Presence, Induction and Possible Role of Glucose 6-Phosphatase in Mammalian Pancreatic Islets

By I.-B. TÄLJEDAL

Department of Histology, University of Umeå, Umeå, Sweden

(Received 12 March 1969)

1. Pancreatic islets from several mammalian species were investigated for hydrolytic activity towards glucose 6-phosphate. Both the total phosphatase activity towards this substrate and the proportion cleaving glucose 6-phosphate in preference to β -glycerophosphate varied widely between species. In pancreatic-islet homogenates prepared from mice and guinea pigs there was a higher rate of liberation of P_i at pH 6.7 from glucose 6-phosphate than from β -glycerophosphate. In these two species cortisone treatment enhanced the enzyme activity towards glucose 6-phosphate but not that towards β -glycerophosphate. Simultaneous injections of ethionine or puromycin blocked this stimulating effect of cortisone. 2. With whole homogenates of mouse pancreatic islets, inverse plots of the relationship between glucose 6-phosphate concentration and enzyme activity suggested the simultaneous action of two enzymes with different K_m values. After fractionation of islets from obese-hyperglycaemic mice by differential centrifugation, one of these enzymes could be shown to be localized in the microsome fraction. It had K_m for glucose 6-phosphate about 0.5 mM and optimum pH 6.7. It split glucose 6-phosphate in preference to β -glycerophosphate, glucose 1-phosphate, fructose 6-phosphate and fructose 1,6-diphosphate. Incubation of the microsomes at pH 5.0 and 37° for 15 min. decreased the enzyme activity by about 80%. Glucose was a potent inhibitor, the type of inhibition being neither strictly competitive nor non-competitive. It is suggested that the results indicate the presence of glucose 6-phosphatase in mammalian endocrine pancreas, and that this enzyme may play a role in the metabolic regulation of release of insulin.

The stimulating action of glucose on the secretion of insulin is blocked by mannoheptulose (Coore, Randle, Simon, Kraicer & Shelesnyak, 1963). This indicates that glucose must be phosphorylated in the β -cell in order to promote secretion of insulin, and suggests that possible regulators of the glucose 6-phosphate pool(s) in the β -cell may be significant for the regulation of release of insulin. Previous histochemical demonstrations that glucose 6-phosphate is rapidly hydrolysed in the endocrine pancreas of some species (Lazarus, 1959; Hellman & Hellerström, 1962; Gepts & Toussaint, 1964) were recently confirmed in a biochemical study of whole-islet homogenates from obese-hyperglycaemic mice and cortisone-injected guinea pigs (Täljedal, 1967). The differential behaviour of glucose 6-phosphate- and β -glycerophosphate-splitting activities under various experimental conditions supports the assumption that a specific glucose 6-phosphatase is present in mammalian β -cells (Täljedal, 1967).

The elaboration of a very sensitive spectrophotometric assay for P_i (Itaya & Ui, 1966) made

possible an extension of our studies to subfractions of β -cells and to normal individuals of species with rather small pancreatic islets. These experiments are reported in the present paper with special reference to the specificity and kinetic properties of a microsomal phosphatase present in the β -cells of obese-hyperglycaemic mice.

MATERIALS AND METHODS

Materials. D(+)-Glucose 6-phosphate (sodium salt), β -glycerophosphate (sodium salt), D(+)-glucose, NADP⁺ and glucose 6-phosphate dehydrogenase were purchased from C. F. Boehringer und Soehne G.m.b.H., Mannheim, Germany. Malachite Green was obtained from E. Merck A.-G., Darmstadt, Germany, bovine serum albumin was from Armour Pharmaceutical Co., Eastbourne, Sussex, cortisone acetate (Cortone) was from Merck, Sharp and Dohme, New York, N.Y., U.S.A., and DL-ethionine and puromycin hydrochloride were from Sigma Chemical Co., St Louis, Mo., U.S.A. Crystalline mouse insulin was prepared by Novo Industry A/S, Copenhagen, Denmark. Reagents for radioimmunological determination of insulin were obtained from The Radiochemical Centre, Amersham,

Bucks. All reagents used were of analytical grade. Triple-distilled water was used throughout the experiments.

Non-treated animals. The following animals were used. (a) Male mice about 5 months old. The animals were of a stock that has been bred at our laboratory since 1959 (Hellman, 1965); it originates from Jackson Memorial Laboratories, Bar Harbor, Maine, U.S.A., and carries a gene *ob*, which in the homozygous state gives rise to a syndrome of obesity and hyperglycaemia (Ingalls, Dickie & Snell, 1950); both obese mice and their normal litter mates were used. (b) Male Sprague-Dawley rats weighing about 125 g. (c) Male guinea pigs weighing about 250 g. (d) Male adult Syrian hamsters weighing about 90 g. (e) Adult Chinese hamsters of both sexes weighing about 25 g.

An ordinary laboratory diet was given *ad libitum* until 12 hr. before killing, when the animals were given only water.

Treated animals. Normal male mice about 5 months old were divided into three groups. (a) 16 animals were injected subcutaneously with a suspension of cortisone acetate (25 mg./ml.) in daily doses of 100 mg./kg. body wt. for 6 days. (b) 11 animals were injected with corresponding volumes of 0.9% NaCl. (c) 12 animals received the same treatment with cortisone as the members of group (a) but were, in addition, given daily intraperitoneal injections of a solution of 1.0% (w/v) DL-ethionine in 0.9% NaCl. This solution was adjusted to neutral pH before use and administered in doses of 250 mg. of ethionine/kg. body wt. The mice were allowed free access to food and water until 12 hr. before being killed, when only water was given. The animals were killed at about 9 a.m. on the day after the last injection.

Male guinea pigs weighing about 250 g. were divided into three groups. (a) Seven animals were injected subcutaneously with cortisone acetate (25 mg./ml.) in daily doses of 50 mg./kg. body wt. for 6 days. (b) Seven animals were injected with corresponding volumes of 0.9% NaCl. (c) Five animals were given the same treatment with cortisone as the members of group (a); on the last 3 days before being killed they were, in addition, given subcutaneous injections of puromycin hydrochloride (25 mg./ml.) dissolved in distilled water; a daily dose of 15 mg. of puromycin was administered in three equal parts at 9 a.m., 2 p.m. and 7 p.m. The guinea pigs were allowed free access to food and water throughout the experiment and were killed at about 9 a.m. on the day after the last injection.

Tissue sampling. The animals were killed by decapitation under ether anaesthesia. The pancreas was quickly excised and immersed in 0.25 M-sucrose-1 mM-EDTA. An appropriate number of pancreatic islets (5-50) were isolated from the exocrine parenchyma by free-hand dissection under a stereo microscope as described by Hellerström (1964). This number of islets has a dry weight of about 10-500 µg. In some cases, small specimens of exocrine pancreatic parenchyma were also taken for comparative purposes. The tissues were homogenized in 30-100 µl. of 0.25 M-sucrose solution, usually supplemented with 1 mM-EDTA. In experiments involving subcellular fractionation of the tissue, EDTA was omitted from both the dissection and homogenization solutions, which were adjusted to pH 7.0 with NaOH. All experimental handling, from the killing of the animal to the incubation (see below), was performed in a 3° cold-room.

Subcellular fractionation. Differential centrifugation of

islet homogenates was performed in a Sorvall refrigerated centrifuge (RC2-B) for forces below 25 000 g and in a Spinco ultracentrifuge (L50) for higher forces. Homogenates to be centrifuged were appropriately diluted with 0.25 M-sucrose solution (pH 7.0) to completely fill a polyethylene micro-tube 45 mm. high with a capacity of 400 µl. (Milian Instrument, Geneva, Switzerland). The tubes were placed in balanced Perspex inserts shaped to fit the ordinary buckets of swing-out rotors HB-4 (Sorvall) and SW 39 (Spinco). The gravitational forces given below refer to the middle of these micro-tubes.

For the routine isolation of pancreatic-islet microsomes, the homogenate was initially spun at 25 000 g for 10 min. After careful withdrawal, the supernatant was centrifuged at 100 000 g for 30 min. and the sediment taken up in 30-60 µl. of a 0.25 M-sucrose solution supplemented with 1 mM-EDTA and 0.2% (w/v) of sodium deoxycholate.

Enzyme assay. The rate of liberation of P_i from glucose 6-phosphate and β -glycerophosphate was studied in a final volume of 4.0 µl. containing 20 mM substrate, 1 mM-EDTA, 50 mM-cacodylate buffer, pH 6.7, and 2.0 µl. of microsomal suspension or homogenate. After incubation in polyethylene micro-tubes for 60 min. at 37°, the reaction was stopped by the addition of 200 µl. of Malachite Green-molybdate reagent prepared as described by Itaya & Ui (1966). Solubilization of the coloured product was achieved by the addition of 10 µl. of 1% (v/v) Triton X-100. The test tubes were kept at 4° for 60 min. and E_{660} values were then measured in a Zeiss PMQ II spectrophotometer equipped with micro-cells (10 mm. optical pathway). It was checked that E_{660} was proportionally related to the amount of enzyme present during incubation and to the incubation time within a period of 60 min.

Phosphate standards as well as medium and tissue blanks were included in each experiment. All analyses were performed on triplicate samples, and the error of a single determination was calculated as 4% at the level of 0.93 nmole of P_i . Deviations from the routine procedure in studies of enzyme kinetics are described in the Results section.

Protein determinations. Protein determinations were carried out on triplicate samples (10 µl. each) of whole homogenates or subcellular fractions by the procedure of Lowry, Rosebrough, Farr & Randall (1951). Bovine serum albumin served as reference. The error of a single determination was calculated as 12% at the level of 1.4 µg. of protein.

Glucose 6-phosphate determinations. In studies of phosphatase kinetics the substrate concentrations of the media were checked enzymically with the aid of glucose 6-phosphate dehydrogenase (Hohorst, 1963). Glucose 6-phosphate concentrations were calculated by using a molar extinction coefficient of 6.22×10^4 for NADPH at 340 nm.

Insulin assays. For the determination of insulin in the serum of cortisone-treated mice and their controls, mixed arterial and venous blood was collected from the neck wound at decapitation. After clotting for about 6 hr. at 3°, the blood was centrifuged at 10 000 g, for 5 min. and the serum withdrawn. It was stored at -90° until assayed by the radioimmunological procedure of Hales & Randle (1963) within 2 months, with crystalline mouse insulin as standard.

Computing. Some kinetic data obtained with whole-islet homogenates were treated hypothetically as reflecting hydrolysis of glucose 6-phosphate catalysed by the simultaneous action of two different enzymes. To estimate the

K_m values of these enzymes, the experimental results were fitted to a composite Michaelis-Menten function [$v = V_1/(1 + K_{m1}/s) + V_2/(1 + K_{m2}/s)$] by computing on a Control Data 3600 digital computer.

RESULTS

Comparison of species. The enzyme activities towards glucose 6-phosphate and β -glycerophosphate in the endocrine pancreas of various mammalian species are given in Table 1. A rather large variation in both the total activity and the enzyme specificity for glucose 6-phosphate is evident. Although the rate of liberation of P_i from glucose 6-phosphate significantly exceeded that from β -glycerophosphate in normal mice ($P < 0.02$), obese mice ($P < 0.001$) and guinea pigs ($P < 0.001$), the latter substrate was the more rapidly split in Syrian ($P < 0.001$) and Chinese ($P < 0.03$) hamsters. The homozygous occurrence of the gene *ob* in mice was associated with an elevated activity towards glucose 6-phosphate as compared with normal

controls ($P < 0.01$). The β -glycerophosphate-splitting activity was, however, the same in the two groups of mice.

Subcellular distribution of phosphatase activities. The subcellular distribution of glucose 6-phosphate- and β -glycerophosphate-splitting activities in the islets of obese mice is shown in Table 2. It is evident that the enzymes hydrolysing glucose 6-phosphate could be partially separated from those acting on β -glycerophosphate. It is also obvious that a major part of the total glucose 6-phosphate-splitting activity is localized in the microsomal fraction, where the specific activity expressed per unit of protein is also the highest. The fraction sedimenting at 600g also displayed a higher specific activity than the whole homogenate. The total recoveries of analysed activities were all above 95%.

Optimum pH. The influence of pH on the hydrolysis of glucose 6-phosphate by islet enzymes is shown in Fig. 1. There is only a hint of an optimum at pH 6.7 with whole-islet homogenates. The masking effect of hydrolases with a more acid pH optimum

Table 1. *Enzyme activities towards glucose 6-phosphate and β -glycerophosphate in islet homogenates prepared from different species*

For each animal one homogenate of micro-dissected islets was prepared and assayed at pH 6.7 for hydrolysis of both glucose 6-phosphate and β -glycerophosphate. The routine procedure described in the text was used. The results are presented as moles of P_i liberated from either substrate/kg. of protein/hr. In addition, the difference and ratio between the rates of hydrolysis of glucose 6-phosphate and β -glycerophosphate are given. Mean values $\pm$ s.e.m. are given, with the numbers of animals studied in parentheses.

Species	Glucose 6-phosphate hydrolysis	β -Glycerophosphate hydrolysis	Difference	Ratio
Obese mice (6)	0.57 ± 0.044	0.19 ± 0.030	0.38 ± 0.038	3.13
Guinea pigs (6)	0.42 ± 0.023	0.14 ± 0.021	0.28 ± 0.021	3.20
Normal mice (6)	0.30 ± 0.056	0.18 ± 0.054	0.12 ± 0.032	1.83
Rats (6)	0.35 ± 0.021	0.32 ± 0.022	0.03 ± 0.032	1.12
Syrian hamsters (6)	0.18 ± 0.019	0.29 ± 0.018	-0.11 ± 0.015	0.61
Chinese hamsters (5)	0.25 ± 0.037	0.37 ± 0.034	-0.12 ± 0.034	0.67

Table 2. *Subcellular distribution of enzyme activities towards glucose 6-phosphate and β -glycerophosphate in pancreatic islets of obese mice*

Enzyme activities are given as moles of P_i liberated/kg. of protein/hr. In addition, the recoveries of enzymes and protein are indicated for each fraction as percentages of values obtained with the whole homogenate. The latter hydrolysed glucose 6-phosphate at a rate of 0.86 mole/kg. of protein/hr. and β -glycerophosphate at a rate of 0.43 mole/kg. of protein/hr. Routine procedures were used in enzyme and protein assays (see the text).

Fraction	Glucose 6-phosphate hydrolysis		β -Glycerophosphate hydrolysis		Protein recovery
	Activity	Recovery	Activity	Recovery	
600g, 10 min.	2.23	9.4	0.90	7.6	3.6
10000g, 10 min.	0.54	12.8	0.53	24.8	20.0
25000g, 10 min.	0.72	18.0	0.53	26.7	21.3
100000g, 30 min.	2.51	46.9	0.69	26.7	16.1
Supernatant	0.19	8.1	0.15	12.4	36.5
Total		95.2		98.2	97.5

Fig. 1. Effect of pH on the rate of hydrolysis of glucose 6-phosphate in (a) islet microsomes, (b) a whole-islet homogenate and (c) a whole homogenate of exocrine pancreas. The islet material was prepared from obese mice. The exocrine pancreatic parenchyma was taken from a normal mouse to minimize the risk of contamination with endocrine cells. The substrate concentrations of the media were checked enzymically (see the text) and varied between 17.2 and 17.6 mM. Apart from variation of the pH, the routine assay procedure was used.

is, however, removed from the microsomal fraction. With this material a clear optimum at about pH 6.7 is shown. By contrast, purely exocrine pancreatic parenchyma showed no evidence of optimum activity at this pH.

Substrate specificity. As shown in Table 3, the islet microsomal fraction split glucose 6-phosphate in preference to some other phosphorylated sugars.

Effect of substrate concentration. The relationships between substrate concentration and enzyme activity in whole homogenates of endocrine and exocrine pancreas are given as double-reciprocal plots in Fig. 2. Although a linear relationship was obtained with acinar tissue from a normal mouse, the results for islet tissue from both normal and obese mice are fitted by curves suggesting the simultaneous action of more than one enzyme. Assuming that the observed activities in the islet material were the sums of two enzymes obeying Michaelis-Menten kinetics, a high K_m in the range 20–50 mM and a low K_m 0.5–0.6 mM could be computed. There is close concordance between the observed reaction velocities and the corresponding values of the computed hyperbola. The former K_m values are of the same order as the values

obtained with exocrine pancreas, and the latter are close to those recorded for isolated microsomes. Although the results varied somewhat between individual animals, K_m about 0.5 mM was found with the latter material (Fig. 3).

Inhibition by glucose. As shown in Fig. 3, glucose markedly inhibited hydrolysis of glucose 6-phosphate in both the whole-islet homogenate and its microsomal fraction. Although the results invariably indicated that the inhibition was not strictly competitive, an increase in the apparent K_m was the most prominent feature. In another experiment with isolated microsomes, the apparent K_m was found to be 0.71 mM in the absence of inhibitor but rose to 3.28 mM and 4.88 mM in the presence of 45 mM- and 90 mM-glucose respectively. Extrapolation to infinite substrate concentration showed that the accompanying decreases in maximum velocity amounted to only 11 and 24%. If these findings are interpreted as approximating competitive inhibition, K_i about 14 mM is obtained.

Enzyme lability. The rate of hydrolysis of glucose 6-phosphate was studied after preincubation of islet microsomes at 37° and pH 5.0 (5 mM-sodium acetate buffer). After periods of 15 and

Table 3. *Substrate specificity of phosphatase present in islet microsomes*

Microsomes were isolated from pancreatic islets of obese mice as described in the text. The routine procedure was used for enzyme assay, with a substrate concentration of 20 mM and pH 6.7 throughout. The rate of liberation of P_i from each substrate is expressed as a percentage of that from glucose 6-phosphate. Means of three experiments are given.

Substrate	Relative activity
Glucose 6-phosphate	100
Glucose 1-phosphate	27
Fructose 6-phosphate	64
Fructose 1,6-diphosphate	43

Fig. 2. Double-reciprocal plots of velocity versus glucose 6-phosphate concentrations for whole-islet homogenates (●) from (a) an obese mouse and (b) a normal mouse. The corresponding curve for purely exocrine pancreas from a normal mouse (□) is also indicated in (b). The results for islet tissue are fitted by hyperbolae representing the simultaneous action of two enzymes with different K_m values. These curves were obtained from the results for islet tissue by the method of least squares, by using a digital computer. The goodness of fit is shown in (a), where theoretical values of $1/v$ predicted by the computed hyperbola for given values of $1/s$ have been indicated (○). The negative branches of the hyperbolae were used for estimations of the low K_m . Apart from variations of the substrate concentration, the routine assay procedure was used.

30 min. of preincubation only 23 and 20% of the initial glucose 6-phosphate-splitting activity was recovered.

Effect of cortisone and inhibitors of protein synthesis on enzyme activities in vivo. Table 4 summarizes the effect of cortisone treatment on the glucose 6-phosphate- and β -glycerophosphate-splitting enzyme activities in mouse islets. A small but probably significant ($P \approx 0.05$) increase in the activity towards the former substrate was found. The simultaneous injection of ethionine diminished this effect of cortisone to an insignificant level. There was no significant difference between the three groups of mice with respect to the rate of hydrolysis of β -glycerophosphate. A stimulating effect of the steroid injections on the function of the β -cells is indicated by the significantly increased starvation concentrations of insulin in the cortisone-treated mice.

An enhanced enzyme activity towards glucose 6-phosphate ($P < 0.01$) was also noted after treatment of guinea pigs with cortisone (Table 5). The degree of enzyme stimulation appeared to be greater in this species than in the mouse despite the fact that the steroid was given in a lower dose. Puromycin exerted an inhibitory effect on the stimulating action of cortisone. In guinea pigs, as in mice, the activity for hydrolysis of β -glycerophosphate was not significantly affected by the steroid.

DISCUSSION

The method described by Itaya & Ui (1966) for the spectrophotometric assay of P_i has in the present study been adapted to the micro scale to permit measurements in the range of 0.1–1.0 nmole. By virtue of this high sensitivity it was possible to measure the hydrolysis of glucose 6-phosphate in subcellular fractions of mammalian β -cells and in whole homogenates of micro-dissected endocrine pancreas from species with rather small islets. The results obtained leave little doubt that a phosphomonoesterase with particularly high activity towards glucose 6-phosphate is present in the pancreatic islets of some species. There is a great similarity between this enzyme and liver glucose 6-phosphatase with regard to subcellular localization, pH optimum, heat-lability, substrate specificity and sensitivity to inhibition by glucose (de Duve, Berthet, Hers & Dupret, 1949; Byrne, 1961).

Different subcellular distributions of the enzyme activities in mammalian islets towards glucose 6-phosphate and β -glycerophosphate have previously been noted after low-speed centrifugation of acidified and aggregated microsomes (Täljedal, 1967). This observation was corroborated in the present study, in which a more elaborate fractionation of the small islet homogenates could be achieved by ultracentrifugation at neutral pH. The localization of a glucose 6-phosphatase mainly in the microsomal fraction was also reflected in a

Fig. 3. Double-reciprocal plots of velocity versus glucose 6-phosphate concentration in the absence (●) and in the presence (O) of 90 mM-glucose. (a) Results for microsomes; (b) results for a whole homogenate prepared from islets of an obese mouse. Routine procedures were used for the preparation of microsomes and for the assay of enzyme activities.

Table 4. *Effects of cortisone and ethionine on the enzyme activities towards glucose 6-phosphate and β -glycerophosphate in pancreatic islets of mice*

Enzyme activities (moles of P_i liberated/kg. of protein/hr.) are presented as mean values $\pm$ s.e.m. The numbers of animals in each group are indicated within parentheses. Cortisone acetate was subcutaneously given in daily doses of 100 mg./kg. body wt. for 6 days. Control animals received corresponding injections of 0.9% NaCl. DL-Ethionine was administered intraperitoneally in doses of 250 mg./kg. body wt. Ten of the controls and seven of the cortisone-treated mice were randomly selected for analyses of serum insulin concentrations at the time of killing. The following mean values $\pm$ s.e.m. were obtained; control mice, 1.38 ± 0.24 ng./ml.; cortisone-treated mice, 3.94 ± 0.73 ng./ml. This difference between the two groups is statistically significant ($P < 0.01$). All animals were starved for 12 hr. before being killed.

Treatment	Glucose 6-phosphate hydrolysis	β -Glycerophosphate hydrolysis
NaCl	0.30 ± 0.028 (16)	0.125 ± 0.010 (13)
Cortisone	0.40 ± 0.043 (11)	0.143 ± 0.018 (8)
Cortisone + ethionine	0.34 ± 0.040 (12)	0.126 ± 0.021 (7)

higher activity per unit of protein in this fraction as compared with the whole homogenate. These observations conform well with the histochemical

Table 5. *Effects of cortisone and puromycin on the enzyme activities towards glucose 6-phosphate and β -glycerophosphate in pancreatic islets of guinea pigs*

Enzyme activities (moles of P_i liberated/kg. of protein/hr.) are presented as mean values $\pm$ s.e.m. The numbers of animals in each group are indicated within parentheses. Cortisone acetate was given subcutaneously in daily doses of 50 mg./kg. body wt. for 6 days. Control animals received corresponding injections of 0.9% NaCl. Puromycin was administered subcutaneously three times a day on the last 3 days before the animals were killed, each injection representing 5 mg. of the drug.

Treatment	Glucose 6-phosphate hydrolysis	β -Glycerophosphate hydrolysis
NaCl	0.45 ± 0.065 (7)	0.15 ± 0.021 (7)
Cortisone	0.72 ± 0.056 (7)	0.15 ± 0.025 (7)
Cortisone + puromycin	0.52 ± 0.043 (5)	0.16 ± 0.035 (4)

results of Petersson (1966), who reported that the staining for hydrolysis of glucose 6-phosphate appeared to be localized to the endoplasmic reticulum of isolated β -cells from guinea-pigs. Petersson's (1966) observation that the nuclear membrane of these cells was also intensely stained is paralleled by the present finding of high activity in the low-speed sediment.

Isolation of the microsome fraction from the islet cells made possible a more detailed study of enzyme kinetics than could be performed with the whole homogenate. To obtain results representative of β -cells, the islets of obese mice, which consist of more than 90% of these cells (Hellman, 1965), were used as the source of microsomes. Although inverse plots of the relationship between substrate concentration and enzyme activity in whole homogenates strongly indicated the simultaneous action of two enzymes with different K_m values, a clear pH optimum for glucose 6-phosphatase could not be obtained with this material. The masking effect of hydrolases with a more acid pH optimum was, however, removed in the microsomal fraction, which also displayed a K_m similar to the low K_m computed from results for the whole homogenate. This K_m , 0.5mM, is lower than that usually found for the glucose 6-phosphatase in liver (Segal, Washko & Lee, 1958; Arion & Nordlie, 1964). It should be pointed out, however, that the concentration of glucose 6-phosphate in the tissue water of the normal β -cell is about 0.1mM (Idahl & Hellman, 1968; Matschinsky & Ellerman, 1968), which suggests that the rate of dephosphorylation *in vivo* is regulated by the intracellular concentration of substrate. The somewhat higher K_m reported in an independent study of hydrolysis of glucose 6-phosphate in mouse islets (Ashcroft & Randle, 1968) may well be explained by the exclusive use of whole-tissue homogenates.

Glucose was shown to be a potent inhibitor of glucose 6-phosphate hydrolysis in the whole homogenate as well as in the microsome fraction. Because only small amounts of microsomes could be obtained for each experiment, a detailed investigation of the mechanism of inhibition was not possible. Repeated experiments demonstrated, however, that glucose affected both the apparent K_m and the maximum velocity of the enzyme, which is in agreement with previous results for liver glucose 6-phosphatase (Byrne, 1961). In contrast with liver, however, the main effect of glucose was an increase in the apparent K_m , maximum velocity being only slightly affected. Especially pertinent to the discussion of islet physiology is the close correspondence between the approximate K_i 14mM and the concentration range for glucose (3–17mM) within the β -cells (Idahl & Hellman, 1968).

There were marked differences in islet glucose 6-phosphatase activity among the various species studied. In Syrian and Chinese hamsters there was a higher rate of liberation of P_i from β -glycerophosphate than from glucose 6-phosphate. This does not prove the absence of glucose 6-phosphatase from the islets of these species, but it may be suggested that the specific enzyme activity is comparatively

low. It is still more doubtful whether the rat should be regarded as lacking glucose 6-phosphatase; its islets display the same activity towards the two substrates under the present assay conditions. Although the specific enzyme has not been histochemically demonstrable in normal β -cells of this species (Gepts & Toussaint, 1964), it has been claimed to appear soon after the injection of alloxan (Petkov, Verne & Wegmann, 1965).

Although our present knowledge does not permit definite conclusions about a role of glucose 6-phosphatase in islet physiology, the increase in the enzyme activity during prolonged stimulation of the β -cells should be borne in mind. An increased glucose 6-phosphate-splitting activity has previously been demonstrated histochemically in the islets of obese mice (Hellman & Hellerström, 1962) and cortisone-treated rabbits (Gepts & Toussaint, 1964). The present biochemical approach showed that the increased enzyme activity displayed by obese mice and cortisone-treated animals was not due to non-specific phosphatases, the rate of hydrolysis of β -glycerophosphate being the same as in control animals. The islet glucose 6-phosphatase of the guinea-pig showed a greater response to a lower dose of cortisone than did that of the mouse. Whether this reflects some metabolic difference between the β -cells of the two species is unknown, since the mechanism underlying the cortisone-induced stimulation of the endocrine pancreas is not fully understood. The fact that ethionine and puromycin inhibited the enhancement of islet glucose 6-phosphatase activity strongly suggests that the latter was at least to some extent due to synthesis of enzyme in both species (Weber, Singhal, Stamm, Fisher & Mentendiek, 1964).

There is now strong evidence for the view that the process of release of insulin is linked to the metabolism of glucose 6-phosphate (Coore *et al.* 1963; Malaisse, Lea & Malaisse-Lagae, 1968). In discussing what role may be envisaged for a specific glucose 6-phosphatase in this context, it must first be pointed out that nothing is known about the concentration of metabolites in different parts of the β -cell. If, however, both glucose and glucose 6-phosphate are accessible to the enzyme, two effects might be conceived. First, the enzyme would tend to drain the glucose 6-phosphate pool. Secondly, according to the kinetic results presented, this drain would be considerably inhibited by glucose in physiological concentrations. The assumption that the glucose 6-phosphate concentration is significantly influenced by glucose 6-phosphatase receives support from recent results showing a much lower glucose 6-phosphate/glucose ratio in the islets and in liver than in the exocrine pancreatic parenchyma (Idahl & Hellman, 1968). On the other hand, Ashcroft & Randle (1968) failed to demonstrate any

conversion of mannose into glucose in whole mouse islets, despite the fact that islet extracts were found to convert mannose into glucose 6-phosphate. They concluded that hydrolysis of glucose 6-phosphate does not play a significant role in regulating the glucose 6-phosphate concentration. The question therefore arises as to whether these cells may contain different pools of glucose 6-phosphate. This possibility has been considered by Samols, Marri & Marks (1966), who suggested that the glucose 6-phosphate involved in the control of the release of insulin is derived from glycogen rather than from glucose. Readily mobilizable stores of glycogen have, indeed, been demonstrated in the endocrine pancreas of both normal and obese mice (Hellman & Idahl, 1969). Interruption of the blood supply to the islets brought about a prompt decrease of their glycogen content. There was, however, no change of the intracellular glucose concentration during the early phases of ischaemia, despite the fact that glucose utilization must be assumed to occur for some time also under anaerobic conditions. These observations are conformable with the idea that the glucose 6-phosphatase is active as a hydrolase in intact β -cells.

This work was financially supported by the U.S. Public Health Service (AM-12535) and the Swedish Medical Research Council (12x-2288). The source programme for the computer calculations was kindly written by Dr S. Wold, Department of Organic Chemistry, University of Umeå. The skilful technical assistance of Miss K. Janze and Miss A.-C. Lindström is also gratefully acknowledged.

REFERENCES

- Arion, W. J. & Nordlie, R. C. (1964). *J. biol. Chem.* **239**, 2752.
- Ashcroft, S. J. H. & Randle, P. J. (1968). *Nature, Lond.*, **219**, 857.
- Byrne, W. L. (1961). In *The Enzymes*, vol. 5, p. 73. Ed. by Boyer, P. D., Lardy, H. & Myrbäck, K. New York: Academic Press Inc.
- Coore, H. G., Randle, P. J., Simon, E., Kraicer, P. F. & Shelesnyak, M. C. (1963). *Nature, Lond.*, **197**, 1264.
- de Duve, C., Berthet, J., Hers, H. G. & Dupret, L. (1949). *Bull. Soc. Chim. biol., Paris*, **31**, 1242.
- Gepts, W. & Toussaint, D. (1964). In *The Structure and Metabolism of the Pancreatic Islets*, p. 357. Ed. by Brolin, S. E., Hellman, B. & Knutson, H. Oxford: Pergamon Press Ltd.
- Hales, C. N. & Randle, P. J. (1963). *Biochem. J.* **88**, 137.
- Hellerström, C. (1964). *Acta endocr., Kbh.*, **45**, 122.
- Hellman, B. (1965). *Ann. N.Y. Acad. Sci.* **131**, 541.
- Hellman, B. & Hellerström, C. (1962). *Acta endocr., Kbh.*, **39**, 474.
- Hellman, B. & Idahl, L.-Å. (1969). *Endocrinology*, **84**, 1.
- Hohorst, H.-J. (1963). In *Methods of Enzymatic Analysis*, p. 134. Ed. by Bergmeyer, H. U. New York: Academic Press Inc.
- Idahl, L.-Å. & Hellman, B. (1968). *Acta endocr., Kbh.*, **59**, 479.
- Ingalls, A. M., Dickie, M. M. & Snell, G. D. (1950). *J. Hered.* **41**, 317.
- Itaya, K. & Ui, M. (1966). *Clin. chim. Acta*, **14**, 361.
- Lazarus, S. S. (1959). *Proc. Soc. exp. Biol., N.Y.*, **101**, 819.
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). *J. biol. Chem.* **193**, 265.
- Malaisse, W. J., Lea, M. A. & Malaisse-Lagae, F. (1968). *Metabolism*, **17**, 126.
- Matschinsky, F. M. & Ellerman, J. E. (1968). *J. biol. Chem.* **243**, 2730.
- Petersson, B. (1966). *Histochemie*, **7**, 116.
- Petkov, P., Verne, J. & Wegmann, R. (1965). *Experientia*, **21**, 530.
- Samols, E., Marri, G. & Marks, V. (1966). *Diabetes*, **15**, 855.
- Segal, H. L., Washko, M. E. & Lee, C. W. (1958). *Science*, **128**, 251.
- Täljedal, I.-B. (1967). *Biochim. biophys. Acta*, **146**, 292.
- Weber, G., Singhal, R. L., Stamm, N. B., Fisher, E. A. & Mentendiek, M. A. (1964). In *Advances in Enzyme Regulation*, vol. 2, p. 1. Ed. by Weber, G. Oxford: Pergamon Press Ltd.