QUANTITATIVE STUDIES ON PHOSPHATASES IN ISOLATED PANCREATIC ISLETS OF MAMMALS

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To Anna-Lena

The present review is based on the following papers as well as on some hitherto unpublished results, which will be presented.

I. Täljedal, I.-B., Hellman, B. and Hellerström, C.: Quantitative studies on isolated pancreatic islets of mammals: adenosine triphosphatase activity in normal and obese-hyperglycemic mice. *J. Histochem. Cytochem. 12,* 491 (1964).

II. Hellerström, C., Täljedal, I.-B. and Hellman, B.: Quantitative studies on isolated pancreatic islets of mammals. 5-nucleotidase and adenosine triphosphatase activities in normal and cortisone-treated rats. *Endocrinology 76,* 315 (1965).

III. Täljedal, I.-B., Hellman, B. and Hellerström, C.: Quantitative studies on isolated pancreatic islets of mammals: enzymic hydrolysis of nucleoside diphosphates and p-nitrophenyl phosphate in normal and cortisone-treated rats. *J. Endocrin. 36,* 115 (1966).

IV. Täljedal, I.-B.: Electrophoretic separation of enzymes from mammalian pancreatic islets. *Med. Pharm. Exp. 15,* 603 (1966).

V. Täljedal, I.-B.: Electrophoretic studies on phosphatases in the pancreatic islets of obese-hyperglycemic mice. *Acta Endocr. (Kbh.) in press.*

VI. Täljedal, I.-B.: Quantitative studies on isolated pancreatic islets of mammals: some aspects of the apparent glucose-6-phosphatase activity in obese-hyperglycemic mice and guinea-pigs. To be printed in condensed form in *Biochim. Biophys. Acta.*

Reference to these papers will be made by citation of the appropriate Roman numerals.

CONTENTS

INTRODUCTION

In the present state of limited knowledge about the etiology of diabetes mellitus, research on this disease has to be carried out on different fronts. One of these is the endocrine pancreas, the special anatomy of which has greatly hampered progress in our insight into its physiology under normal and pathological conditions. The central position held by this organ in carbohydrate metabolism by being the source of both insulin and glucagon contrasts sharply to the scanty information available about the mechanisms behind the normal synthesis and release of these hormones.

Until recently, the biochemical characteristics of the mammalian islet cells could be explored almost exclusively by histochemical staining techniques. Although many significant observations have been made with methods of that kind, quantitative information seems indispensable for the promotion of a more thorough understanding. The great value of using certain bony fish in order to secure enough islet material for biochemical studies is by now well documented (*Falkmer* 1961; *Lazarow et al.* 1964; *Renold et al.* 1964). It meant a further advance when *Lacy* (1962) introduced chemical micro-assays to the study of mammalian islet tissue dissected from frozen-dried sections of pancreas. This technique has since then been employed for quantitative studies of oxidative enzymes in a number of species including man (*Kissane et al.* 1964; *Brolin et al.* 1964). Recently *Hellerström* (1964) devised a means for the isolation of fresh mammalian islet tissue. The amount of islet material available by this technique exceeds considerably what can be obtained from frozen-dried sections, and it has been shown that the respiration of the fresh islets can be kept intact for several hours (*Hellerström* 1966).

The increased yield of islet material offered by the new isolation procedure extended the range of conveniently applicable micro-chemical techniques to include assays of hydrolytic enzymes which are much less sensitive than those commonly used for the study of oxidative enzymes. The principal subject of the present investigation was to add quantitative data to previous histochemical observations which suggest that the activities of various phosphatases are correlated to the functional state of the islet β cells (*Lazarus* and *Barden* 1961; *Lazarus* and *Volk* 1962; *Hellman* and *Hellerström* 1962; *Gepts* and *Toussaint* 1964). In pursuing this line of investigation, the need for methods capable of separating different enzymes present in pancreatic islet homogenates grew increasingly evident. It was therefore considered within the scope of this study also to examine the usefulness of disc electrophoresis for this purpose.

EXPERIMENTAL

Animals

The following animals were used:

1. About 150 adult obese-hyperglycemic mice of the American variety originally described by *Ingalls et al.* (1950) and 10 of their lean litter-mates (I, IV, V, VI).
2. About 100 Sprague-Dawley rats weighing approximately 95 g (II, III).
3. Eleven guinea-pigs weighing approximately 350 g (VI).

Two different modes of influencing the rate of insulin release from the pancreatic islet β cells were used: starvation of the obese-hyperglycemic mice for 7 days and prolonged administration of cortisone to rats and guinea-pigs. While fed obese-hyperglycemic mice are characterized by a degranulation of the islet β cells (*Wrenshall et al.* 1955) and an increased circulating insulin-like activity (*Christophe et al.* 1959; *Christophe* 1961), starvation induces normalization of the blood sugar (*Christophe* and *Mayer* 1959), suppression of the insulin release (*Christophe* 1961), and regranulation of the β cells (*Hellerström* and *Hellman* 1963 a). Cortisone acetate (Cortone®; Merck, Sharp and Dohme), which was given once (II, III) or twice (VI) daily in subcutaneous doses of 50—60 mg/kg body weight, is known to induce morphological signs of a raised β cell function in both rats (*Gepts* 1957; *Christophe et al.* 1958) and guinea-pigs (*Hausberger* and *Ramsay* 1953; *Abelove* and *Paschkis* 1954; *Hellerström* 1963).

Tissue Sampling

The pancreas was rapidly excised and suspended in 0.25 M sucrose solution maintained at +2°C. Ten to 40 islets were carefully isolated from surrounding exocrine parenchyma as described by *Hellerström* (1964) and homogenized either in distilled, deionized water, (I, II, III, V), a buffered (pH 6.5) solution of ethylenediaminotetra-acetate (VI) or 5 % (v/v) Triton X-100 (V) by means of a lucite micro-homogenizer with a maximal capacity of 200-250 µl. The possibility that leakage of proteins from the cells to the dissection medium could significantly affect the results was examined. For this purpose obese-hyperglycemic mice were used, since sufficiently large samples of islet tissue can be isolated from these animals within relatively short periods of time. Table 1 illustrates an experiment in which islets and tiny pieces of exocrine pancreas were left in the dissection medium for different time intervals before the assay for adenosine triphosphatase (ATPase) activity. As can be seen, the ATPase activity per unit of protein was not apparently influenced by the micro-dissection procedure for up to 60 minutes. In the present series of investigations, the isolation of the islets was usually completed within 20 minutes.

Microchemical Enzyme Assays

The enzyme assays were performed with triplicate samples (10 µl each) from each homogenate. Substrates of analytical grade were used.The liberation of inorganic phosphate

was measured after incubation of whole tissue homogenates with relevant media at +37° C. For this purpose the molybdate-reagent described by *Bonting et al.* (1960) was used, and the optical densities of the phosphate-molybdate complex read at 700 mμ in a Beckman DU (I, II, III) or Zeiss PMQ II (VI) spectrophotometer equipped with micro cells (10 mm optical pathway). The methodological error involved in these determinations varied between 4 and 24 % as calculated (see *Eränkö* 1955) for the mean of triplicates. For the assay of non-specific acid phosphatase activity (III, IV) p-nitrophenyl phosphate was used as substrate (*Bessey et al.* 1946), and the optical densities of liberated p-nitrophenol in alkaline solution read at 410 mμ in a Beckman DU spectrophotometer. The error of this method was calculated as 8 % for the mean of triplicates.

TABLE 1. The pancreas from an obese-hyperglycemic mouse was immersed in the ordinary dissection medium (0.25 M sucrose; +2° C) and islets and tiny pieces of exocrine parenchyma were isolated after different periods of time. The figures denote ATPase activity per unit of protein in per cent of the values obtained for the most rapidly collected samples.

Pancreatic islets		Acinar tissue	
Dissection time (minutes)	ATPase activity	Dissection time (minutes)	ATPase activity
0 — 6	100	0	100
10 — 15	84	15	118
20 — 27	116	30	94
30 — 39	91	45	109
		60	111

Protein Determinations

Micro-determinations of protein were performed with the aid of the Folin phenol reagent (*Lowry et al.* 1951) on triplicate samples (10 μl each) from each homogenate. Spectrophotometric measurements were performed at 750 mμ and the error of the method calculated as 11 % for the mean of triplicates. The enzyme activities of the homogenates were expressed either per unit of protein (VI) with bovine serum albumin (Armour Pharmaceutical Company, England) as reference, or per unit dry weight of tissue (I, II, III). The latter values were calculated on the basis of a separate study of the relation between dry weight and protein content of the tissues. In control experiments, the accuracy of this technique was carefully checked by comparison with enzyme data obtained for large tissue samples, which were weighed before the homogenization. As can be seen in Table 2, the data presented in papers I and II are not significantly different from those obtained in the control experiments.

Histochemical Staining

Sections, 10 μ thick, were prepared in a cryostat-microtome from small blocks of fresh frozen (—70°C) pancreatic tissue and attached to glass microscope-slides (I, II, III). For the demonstration of different nucleoside phosphate-splitting enzymes, the sections were incubated at +37°C in media slightly modified from *Padykula* and *Herman* (1955), *Naidoo* (1962), and *Allen* (1963 a). After incubation for 15—60 minutes, the sections were briefly rinsed and mounted in glycerine jelly.

TABLE 2. ATPase and 5-nucleotidase activities in the exocrine pancreas of lean mice and non-diabetic rats as calculated in papers I and II, and corresponding values for homogenates of large, pre-weighed samples of pancreas. In calculating the enzyme activites for the latter, control samples, the water content of the pancreas has been assumed to be 70 % (w/w). The figures denote moles of inorganic phosphate liberated per kg dry weight and hour. Mean values ± S.E.M. The numbers of experiments have been given within parantheses.

Source of data	ATPase (lean mice)	5-nucleotidase (rats)
Papers I and II	7.42±0.89 (10)	0.61±0.05 (10)
Control experiments	6.63±0.70 (7)	0.58±0.04 (5)

Electrophoresis

Disc electrophoresis on polyacrylamide gels (*Ornstein* 1964; *Davis* 1964) was performed (IV, V) according to the modified procedures of *Allen* (1963 b) and *Allen* and *Gockerman* (1964). Ordinary histochemical staining techniques were applied to the gels for the visualization of enzyme activity. For quantitative determinations of nucleoside diphosphatase activity (V), the gels were scanned in a densitometer (Model 52-C, Photovolt Corp., New York) and the degree of staining evaluated with the aid of a variable-response recorder (Varicord Model 42 B, Photovolt Corp., New York). The latter instrument is equipped with a response selector which determines the shape and slope of the function which the pen follows with a given signal. With a given type of sample there is an optimum setting of the response switch that yields a record approximating the actual concentration of the sample, although the optical properties of the latter may not follow Beer's law. For quantitative determinations of non-specific acid phosphatase activity (IV, V), the stained zones were removed by transversely cutting the gels into sections of equal sizes by means of razor blades. The unstained central part of each gel disc was subsequently isolated and homogenized, and the enzyme activity of the extract spectrophotometrically assayed.

RESULTS

Studies on Crude Homogenates

Nucleoside phosphate-splitting enzyme activities. — The enzymatic hydrolysis of adenosine triphosphate (ATP) in islet homogenates from obese-hyperglycemic mice (I) was characterized by an activity optimum at about pH 9.1. At this pH value the liberation of inorganic phosphate was at least 10 times higher from ATP than from adenosine diphosphate (ADP), adenosine monophosphate (AMP), or β-glycerophosphate. The cleavage of cytidine, guanosine, uridine and inosine triphosphates lay in the range of 30—80 % as compared to that of ATP. A considerable enzyme activation was exerted by magnesium ions, while ADP, sodium azide and p-chloromercuribenzoic acid acted as strong inhibitors.

The islets displayed a lower rate of ATP dephosphorylation than the acinar tissue and the liver. The enzyme activity was, however, higher in the islets of obese-hyperglycemic mice than in those of lean litter-mates. Starvation for 7 days did not affect the rate of ATP hydrolysis in either the endocrine or exocrine pancreas of the obese-hyperglycemic mice.

The enzyme activities towards AMP, ADP, inosine diphosphate (IDP) and ATP were studied in rats (II, III). The cleavage of AMP (II) was most rapid at about pH 7.1. It was inhibited by sodium fluoride and nickel chloride, while manganese chloride exerted a slight activation. Optimal activities towards ADP and IDP (III) occurred at about pH 8.0. Magnesium and manganese ions strongly activated the cleavage of both substrates. These cations enhanced the rate of ADP hydrolysis considerably more than that of IDP in the islet homogenates, while the opposite result was obtained for the liver. Sodium fluoride, potassium cyanide and ethylenediaminotetra-acetate acted as inhibitors.

Administration of cortisone for 15 days greatly influenced the enzyme activities towards these nucleoside phosphates in the pancreatic islets. While the AMP-splitting enzyme activity (II) was increased as compared to that of normal rats, there was a significant reduction of the activities towards ADP, IDP (III), and ATP (II). In the acinar tissue there was a reduced cleavage of AMP as well as of the other nucleoside phosphates after cortisone-treatment. No influence was, however, observed on the hydrolysis of these substrates in the heart or liver. Nor was the non-specific acid phosphatase activity affected by the cortisone-treatment in any of the tissues studied, including the pancreatic islets (III).

Glucose-6-phosphate-splitting enzyme activities (VI). Obese-hyperglycemic mice were used for the study of some properties of the glucose-6-phosphate-splitting enzyme activity in the pancreatic islets. Relative to β-glycerophosphate, more glucose-6-phosphate was split at pH 6.5 than at pH 5.2. While the enzyme activity towards β-glycerophosphate was not influenced by preincubation of the homogenates at pH 5.0 and +37°C,

the rate of glucose-6-phosphate hydrolysis was markedly inhibited by such treatment. Resuspended particles, which had been sedimented after aggregation of microsomes at pH 5.0, displayed a higher enzyme activity towards glucose-6-phosphate than towards β-glycerophosphate. In the supernatant, however, β-glycerophosphate was most intensely split.

In both obese-hyperglycemic mice and cortisone-injected guinea-pigs the β-glycerophosphate-splitting enzyme activity was of the same magnitude in the endocrine as in the exocrine pancreas. The glucose-6-phosphate-splitting enzyme activity, on the other hand, was significantly higher in the pancreatic islets than in the acinar tissue.

Localization of Enzyme Activities in Sections

In the obese-hyperglycemic mice there was a positive reaction for ATP-splitting enzyme activity throughout the pancreas, a particularly strong activity being noted in the walls of capillaries as well as larger blood vessels (I). The islet cells displayed a moderate reaction which appeared diffusely distributed within the cytoplasm. When β-glycerophosphate was substituted for ATP as substrate, there was no enzyme staining of the islet cells.

Enzymatic cleavage of AMP was demonstrated in the endocrine and exocrine pancreas of the normal rat (II). While there was a weak reaction in the acini and capillary walls, the islets displayed a strong enzyme staining which could be localized at least to the β cells. Cortisone administration considerably increased the staining intensity of the islet cells, no changes being noted in the exocrine tissue. A positive staining reaction for the cleavage of ADP and IDP also occurred throughout the pancreas of rats (III). Particularly strong activity was found in the walls of capillaries and larger blood vessels. The islet cells gave a moderate reaction with either substrate, the same staining intensity being obtained with ADP as with IDP.

Electrophoretic Studies

Disc electrophoresis on polyacrylamide gels was employed for the separation of various phosphatases from a minimum of 10 pancreatic islets of obese-hyperglycemic mice. With the use of a simple technique, described in detail in paper IV, two isoenzymes of non-specific acid phosphatase could be extracted for quantitative assay subsequent to histochemical visualization of the enzyme activity in the gels. In both the islets and the liver (V) the most anodal isoenzyme had the highest activity, while the most cathodal isoenzyme was most active in the exocrine pancreas.

Three different gel sites with enzyme activity towards IDP could be demonstrated with islet homogenates. ADP and thiamine pyrophosphate were also split at these sites. While all the three substrates appeared to be hydrolyzed at approximately the same rate at the most anodal site, thiamine pyrophosphate and IDP were preferentially split at the intermediate and anodal sites, respectively. By densitometric scanning of the stained gels, it proved possible to measure the IDP-splitting enzyme activity at the anodal gel site. This activity was about 4 times higher in the pancreatic islets than in the acinar tissue as expressed per unit of protein. Starvation of the obese-hyperglycemic mice did not influence the activity of this phosphatase.

DISCUSSION

Nucleoside Phosphate-Splitting Enzyme Activities

The present results (I) indicate that the endocrine pancreas of obese-hyperglycemic mice contains a sulfhydryl-dependent ATPase which can be assayed rather specifically by the employed technique. As evidence for this, there was a more pronounced cleavage of ATP than of any of the other nucleoside triphosphates tested, and the activity towards other substrates, including mono- and diphosphates, was conspicuously weak. Furthermore, ADP, sodium azide and p-chloromercuribenzoic acid, each of which has been reported as a potent ATPase inhibitor in purified enzyme preparations (*Novikoff et al.* 1952: *Kielley* and *Kielley* 1953; *Robertsson* and *Boyer* 1955; *Myers* and *Slater* 1957), exerted marked suppression of the rate of ATP hydrolysis in the present assay system. The observation of a strong enzyme activation by magnesium ions at an optimal concentration of about 1 mM also accords well with previous reports on ATPase in mouse liver mitochondria (*Kielley* and *Kielley* 1953).

The importance of these observations may be evaluated against the background that previous attempts to demonstrate the specificity of this enzyme by means of histochemical staining techniques did not meet with success (*Hellman* and *Hellerström* 1962). Whether the discordant ATPase levels in the obese-hyperglycemic mice and their lean litter-mates were related to the different functional activities of their β cells remains uncertain in view of the fact that suppression of the insulin release by starvation did not induce any changes in the enzyme activity. The present histochemical staining revealed a high and distinct ATP-splitting enzyme activity in the walls of capillaries and larger blood vessels. The possibility that the lower enzyme activity in the lean litter-mates may be explained by the lower capillary density in the islets of these animals as compared to the obese-hyperglycemic mice (*Hellerström* and *Hellman* 1961) should therefore be considered. The present data have so far served as a prerequisite for studies on the effects of alloxan on sulfhydryl-dependent enzymes in the islets of mice (*Hellerström et al.* 1964 a; *Hellman et al.* to be published).

Previous histochemical observations on the AMP-splitting enzyme activity of the pancreatic islets in rats (*Gepts* and *Toussaint* 1964) have been confirmed and extended (II). Recent experiments in mammals have demonstrated that certain enzymes can be selectively induced by hormones without affecting other enzymes involved in the utilization of the same substrates (*Weber* 1963). The question of whether the increased rate of AMP hydrolysis after treatment with cortisone was due to a direct effect of the hormone remains to be answered. The observation that not only cortisone but also glucagon enhances the AMP-splitting enzyme activity in the rat islets (*Johansson* and *Täljedal* to be published) emphazises, however, the possibility that the increased rate of AMP hydrolysis reflects an adaptation of the stimulated β cell to an enhanced intracellular formation

of the nucleotide. With regard to the synthesis of insulin it should be remembered that AMP is formed in the reaction sequence of amino acid activation at the step of transfer of the amino acid to soluble RNA (*Stulberg* and *Novelli* 1962). AMP may also arise by enzymatic cleavage of cyclic 3',5'-AMP (*Butcher* and *Sutherland* 1962), and there are recent data to indicate that the latter compound stimulates the release of insulin from rat pancreas *in vitro* (*Sussman et al.* 1966). The fact that the cleavage of p-nitrophenyl phosphate remained unaffected by the prolonged steroid treatment (III), together with the data on pH-dependency, substrate specificity, and activating and inhibiting compounds (II), strongly support the view that the observed AMP-splitting enzyme activity was due to a specific enzyme, most probably 5-nucleotidase.

Since nucleoside triphosphate-nucleoside monophosphokinases are known to be widely distributed in different organs (*Noda* 1962), the liberation of phosphate from ADP in the islet homogenates (III) might have been mediated by a two-step reaction involving transphosphorylation and subsequent hydrolysis of ATP and AMP. Therefore, the lower rate of apparent ADP hydrolysis in the cortisone-treated rats (III) might at least in part be interpreted as reflecting the lower ATP-splitting enzyme activity that was also found in cortisone-treated rats (II). The observation of a lower ATP-splitting enzyme activity after administration of cortisone is in line with histochemical data presented by *Lazarus* and *Barden* (1961), who reported a reduced extra-mitochondrial ATPase activity in the β cells of cortisone-treated rabbits.

The usefulness of disc electrophoresis for the separation of enzymes from the pancreatic islets of mammals was demonstrated (IV, V). Different molecular varieties of non-specific acid phosphatase could not only be visualized by staining the acrylamide gels but also subsequently extracted for quantitative measurements. A distinctly different isoenzyme pattern was obtained for the pancreatic islets as compared with the exocrine parenchyma of obese-hyperglycemic mice. Since the different enzyme fractions recovered from the islet homogenates had too little activity to permit a more extensive characterization, it cannot yet be decided to what extent the present data indicate a true physiological difference between the islets and the acinar tissue. Some evidence for the assumption that the endocrine and exocrine pancreas differ with respect to the presence of different molecular varieties of acid phosphatase has previously emerged. While only one pH optimum (pH 5.3) has been found for the enzyme in exocrine pancreatic parenchyma, crude islet homogenates seem to display two pH optima (pH 3.5 and 5.3; *Hellerström et al.* 1964 b).

Three IDP-splitting enzymes were also separated from the endocrine pancreas of obese-hyperglycemic mice (V). The electrophoretic mobility, substrate specificity and sensitivity to various ions of the different enzymes were apparently the same in this tissue as in the exocrine parenchyma and the epididymis. In close agreement with previous results for mouse epididymis (*Allen* 1963 b), the most anodal enzyme displayed its highest activity towards IDP, while the intermediate enzyme was most active with thiamine pyrophosphate. The cathodal enzyme appeared to be approximately equally active towards all the three substrates. The identification of these enzymes has been established for epididymis by combining electrophoresis with staining histochemistry on the ultrastructural level (*Allen* 1963 a, b). In view of the latter studies, the present data are best interpreted as indicating the presence of alkaline phosphatase (cathodal site), thiamine pyrophosphatase (intermediate site) and Golgi-associated nucleoside diphosphatase (anodal site) in the pancreatic islets. A low alkaline phosphatase activity has previously been observed in homogenates of this tissue (*Hellerström et al.* 1964 a). The present data are also in agreement with the previous demonstration of IDP- and thiamine pyrophosphate-splitting enzymes in the Golgi zone of the β cells in other species by means of staining histochemistry

on both the light- and electron-microscopical levels (*Lazarus* and *Barden* 1962; *Lazarus* and *Volk* 1962; *Lazarus et al.* 1964; *Petersson* 1966).

An attempt was made to measure the nucleoside diphosphatase, supposedly orginating from the Golgi apparatus, without apparent interference from other phosphatases (V). By means of densitometric scanning of the stained gels, this enzyme was found to have a much higher activity in the endocrine than in the exocrine pancreas. It is worthy of note that starvation of the animals for 7 days did not influence the enzyme activities, since an abundance of data indicate a relationship between the Golgi complex and the secretory mechanisms in several kinds of cells (*Dalton* 1961). Although it is not known to what extent this organelle is involved in the formation of insulin granules, an enlarged Golgi complex has been demonstrated in the β cells of obese-hyperglycemic mice (*Hellman et al.* 1961; *Björkman et al.* 1963), dogs injected with growth hormone (*Volk* and *Lazarus* 1964), and mice treated with anti-insulin serum (*Logothetopoulos* and *Bell* 1966).

Glucose-6-Phosphate-Splitting Enzyme Activities

On the basis of staining histochemistry, several authors have suggested that the islet β cells contain a specific glucose-6-phosphatase, at least in some species (*Lazarus* 1959; *Hellman* and *Hellerström* 1962; *Hellerström* and *Hellman* 1963 b; *Hellerström* 1963; *Gepts* and *Toussaint* 1964; *Petersson* 1966). Paper VI was designed to check this hypothesis by a quantitative biochemical approach. In the islets of obese-hyperglycemic mice the ratio between the enzyme activities towards glucose-6-phosphate and β-glycerophosphate was higher at pH 6.5 than at pH 5.2, considerably reduced by pre-incubation of the homogenates for 30 minutes at pH 5.0 and +37°C, and higher in the particulate fraction than in the supernatant after sedimentation of aggregated microsomes. All these observations would be expected if a glucose-6-phosphatase with properties similar to those in the liver and small intestine were present in the pancreatic islets (*De Duve et al.* 1949; *Öckerman* 1965). Strong support for this hypothesis was also derived from the observation that both obese-hyperglycemic mice and cortisone-treated guinea-pigs displayed much higher activities towards glucose-6-phosphate in the endocrine than in the exocrine pancreas, despite the fact that the rate of β-glycerophosphate hydrolysis at pH 6.5 was about the same in the two tissues.

SUMMARY AND CONCLUSIONS

Quantitative chemical micro-methods, staining histochemistry and disc electrophoresis were employed for the characterization, localization and assay of various phosphatases in homogenates and sections of the endocrine pancreas from mice, rats and guinea-pigs. The principal observations and interpretations are as follows:

1. A sulfhydryl-dependent adenosine triphosphatase, which is present in the pancreatic islets of obese-hyperglycemic mice, can be assayed rather specifically in crude islet homogenates. Previous attempts to demonstrate the specificity of this enzyme by means of histochemical staining techniques were unsuccessful. A lower adenosine triphosphatase activity in the islets of normal mice as compared to obese-hyperglycemic litter-mates may be explained by the difference in capillary density between the two groups of animals, since staining histochemistry revealed a high and distinct adenosine triphosphate-splitting enzyme activity in the walls of islet capillaries.

2. Prolonged administration of cortisone to rats induced an increased adenosine monophosphate-splitting enzyme activity but a reduced rate of phosphate liberation from adenosine diphosphate and adenosine triphosphate in crude islet homogenates. By contrast, the steroid-treatment was without effect on the non-specific acid phosphatase activity. Data on pH-dependency, substrate specificity, and inhibiting and activating compounds are consistent with the assumption that the increased rate of adenosine monophosphate hydrolysis was due to 5-nucleotidase.

3. The possibility of using disc electrophoresis for the separation of enzymes from homogenates of only a few mammalian islets was demonstrated. Two molecular varieties of non-specific acid phosphatase were identified in obese-hyperglycemic mice, and their relative activities measured after extraction of the isoenzymes from the gels.

Three different enzymes acting on inosine diphosphate were separated from the islets of obese-hyperglycemic mice. With regard to their electrophoretic mobility, substrate specificity and sensitivity to ions, they were tentatively identified as alkaline phosphatase, thiamine pyrophosphatase and Golgi-associated nucleoside diphosphatase. The activity of the phosphatase supposedly originating in the Golgi apparatus was measured by photometric scanning of the stained gels. The enzyme activity was found to be about 4 times higher in the endocrine than in the exocrine pancreas as expressed per unit of protein. Starvation for 7 days did not affect this enzyme activity in the islets or the acinar tissue.

4. An attempt was made to elucidate the specificity of the apparent glucose-6-phosphatase activity in the pancreatic islets of mice. The dephosphorylating activities towards glucose-6-phosphate and β-glycerophosphate were found to differ with respect to pH-dependency, lability an sub-cellular distribution. Both obese-hyperglycemic mice and cortisone-injected guinea-pigs displayed much higher activities towards glucose-6-phosphate in the endocrine than in the exocrine pancreas, despite the fact that the rate of β-glycerophosphate hydrolysis at pH 6.5 was about the same in the two tissues. The data support the view that a glucose-6-phosphatase in present in the pancreatic islets.

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REFERENCES

Abelove, W. A. and Paschkis, K. E., *Endocrinology 55:* 637, 1954.
Allen, J. M., *J. Histochem. Cytochem. 11:* 529, 1963 a.
Allen, J. M., *J. Histochem. Cytochem. 11:* 542, 1963 b.
Allen, J. M. and Gockerman, J., *Ann. N. Y. Acad. Sci. 121:* 616, 1964.
Bessey, O. A., Lowry, O. H. and Brock, M. J., *J. Biol. Chem. 164:* 321, 1946.
Björkman, N., Hellerström, C. and Hellman, B., *Z. Zellforsch. 58:* 803, 1963.
Bonting, S. L., Tsoodle, A. D., de Bruin, H. and Mayron, B. R., *Arch. Biochem. Biophys. 91:* 130, 1960.
Brolin, S. E., Borglund, E. and Ohlsson, A., In: *The Structure and Metabolism of the Pancreatic islets,* p. 289. Eds. Brolin, S. E. Hellman, B. and Knutson, H. Oxford: Pergamon Press, 1964.
Butcher, R. W. and Sutherland, E. W., *J. Biol. Chem. 237:* 1244, 1962.
Christophe, J., *Contribution a la biochimie des obésités expérimentales.* Bruxelles: Editions Arscia, 1961.
Christophe, J., Bellens, R., Demanet, J. C., Conard, V. and Gepts, W., *Ann. Endocr. (Paris) 18:* 204, 1958.
Christophe, J., Dagenais, Y. and Mayer, J., *Nature 184:* 61, 1959.
Christophe, J. and Mayer, J., *Endocrinology 64:* 664, 1959.
Dalton, A. J., In: *The Cell,* vol. II, p. 603. Eds. Brachet, J. and Mirsky, A. E. New York: Academic Press, 1961.
Davis, B. J., *Ann. N. Y. Acad. Sci. 121:* 404, 1964.
De Duve, C., Berthet, J., Hers, H. G. and Dupret, L., *Bull. Soc. Chim. Biol. 31:* 1242, 1949.
Eränkö, O., *Quantitative Methods in Histology and Microscopic Histochemistry.* Basel: S. Karger A. G., 1955.
Falkmer, S., *Acta Endocr. (Kbh.)* suppl. 59, 1961.
Gepts, W. *Ann. Endocr. (Paris) 18:* 204, 1957.
Gepts, W. and Toussaint, D., In: *The Structure and Metabolism of the Pancreatic Islets,* p. 357. Eds. Brolin, S. E., Hellman, B. and Knutson, H. Oxford: Pergamon Press, 1964.
Hausberger, F. X. and Ramsey, A. J., *Endocrinology 53:* 637, 1953.
Hellerström, C., *Acta Soc. Med. Upsal. 68:* 1, 1963.
Hellerström, C., *Acta Endocr. (Kbh.) 45:* 122, 1964.
Hellerström, C., *Biochem. J. 98:* 7 C, 1966.
Hellerström, C. and Hellman, B., *Acta Soc. Med. Upsal. 66:* 88, 1961.
Hellerström, C. and Hellman, B., *Acta Endocr. (Kbh.) 42:* 615, 1963 a.
Hellerström, C. and Hellman, B., *Metabolism 12:* 527, 1963 b.
Hellerström, C., Hellman, B. and Täljedal, I.-B., In: *The Structure and Metabolism of the Pancreatic Islets,* p. 311. Eds. Brolin, S. E., Hellman, B. and Knutson, H. Oxford: Pergamon Press, 1964 a.
Hellerström, C., Täljedal, I.-B. and Hellman, B., *Acta Endocr. (Kbh.) 45:* 476, 1964.

Hellman, B. and Hellerström, C., *Acta Endocr. (Kbh.) 39:* 474, 1962.
Hellman, B., Hellerström, C., Larsson, S. and Brolin, S., *Z. Zellforsch. 55:* 235, 1961.
Ingalls, A. M., Dickie, M. M. and Snell, G. D., *J. Hered. 41:* 317, 1950.
Kielley, W. W. and Kielley, R. K., *J. Biol. Chem. 200:* 213, 1953.
Kissane, J. M., Lacy, P. E., Brolin, S. E. and Smith, C. H., In: *The Structure and Metabolism of the Pancreatic Islets,* p. 281. Eds. Brolin, S. E., Hellman, B. and Knutson, H. Oxford: Pergamon Press, 1964.
Lacy, P. E., *Diabetes 11:* 96, 1962.
Lazarow, A., Dixit, P. K., Lindall, A., Moran, J., Hostetler, K. and Cooperstein, S. J., In: *The Structure and Metabolism of the Pancreatic Islets,* p. 249. Eds. Brolin, S. E., Hellman, B. and Knutson, H. Oxford: Pergamon Press, 1964.
Lazarus, S. S., *Proc. Soc. Exp. Biol. Med. (N. Y.) 101:* 819, 1959.
Lazarus, S. S. and Barden, H., *J. Histochem. Cytochem. 9:* 628, 1961.
Lazarus, S. S. and Barden, H., *J. Histochem. Cytochem. 10:* 368, 1962.
Lazarus, S. S., Barden, H. and Volk, B. W., *Excerpta Medica Intl. Congress Series 74:* 68, 1964.
Lazarus, S. S. and Volk, B. W., *Diabetes 11:* (suppl.) 2, 1962.
Logothetopoulos, J. and Bell, E. G., *Diabetes 15:* 205, 1966.
Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., *J. Biol. Chem. 193:* 265, 1951.
Myers, D. K. and Slater, E. C., *Biochem. J. 67:* 572, 1957.
Naidoo, D., *J. Histochem. Cytochem. 10:* 421, 1962.
Noda, L., In: *The Enzymes,* vol. VI, p. 139. Eds. Boyer, P. D., Lardy, H. and Myrbäck, K. New York: Academic Press, 1962.
Novikoff, A. B., Hecht, L., Podber, E. and Ryan, J., *J. Biol. Chem. 194:* 153, 1952.
Ornstein, L., *Ann. N. Y. Acad. Sci. 121:* 321, 1964.
Padykula, H. and Herman, E., *J. Histochem. Cytochem. 3:* 161, 1955.
Petersson, B., *Histochimie 7:* 116, 1966.
Renold, A. E., DiPietro, D. L. and Williams, A. K., In: *The Structure and Metabolism of the Pancreatic Islets,* p. 269. Eds. Brolin, S. E., Hellman, B. and Knutson, H. Oxford: Pergamon Press, 1964.
Robertson, H. E. and Boyer, P. D., *J. Biol. Chem. 214:* 295, 1955.
Stulberg, M. P. and Novelli, G. D., In: *The Enzymes,* vol. VI, p. 401. Eds. Boyer, P. D., Lardy, H. and Myrbäck, K. New York: Academic Press, 1962.
Sussman, K. E., Vaughn, G. D. and Timmer, R. F., *Diabetes 15:* 521, 1966.
Volk, B. W. and Lazarus, S. S., In: *The Structure and Metabolism of the Pancreatic Islets,* p. 143. Eds. Brolin, S. E., Hellman, B. and Knutson, H. Oxford: Pergamon Press, 1964.
Weber, G., In: *Advances in Enzyme Regulation,* vol. I, p. 1. Ed. Weber, G. Oxford: Pergamon Press, 1963.
Wrenshall, G. A., Andrus, S. B. and Mayer, J., *Endocrinology 56:* 335, 1955.
Öckerman, P. A., *Biochim. Biophys. Acta 105:* 22, 1965.