substrate-site and regulatory-site hypothesis, attention is drawn to the observation that α-D-glucose is more effective than β-D-glucose in stimulating the accumulation of radioactive cyclic AMP in islets prelabeled with tritiated adenine.[34] This observation could signify that the anomers have a correspondingly different capacity to activate adenylate cyclase in the β-cells, an interpretation in accord with the assumption that cyclic AMP plays a role in mediating the acute insulin-releasing action of glucose.[23,33,34] In view of the effects of cholera toxin on insulin release and islet cyclic

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AMP,[35] we would contest the idea that the acute effect of glucose on insulin release is essentially mediated through activation of adenylate cyclase; but there is no strong evidence against such activation's playing a role in parallel with some other mechanism. However, the effect of glucose on islet cyclic AMP resembles that on insulin release in being inhibited by Ca^{2+} deficiency,[36,37] and release of cyclic AMP[33] may occur as the result of, rather than as the cause of, insulin secretion.[38] Therefore, caution is warranted in concluding that the different insulin-releasing activities of D-glucose anomers are necessarily due to their having different capacities to activate adenylate cyclase.

When a metabolic mechanism that could complement glycolysis in forming a dual system for stimulus recognition entirely within the framework of the substrate-site hypothesis is sought, the anomer data rule out all pathways that branch off from glucose-6-phosphate. This leaves only the sorbitol pathway, and it is interesting that sorbitol can potentiate insulin release when stimulated by low glucose concentrations.[39] An auxiliary role for the sorbitol pathway may exist, even if the proposal that metabolism through this pathway is requisite for glucose to stimulate secretion[31] seems to be too far-reaching.[39] Like 3-phosphoglyceraldehyde oxidation in glycolysis, the oxidation of sorbitol to fructose is linked to NAD+ reduction, which is noteworthy in view of previous speculations that NADH may be a factor in transmitting the secretagogic signal from glycolysis.[20,26] We do not yet know whether the sorbitol pathway in the β-cells prefers α-D-glucose to β-D-glucose. In view of the present data it would seem highly relevant to study the rate of formation of sorbitol and fructose from the radioactive anomers, but the required methods have still to be developed.

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