with values determined from other enzyme sources[4,8,12] while the value for *N*-acetylglucosamine differs by an order of magnitude[8,11].

At a final concentration of 4 mM, Mn^{2+}, Ca^{2+}, and EDTA had no effect on enzyme activity, while the same concentration of Mg^{2+}, Cu^{2+}, Pb^{2+}, and Co^{2+} reduced activity by approximately 14%. No enzyme activity could be detected in the presence of 1 mM of Ag^{+} or Hg^{2+}. At 10 mM, CN^{-} reduced activity 14%, and NaCl enhanced activity by 10%. Bovine serum albumin (0.01%) stimulated activity by 25%. In general, there was little to no effect on β-*N*-acetylglucosaminidase activity by most of the metals tested. An exception appeared among the heavy metals, where the enzyme was relatively insensitive to Pb^{2+} as compared to Ag^{+} and Hg^{2+}. The sensitivity of the uterine enzyme to various metals was found to be similar to that of spleen and arterial tissues (*cf.* BUDDECKE AND WERRIE[3,4]).

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Some aspects of the apparent glucose-6-phosphatase activity in the pancreatic islets of mammals

On the basis of staining histochemistry it has been claimed that glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) occurs in the pancreatic islets of several species[1,2]. There are, however, no biochemical data available to corroborate this view. On the contrary, it has been shown in toadfish[3] that glucose 6-phosphate is hydrolyzed at a lower rate than glycerophosphate in the

endocrine pancreas. Since glucose-6-phosphatase, if really present in mammalian β cells, may be involved in the regulation of the synthesis and/or release of insulin, the apparent activity of this enzyme in the endocrine pancreas of obese–hyperglycemic mice and guinea pigs has been subjected to a biochemical analysis.

The following animals were used: About 30 adult, female obese–hyperglycemic mice of the American variety (gene symbol "obob"), which were given free access to food and water (*i.e.*, AO-mice); and eleven male guinea pigs, weighing about 400 g, which received two daily subcutaneous injections of a saline suspension of cortisone acetate (Cortone; Merck, Sharp and Dohme) in doses of 60 mg/kg body weight for 14–29 days. The guinea pigs were given free access to food and water until 12 h before death. All animals were killed by decapitation and the pancreases rapidly removed and placed in a solution of 0.25 M sucrose and 1 mM EDTA, maintained at a temperature of 2°. 20–30 pancreatic islets were isolated from each pancreas[4] and homogenized in 100 μl of Tris–maleate buffer (50 mM; pH 6.5) containing 1 mM EDTA. Small pieces of exocrine pancreas and liver were similarly homogenized. All preparative handling was performed at 0–4°.

For the assay of enzyme activities, triplicate samples of homogenate (10 μl each) were incubated at 37° with 50 μl of substrate–reagent media consisting of 40 mM glucose 6-phosphate (Boehringer; disodium salt) or 40 mM β-glycerophosphate (Sigma; disodium salt) dissolved in Tris–maleate buffer (50 mM; pH 6.5). The reaction was arrested by the addition of 100 μl of 10% trichloroacetic acid and determinations of the P_i liberated performed as previously[2]. The protein concentrations of the homogenates were measured in triplicate samples (10 μl each) according to LOWRY *et al.*[5] using bovine serum albumin as reference (Armour Pharmaceutical Co.). It was ascertained that the hydrolysis of glucose 6-phosphate was linearly related to the concentration of tissue homogenate and the incubation time for at least 30 min. In four different experiments with islets of AO-mice the ratio between the glucose 6-phosphate- and β-glycerophosphate-splitting enzyme activities was found to be higher at pH 6.5 than at pH 5.2. The mean values for this ratio were 3.1 and 2.3, respectively.

The effect of preincubating homogenates of endocrine pancreas from AO-mice at 37° was tested in three different experiments. The islets were homogenized in acetate buffer (100 mM; pH 5.0) containing 1 mM EDTA, and samples were drawn for parallel assays of glucose 6-phosphate- and β-glycerophosphate-splitting enzyme activities after different periods of time. In all experiments the enzyme activity towards glucose 6-phosphate was markedly inhibited by 15–30 minutes of preincubation. The mean values for the degree of inhibition were 36 and 46% after 15 and 30 min, respectively. The rate of β-glycerophosphate hydrolysis was not inhibited by this treatment, but rather slightly activated, the mean values for the degree of activation being 23 and 16% after the corresponding intervals.

An attempt to separate the enzyme activity towards glucose 6-phosphate from that towards β-glycerophosphate was made according to the technique described by DE DUVE *et al.*[6]. Islets from AO-mice were homogenized in acetate buffer (100 mM; pH 5.0) containing 1 mM EDTA and 0.25 M sucrose. After centrifugation at 750 $\times$ *g* for 10 min, the aggregated particles were washed in the buffered EDTA–sucrose solution and once more sedimented. After resuspension of the particles in Tris–maleate buffer (50 mM; pH 6.5), the enzyme activities towards glucose 6-phosphate

and β-glycerophosphate were measured in the particles and the first supernatant. The distribution of glucose 6-phosphate- and β-glycerophosphate-splitting enzyme activities after the aggregation of microsomes is presented in Table I. Glucose 6-phosphate was more rapidly hydrolyzed than β-glycerophosphate in the particulate fraction, while the reverse result was obtained for the supernatant.

TABLE I

SUBCELLULAR DISTRIBUTION OF PHOSPHATASES

The ratio between the glucose 6-phosphate- and β-glycerophosphate-splitting enzyme activities (pH 6.5) in the supernatant and the particulate fraction after aggregation of microsomes in pancreatic islet homogenates. The results are of three different experiments.

Experiment	*Supernatant*	*Particles*
1	0.72	1.21
2	0.65	1.70
3	0.59	1.76
Mean	0.65	1.56

The enzyme activities towards glucose 6-phosphate and β-glycerophosphate in islets, acinar tissue and liver of AO-mice and cortisone-treated guinea pigs are presented in Table II. For neither group of animals was there any significant difference between the tissues with regard to the rate of β-glycerophosphate hydrolysis. The activity towards glucose 6-phosphate was, however, greater in the islets than in the exocrine parenchyma for both AO-mice ($P<0.01$) and guinea pigs ($P<0.02$). For each animal and tissue the difference and ratio between the enzyme activities towards glucose 6-phosphate and β-glycerophosphate were also calculated. In the AO-mice this difference was significant for both the endocrine (0.49 ± 0.10 moles of P_i liberated/kg protein per h; $P<0.01$) and exocrine pancreas (0.12 ± 0.03 moles of P_i liberated/kg protein per h; $P<0.01$), while in the guinea pigs it was significant only for the islets (0.56 ± 0.21 moles of P_i liberated/kg protein per h; $P<0.05$). In both groups of animals the ratio was also much higher for the islets than for the acinar tissue (AO-mice: islets, 3.29; acinar tissue, 1.52. Guinea pigs: islets, 2.43; acinar tissue, 1.37).

This study was designed to check by quantitative chemical microtechniques the hypothesis that glucose-6-phosphatase is present in mammalian endocrine pancreas. Although crude homogenates had to be used because of the small amounts of islet tissue available from mammals, the enzyme activity towards glucose 6-phosphate could be clearly distinguished from the non-specific activity acting on β-glycerophosphate. As evidence for this, the non-specific phosphatase activity at pH 6.5 was not significantly different in the endocrine and exocrine pancreas, while the islets displayed greater activity towards glucose 6-phosphate than the acinar tissue in both groups of animals. It therefore seems reasonable to conclude either that the acid phosphatases in islets and acinar tissue have different affinities for glucose 6-phosphate, or that the endocrine pancreas contains another enzyme in addition to non-specific acid phosphatases acting on glucose 6-phosphate. In support of the

TABLE II

DISTRIBUTION OF PHOSPHATASE ACTIVITIES IN TISSUES

Glucose 6-phosphate- and β-glycerophosphate-splitting enzyme activities (pH 6.5) in homogenates of the endocrine and exocrine pancreas and the liver of AO-mice and cortisone-injected guinea pigs, expressed in moles of P_i liberated/kg protein per h. The numbers of animals in each group are given within parentheses. Mean values ± S.E.

Animals	*Islets*		*Acinar tissue*		*Liver*	
	Glucose 6-phosphate	*β-glycero-phosphate*	*Glucose 6-phosphate*	*β-glycero-phosphate*	*Glucose 6-phosphate*	*β-glycero-phosphate*
AO-mice	0.72 ± 0.11 (8)	0.24 ± 0.03 (8)	0.38 ± 0.04 (9)	0.26 ± 0.03 (9)	6.72 ± 1.05 (11)	0.39 ± 0.08 (11)
Guinea pigs	1.21 ± 0.21 (11)	0.66 ± 0.20 (11)	0.62 ± 0.07 (10)	0.57 ± 0.16 (10)	8.61 ± 2.72 (11)	0.69 ± 0.12 (11)

latter alternative, the ratio between the enzyme activities towards the two substrates was higher at pH 6.5, which is known to be optimal for glucose-6-phosphatase[6], than at pH 5.2 which is optimal for acid phosphatases[2]. In accordance with the great lability of glucose-6-phosphatase[6], the enzyme activity towards glucose 6-phosphate was drastically inhibited by preincubation at pH 5.0 and 37°. By contrast, the β-glycerophosphate-splitting enzyme activity was not inhibited, which conforms well with the stability of acid phosphatases in pancreatic islet homogenates[2]. Finally, the subcellular distribution of the phosphatase activities indicates that glucose-6-phosphatase was sedimented with the aggregated microsomes, while non-specific acid phosphatases remained in the supernatant[6].

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