The Metabolism of Lipids in Mouse Pancreatic Islets

THE BIOSYNTHESIS OF TRIACYLGLYCEROLS AND PHOSPHOLIPIDS

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The rate of incorporation of [U-¹⁴C]glucose and [U-¹⁴C]palmitate into the lipids of the pancreatic islets of obese-hyperglycaemic mice was examined. The following main observations were made. 1. Both glucose and palmitate were incorporated into lipids in the islets. The fraction of glucose utilized for lipid biosynthesis was calculated to be 3-6% of that oxidized at high and low glucose concentrations, whereas palmitate was about equally divided between oxidation and esterification into lipids. 2. Glucose was primarily incorporated from *sn*-glycerol 3-phosphate. Of the total glucose carbon incorporated, only 2-4% was recovered as fatty acids. 3. A major portion of both glucose and palmitate was incorporated into phospholipids, whereas 10-30% went into triacylglycerols, depending on the extracellular glucose concentrations. 4. An increase in the glucose concentration from 3.5 to 17 mM caused a twofold increase in the rate of glucose incorporation into triacylglycerols and a fivefold increase in the rate of incorporation into phospholipids. Similar effects were also obtained with normal mouse islets. Palmitate was also preferentially directed into phospholipids by an increased glucose concentration. 5. Islets pre-labelled with radioactive palmitate showed a decrease in triacylglycerol radioactivity when they were subsequently incubated in the absence of exogenous sources of energy. 6. Mannoheptulose inhibited the rate of glucose incorporation into phospholipids, whereas omission of Ca²⁺ and adrenaline left phospholipid biosynthesis unimpaired. The results suggest that pancreatic B-cells have the capacity to store and utilize energy in the form of triacylglycerols. A stimulation of the B-cells by glucose is followed by an increased rate of phospholipid biosynthesis. However, this does not seem to be directly linked to the release of secretory granules.

Studies of the regulation of glucose metabolism in the pancreatic B-cells have added much to our knowledge on possible mechanisms behind the release of insulin (Ashcroft *et al.*, 1970; Hellman *et al.*, 1974). By comparison much less attention has been paid to the role of lipids in both insulin secretion and islet metabolism.

Intracellular lipids serve two important functions, as storage depots for metabolic fuel and as structural components of membranes. The fuel function is met by triacylglycerols dispersed in the cytoplasm or contained in lipid droplets, whereas phospholipids are mainly located in the cellular membranes. The relationship between these two aspects of lipid function and glucose metabolism in the pancreatic B-cells deserves special consideration in view of the central role occupied by glucose in the control of insulin secretion, a process that involves both the expenditure of cellular energy (Milner & Hales, 1969) and the turnover of cell membranes (Lacy, 1962).

A substrate reserve in the B-cells is present either as deposits of non-particulate glycogen (Hellman & Idahl, 1972) or as 'masked' lipids (usually triacyl-

glycerols which occur in such small amounts that they are not stained by the usual lipid stains but can be detected by sensitive fluorescent techniques) (Hellman *et al.*, 1961) and rarely seen lipid droplets (O'Leary, 1930; Westman *et al.*, 1970) in the B-cells of mouse islets. Although the mechanisms for the formation of glycogen have been elucidated (Matschinsky & Ellerman, 1968; Hellman & Idahl, 1969; Brodin & Berne, 1970), little is known of the synthesis and turnover of the lipid stores in the B-cells.

The turnover of cellular membranes, including the plasma membrane, is intimately associated with the secretory process as a continuous formation of membrane-enclosed granules takes place. The final fusion of the granule and plasma membranes may lead to a certain degree of endocytosis and recirculation of membranes (Orci *et al.*, 1973), and phospholipids are known to turn over rapidly in exocrine and endocrine cells in response to their appropriate secretory stimuli (Hokin, 1968).

The aim of the present study was to evaluate the role of lipids in B-cell metabolism by investigating the rate of incorporation of [U-¹⁴C]glucose and

[U-¹⁴C]palmitate into triacylglycerols and phospholipids in isolated pancreatic islets. In addition the effect on the incorporation rate of some inhibitors of the glucose-induced insulin release was studied. Most of the experiments were performed with islets from obese-hyperglycaemic mice, which are known to contain over 90% B-cells (Gepts *et al.*, 1960; Hellman 1961), thus minimizing interference from other islet cell types.

Experimental

Chemicals

Calf thymus DNA, EGTA [ethanedioxybis-(ethylamine)tetra-acetic acid], adrenaline, tripalmitoylglycerol, 1,2-dipalmitoylglycerol, mannoheptulose and palmitic acid were obtained from Sigma Chemical Co., St. Louis, Mo., U.S.A. Fatty acid-poor bovine albumin, which was prepared as described by Chen (1967), was obtained from Miles Laboratories, Kankakee, Ill., U.S.A. An emulsion of egg-yolk lipids was kindly given by AB Vitrum, Stockholm, Sweden. InstaGel, 2,5-diphenyloxazole and 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene were obtained from Packard Instrument Co., Downers Grove, Ill., U.S.A. 3,5-Diaminobenzoate dihydrochloride was obtained from EGA-Chemie, Steinheim, Germany. 2',7'-Dichlorofluorescein, all organic solvents and inorganic salts were of analytical grade and were purchased from E. Merck A.G., Darmstadt, Germany. [U-¹⁴C]Glucose (268–297 mCi/mmol), [U-¹⁴C]palmitate (237 mCi/mmol) and tri[1-¹⁴C]palmitoylglycerol (63 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. Deionized double-distilled water was used in all experiments.

Animals

Female obese-hyperglycaemic mice (gene symbol, *ob/ob*) and lean female mice (*Ob/ob*, *Ob/Ob*), 6–8-months old, were used. The animals belonged to a non-inbred colony which has been kept at the Histological Department, University of Uppsala, since 1959 and which originated from The Jackson Laboratory, Bar Harbor, Maine, U.S.A. Before the experiments, the animals were starved overnight in individual cages with a perforated metal floor to prevent coprophagy. The mice were killed by hyperextension of the neck.

Methods

Isolation of the islets and incubation procedures. The islets were isolated by the procedure described by Howell & Taylor (1968) by using crude collagenase (code CLS; Worthington Biochemical Corp., Freehold, N.J., U.S.A.; 6 mg/ml) in a Krebs–Ringer

phosphate buffer, pH 7.4 (Umbreit *et al.*, 1964). Some 100–300 islets were obtained from a single animal. The time between killing of the mouse and the start of the incubations was about 45 min. The islets were transferred in groups of 10–15 in 50 µl of a bicarbonate-buffered salt mixture (Gey & Gey, 1936), pH 7.4, equilibrated with O₂+CO₂ (95:5) which contained radioactive substrates and other additions as indicated in the Tables and the Figures. The islets were incubated in a centre well hanging inside a scintillation vial (Keen *et al.*, 1963). Double-distilled water (1 ml) was added to the vial to prevent evaporation of the incubation medium. Before closure, the vial was thoroughly gassed with O₂+CO₂ (95:5). Incubations were then carried out in a shaking water bath (100 strokes/min) for 3 h at 37°C. At the end of the 3 h incubation, 200 µl of Gey & Gey (1936) buffer (warmed to 37°C) containing glucose (16.7 mM) was injected and the incubation was continued for another 10 min, to dilute the radioactivity.

Palmitate was saponified with 15 mM-NaOH as described (Berne, 1975b), and was then complexed to fatty acid-poor albumin (10 mg/ml) before it was added to the incubation vessels. The radioactive glucose was freeze-dried in portions on each day of experimentation to remove ethanol and water from the stock solution.

Extraction of lipids. After incubation the islets were rinsed twice in double-distilled water and transferred to 125 µl of a salt solution with the composition described by Folch *et al.* (1957). After disruption of the islets by sonication in an MSE Ultrasonic Disintegrator, model 60 W (MSE, London S.W.1, U.K.) for 10 s, duplicate samples (10 µl) were taken for determination of the DNA content (see below). The rest of the islet homogenates were stored overnight at –30°C. The lipids were extracted with 2 ml of chloroform–methanol (2:1, v/v), giving a one-phase system. A solution (40 µl) of carrier lipids composed of tripalmitoylglycerol, palmitic acid and a mixture of egg-yolk phospholipids (3 mg/ml of each) was added to each chloroform–methanol extract, which was then washed by the procedure described by Folch *et al.* (1957) to remove water-soluble radioactive substances. Control experiments showed that four washings left no interfering traces of radioactivity in the lower chloroform phase. A sample of the lipid-containing lower phase was evaporated to dryness and redissolved in 40 µl of chloroform–methanol and was then applied to t.l.c. plates. T.l.c. was run on precoated plates of silica gel G (E. Merck A.G., Darmstadt, Germany) and the lipids were separated in a solvent system of *n*-hexane–diethyl ether–methanol–acetic acid (90:20:2:3, by vol.) (Garland & Randle, 1963). The plates were activated for 1 h at 110°C before use. The lipid spots were detected in u.v. light after spraying the plates with an ethanolic solution of 2',7'-dichlorofluorescein (0.2 mg/ml).

The spots corresponding to the triacylglycerol and phospholipid markers were scraped off as well as the remaining silica gel which was located between the origin and the solvent front. The spots were scraped into polyethylene scintillation vials containing 0.5 ml of methanol, which minimized adsorption of the polar lipids to the silica particles (Boberg, 1966) and 10 ml of a toluene-based scintillation mixture [2,5-diphenyloxazole, 5 g; 1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene, 0.05 g; toluene, 1 litre]. Controls were obtained from scrapings of sprayed plates with only carrier lipids, run in the same solvent system. The radioactivity was assayed in a Packard liquid-scintillation counter, model 3380. The quenching was as a routine controlled by an external-standard channels-ratio method.

Hydrolysis of the lipid extract. The washed lipid extracts were dried by evaporation of the solvent, and 5M-KOH in 70% (v/v) ethanol was then added. After 3 h at 80°C the pH was adjusted to 1 with conc. HCl and the fatty acids were extracted three times with light petroleum (b.p. 40–60°C). These extracts were pooled, the solvent was evaporated and finally they were fractionated by t.l.c. as described above. The aqueous phase containing the non-lipid residues was placed in 7 ml of InstaGel and its radioactivity was measured.

Recovery. Tests were performed in model experiments with tri[1-¹⁴C]palmitoylglycerol. The absolute recovery from application of the extracts to the t.l.c. plates to scraping off the silic acid areas was over 95%. Over 80% of the radioactivity from the islet lipid extracts was recovered from the plates. Hydrolysis liberated 93% of the radioactive palmitate from tri[1-¹⁴C]palmitoylglycerol.

Expression of results. The incorporation rates were calculated on the basis of the specific radioactivities of [U-¹⁴C]glucose and [U-¹⁴C]palmitate in the incubation media. These were obtained by estimating the radioactivities of 5 µl samples from the incubation media in 7 ml of InstaGel. Differences between means were tested by the Student's *t* test (Snedecor, 1956). The term 'experiment' denotes one batch of islets. Two to four batches were obtained from each

animal. Islets isolated from four to six animals were used to calculate the means.

Determination of DNA. DNA was measured in 10 µl duplicate samples from the islet homogenates by the method of Kissane & Robins (1958) as modified by Hinegardner (1971). Calf thymus DNA was used as a standard. The fluorescence was measured in a Farrand ratio fluorimeter (Farrand Optical Co., Mt. Vernon, N.Y., U.S.A.) in a 150 µl volume.

Results

The rate of incorporation of both [U-¹⁴C]glucose and [U-¹⁴C]palmitate into islet lipids proceeded at a constant rate for up to 5 h of incubation.

The fate of the glucose carbon incorporated into islet lipids was examined by alkaline hydrolytic cleavage of the chloroform-methanol extracts. At 3.5 mM-glucose the rate of incorporation of glucose carbon into products water-soluble after hydrolysis was 6.8 ± 0.6 (mean $\pm$ S.E.M.) pmol/h per µg of DNA as compared with 19.7 ± 0.7 pmol/h per µg of DNA at 17 mM-glucose. A much smaller fraction of glucose carbon was recovered in the fatty acids, in which the rates of incorporation were 0.12 ± 0.01 and 0.76 ± 0.07 pmol/h per µg of DNA at 3.5 and 17 mM respectively. Thus only a minor portion (<4%) of the glucose carbon was incorporated into fatty acids by the B-cells. It should be pointed out, however, that the high glucose concentration (17 mM) exerted a more pronounced stimulatory effect on the rate of incorporation into fatty acids, which increased sixfold, than on the incorporation into the water-soluble part of the lipids which showed only a threefold increase.

Of the radioactivity recovered from the t.l.c. plates, 80–90% was present in the triacylglycerol and phospholipid spots. Further analysis of the plates by radioactivity scanning (Packard radiochromatogram scanner) and radioautography showed that, in addition to the high peaks corresponding to triacylglycerols and phospholipids, there were smaller peaks corresponding to both a 1,2-dipalmitoylglycerol marker and the palmitic acid marker, which were as a routine added to the islet lipid extracts.

Table 1. Rate of incorporation of glucose into triacylglycerols and into phospholipids by islets from obese-hyperglycaemic and lean mice

Collagenase-isolated islets were incubated for 3 h in media containing 3.5 or 17 mM-[U-¹⁴C]glucose (6–28 mCi/mmol). Details of the analytical procedure and of the incubation techniques are given in the Experimental section. Values denote means $\pm$ S.E.M. * $P < 0.005$; ** $P < 0.001$ for the difference between 3.5 and 17.0 mM-glucose.

Mouse strain	Concentration of glucose in the incubation medium (mM)	n	Incorporation of glucose (pmol/h per µg of DNA)	
			Triacylglycerols	Phospholipids
Lean (<i>Ob/Ob</i> , <i>Ob/ob</i>)	3.5	5	1.4 ± 0.1	2.1 ± 0.2
	17.0	5	$3.3 \pm 0.4^*$	$8.5 \pm 1.1^{**}$
Obese (<i>ob/ob</i>)	3.5	15	1.9 ± 0.1	3.7 ± 0.2
	17.0	15	$3.5 \pm 0.2^{**}$	$17.4 \pm 0.6^{**}$

Fig. 1. Dose-response curve relating the rate of glucose incorporated into phospholipid to the rate of incorporation into total islet lipids

Collagenase-isolated islets from two obese-hyperglycaemic mice were incubated for 3 h in media containing 0.7–28 mM-[U- 14 C]glucose (1.3–65 mCi/mmol). Details of the analytical procedure are given in the Experimental section. After corrections for controls the radioactivity in the phospholipid fraction has been divided by the total radioactivity recovered from the t.l.c. plates between the origin and the solvent front. Each point represents three batches of islets; S.E.M. is indicated by bars.

When the rate of incorporation of glucose into islet lipids of obese-hyperglycaemic and lean mice was further examined at different concentrations of glucose the following observations were made (Table 1). The islets incorporated between 1.5 and 5 times more carbon from glucose into phospholipids than into triacylglycerols. The pattern of glucose incorporation did not differ between the two strains of mice when tested in the presence of a low (3.5 mM) or a high (17 mM) glucose concentration (Table 1). A twofold increase in the rate of glucose incorporation was found in triacylglycerols and a fourfold increase in phospholipids. The rate of incorporation into phospholipids was about 50% greater in the obese mice than in the lean ones. The fraction of glucose carbon in phospholipids of obese-hyperglycaemic mice increased when the glucose concentration was raised progressively (Fig. 1). The dose-response curve was sigmoid, with the steepest slope in the concentration range 3–7 mM-glucose.

The rates of esterification of fatty acids into triacylglycerols and into phospholipids was measured by following the rate of incorporation of 1 mM-palmitate which had been complexed to bovine albumin. With the fatty acid as a precursor, glucose

Table 2. Effect of glucose on the rate of incorporation of palmitate into triacylglycerols and phospholipids of islets from obese-hyperglycaemic mice

Collagenase-isolated islets were incubated for 3 h in a medium containing albumin-bound 1 mM-[U- 14 C]palmitate (3.0 mCi/mmol). Details of the analytical procedures are given in the Experimental section. Values denote means $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.001$, for the difference between 0 and 17.0 mM-glucose.

Concentration of glucose in the incubation medium (mM)	n	Incorporation of palmitate (pmol/h per μ g of DNA)	
		Triacylglycerols	Phospholipids
—	11	11.8 $\pm$ 1.3	31.8 $\pm$ 1.5
17.0	11	15.7 $\pm$ 1.7*	72.1 $\pm$ 4.9**

Fig. 2. $^{14}\text{CO}_2$ production from islets pre-labelled with [U- 14 C]palmitate

After a pre-labelling with 1 mM-[U- 14 C]palmitate for 1 h 45 min, islets from obese-hyperglycaemic mice were further incubated for 1 h with non-radioactive 1 mM-palmitate. They were then extensively washed and further incubated for up to 4 h in a medium devoid of exogenous substrate. After the final incubation the medium containing the islets was acidified and the $^{14}\text{CO}_2$ formed was recovered in methanolic Hyamine (Berne, 1975b).

was found to increase the rate of synthesis of both triacylglycerols and phospholipids, with a greater stimulation of the latter (Table 2).

To investigate whether triacylglycerols are utilized as substrates to any appreciable extent, islets were pre-labelled for 1 h 45 min with 1 mM-[U- 14 C]-palmitate. One-half of the islets from each incubation vessel were taken for analysis after the pre-labelling period. The remainder were incubated for another 4 h in a non-radioactive medium and in the absence of exogenous substrates. This resulted in a decrease in radioactivity in the triacylglycerol fraction from an initial value of 146 ± 15 d.p.m./ μ g of DNA down to

Table 3. *Effect of mannoheptulose on the incorporation of glucose into triacylglycerols and phospholipids of islets from obese-hyperglycaemic mice*

Collagenase-isolated islets were incubated for 3 h in media containing 1.8 or 17 mM-[U-¹⁴C]glucose (3–28 mCi/mmol). Details of the analytical procedure and incubation techniques are given in the Experimental section. Values denote means $\pm$ S.E.M. * Not significant; ** $P < 0.001$.

Concentration of glucose in the incubation medium (mM)	Mannoheptulose (10 mM)	n	Incorporation of glucose (pmol/h per μ g of DNA)	
			Triacylglycerols	Phospholipids
1.8	—	11	0.9 $\pm$ 0.1	2.1 $\pm$ 0.1
	+	11	0.8 $\pm$ 0.1*	1.6 $\pm$ 0.1**
17.0	—	7	2.8 $\pm$ 0.4	6.4 $\pm$ 0.3
	+	7	2.7 $\pm$ 0.6*	16.0 $\pm$ 0.6**

Table 4. *Effects of inhibitors of insulin release on the rate of incorporation of glucose into lipids in the pancreatic islets of obese-hyperglycaemic mice*

Collagenase-isolated islets were incubated for 3 h in media containing 17 mM-[U-¹⁴C]glucose (3.7 mCi/mmol). Modifications of the media are indicated below. Details of the analytical procedures are given in the Experimental section. Values denote means $\pm$ S.E.M. * $P < 0.01$.

	n	Incorporation of glucose (pmol/h per μ g of DNA)	
		Triacylglycerols	Phospholipids
Control	11	1.7 $\pm$ 0.1	14.9 $\pm$ 0.9
Ca ²⁺ -free medium + 0.5 mM-EGTA	11	2.7 $\pm$ 0.3*	16.0 $\pm$ 1.3
Control	10	1.8 $\pm$ 0.3	18.4 $\pm$ 1.0
Medium + 2 μ g of adrenaline/ml	10	1.7 $\pm$ 0.1	21.3 $\pm$ 1.5

67 $\pm$ 10 d.p.m./ μ g of DNA ($P < 0.001$), indicating an appreciable utilization of the fatty acids in the islet triacylglycerol stores. Further, it was found that ¹⁴CO₂ production occurred in the islets pre-labelled with [U-¹⁴C]palmitate during a subsequent 4 h incubation in the absence of exogenous oxidizable fuels (Fig. 2).

Effect of inhibitors of insulin secretion. The rate of incorporation of glucose into islet phospholipids was inhibited by 10 mM-mannoheptulose at two different concentrations of glucose, i.e. 1.8 and 17 mM (Table 3). No significant effects were, however, found in the rate of incorporation into triacylglycerols. The maximum inhibition of the incorporation into phospholipids was 60% at the highest concentration of glucose.

As Table 4 shows neither the presence of adrenaline nor the omission of Ca²⁺ from the incubation medium affected the stimulation of glucose incorporation into phospholipids at a high glucose concentration (17 mM). A small increase in the rate of incorporation of glucose into triacylglycerols was found after omission of Ca²⁺.

Discussion

The present investigation clearly demonstrated that the pancreatic islets possess the capacity to incorporate both glucose and fatty acids into triacylglycerols and phospholipids. The rate of glucose incorporation into total islet lipids was calculated to be about 3–6% of the glucose-oxidation rate at the high and low glucose concentration respectively. This would represent a smaller share of the total glucose utilization of the islets, since both glycogen formation and lactate production are known to take place in the islets (Hellman & Idahl, 1969; Ashcroft *et al.*, 1970). The radioactivity from uniformly labelled glucose was mainly found in products that were water-soluble after the alkaline hydrolysis and that are likely to be derived from *sn*-glycerol 3-phosphate (Strickland, 1973), generated from glycolytic dihydroxyacetone phosphate. A minor portion of the radioactivity (2–4%) was incorporated into the fatty acids that were extracted and fractionated after the alkaline hydrolysis. Fatty acid synthesis in the islets of obese-hyperglycaemic mice also proceeds with

sodium [$1\text{-}^{14}\text{C}$]acetate as the precursor (C. Berne, unpublished work). These results agree with the previous demonstrations in islets of considerable activities of several enzymes that are important for the synthesis of acylglycerols and fatty acids. Thus glycerol 3-phosphate dehydrogenase (EC 1.1.1.8), ATP citrate lyase (EC 4.1.3.8) and the NADPH-regenerating enzymes glutathione reductase (EC 1.6.4.2), isocitrate dehydrogenase (NADP^+) (EC 1.1.1.42) and malate dehydrogenase (decarboxylating) (NADP^+) (EC 1.1.1.40) were all found to be present in the islets of New Zealand Obese (NZO) mice (Brolin & Berne, 1967; Berne, 1975a).

As in our experiments only 0.1–0.8 pmol of glucose/h per μg of DNA was converted into fatty acids in islet lipids, it is obvious that the major route for fatty acid accumulation in the islets is extracellular uptake. The total rate of palmitate incorporation into both triacylglycerols and phospholipids was 43.7 pmol/h per μg of DNA and the palmitate oxidation rate measured at the same concentration has been found to be 42.1 pmol/h per μg of DNA (Berne, 1975b). Thus apparently the esterification process represents an important part of the total fatty acid utilization in the B-cells.

Energetic aspects

The role of the triacylglycerol energy stores for the B-cell function is difficult to assess. We have obtained evidence to show that a low rate of insulin synthesis occurs in the islets in the absence of exogenous sources of energy. Moreover, in studies on the regulation of islet respiration, Hellerström (1967) found that a substantial oxygen consumption will continue for several hours in the absence of exogenous substrates in islets from obese-hyperglycaemic mice. From knowledge of the amounts of glycogen present in the islets of obese-hyperglycaemic mice it was concluded that other storage forms of respiratory fuel must also be present (Hellerström & Gunnarsson, 1970). The incorporation of both glucose carbon and fatty acids into triacylglycerols of the islets suggests that lipid stores could be important in islet metabolism. When islets pre-labelled with palmitate were incubated without exogenous substrate a decrease in total radioactivity in the triacylglycerol fraction indicates that islet triacylglycerols indeed are utilized as a metabolic substrate. That the triacylglycerols were utilized for oxidation was further supported by the finding of an appreciable production of $^{14}\text{CO}_2$ from islets which had incorporated radioactive palmitate into their triacylglycerol stores. The observation that glucose stimulated the biosynthesis of triacylglycerols further indicates that the B-cells are well adapted to store energy in the form of lipids in response to an increased glucose concentration in the extracellular fluid.

Glucose stimulation of phospholipid biosynthesis

The present investigation showed that both glucose and palmitate were incorporated into islet phospholipids in preference to triacylglycerols when the glucose concentration of the medium was increased. Since phospholipids are mainly membrane constituents in mammalian cells these data indicate that the increased turnover of membranes, which accompanies the synthesis, storage and release of insulin (Lacy, 1962; Orci *et al.*, 1973), also involves a potent effect on their phospholipid components. The results of the present study, using radioactive glucose and palmitate as precursors of lipids, conform well with the observations by Lernmark & Fex (1972), who found a similar degree of glucose stimulation of the rate of incorporation of [^{32}P]P_i into phospholipids in islets of obese-hyperglycaemic mice. We have obtained evidence to show that the rate of incorporation of [$\text{U-}^{14}\text{C}$]glucose is similarly increased by an elevated glucose concentration both in foetal and adult rat islets (Andersson *et al.*, 1975).

The present study also showed that a mere inhibition of the insulin secretory process by the omission of Ca^{2+} or the presence of adrenaline failed to abolish the stimulatory effect of an increased glucose concentration on the rate of incorporation of [$\text{U-}^{14}\text{C}$]glucose. This was despite the fact that insulin release is blocked in these situations (Milner & Hales, 1967; Coore & Randle, 1964). This suggests that the release process itself is not a major regulating factor for the metabolism of membrane phospholipids. The increased phospholipid formation in response to glucose might therefore rather reflect an increased membrane formation after the glucose stimulation of insulin biosynthesis, which has been shown to remain unimpaired by both Ca^{2+} omission and adrenaline (Lin & Haist, 1973).

Mannoheptulose, a potent inhibitor of both insulin release (Simon & Kraicer, 1966; Coore *et al.*, 1963) and biosynthesis (Lin & Haist, 1969), partially abolished the stimulatory effect of glucose on the phospholipid biosynthesis. It is noteworthy, however, that, although the maximum inhibitory effect was about 60% at the high glucose concentration, the rate of oxidation of glucose measured in parallel experiments (results not shown) was inhibited by over 80%, in close agreement with those reported by Hellman *et al.* (1972). Since glucose is incorporated into islet lipids mainly via *sn*-glycerol 3-phosphate, the additional effect on the rate of glucose oxidation cannot be explained by an inhibition of glucose phosphorylation alone. This evidence of a direct action of mannoheptulose also on the oxidative metabolism of the islets conforms with the corresponding observations made by Hedeskov *et al.* (1972), in studies of the effect of mannoheptulose on pyruvate oxidation.

An enhanced rate of incorporation of several precursors of phospholipids in response to an appro-

priate extracellular stimulus, in this case glucose, is a property that the B-cells share with other secreting glands (Hokin, 1968; Basinska *et al.*, 1973; Batzri & Selinger, 1973; Eichberg *et al.*, 1973). As in other glandular cells subjected to extracellular stimuli it appears that the major incorporation of [^{32}P]P_i in the B-cell is into the phosphatidylinositol fraction (Lernmark & Fex, 1972), which composes only 12% of total islet phospholipids, whereas a less significant effect is seen in other phospholipids. We have obtained evidence that both glucose and D-glyceraldehyde are potent stimulators of the incorporation of myo-[^3H]inositol into phosphatidylinositol in the B-cells (C. Berne, unpublished work). The precise significance of this increased turnover of particular phospholipids in a wide range of functionally stimulated tissues is so far unknown, but Hawthorne (1973) has suggested that it involves local changes in the structure and function of the membranes during intracellular transport processes.

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References

- Andersson, A., Grill, V., Asplund, K., Berne, C., Ågren, A. & Hellerström, C. (1975) in *III International Symposium: Early Diabetes Early in Life* (Camerini-Davalos, R. A., ed.), Academic Press, New York, in the press
- Ashcroft, S. J. H., Hedekov, C. J. & Randle, P. J. (1970) *Biochem. J.* **118**, 143–154
- Basinska, J., Sastry, P. S. & Stancer, H. C. (1973) *Endocrinology* **92**, 1786–1789
- Batzri, S. & Selinger, Z. (1973) *J. Biol. Chem.* **248**, 356–360
- Berne, C. (1975a) *J. Histochem. Cytochem.* **23**, 652–659
- Berne, C. (1975b) *Biochem. J.* **152**, 661–666
- Boberg, J. (1966) *Clin. Chim. Acta* **14**, 325–334
- Brolin, S. E. & Berne, C. (1967) *Metab. Clin. Exp.* **16**, 1024–1028
- Brolin, S. E. & Berne, C. (1970) in *The Structure and Metabolism of the Pancreatic Islets* (Falkmer, S., Hellman, B. & Täljedal, I.-B., eds.), pp. 245–252, Pergamon Press, Oxford
- Chen, R. F. (1967) *J. Biol. Chem.* **242**, 173–181
- Coore, H. G. & Randle, P. J. (1964) *Biochem. J.* **93**, 66–78
- Coore, H. G., Randle, P. J., Simon, E., Kraicer, P. F. & Shelesnyak, M. C. (1963) *Nature (London)* **197**, 1264–1266
- Eichberg, J., Schein, H. M., Schwartz, M. & Hauser, G. (1973) *J. Biol. Chem.* **248**, 3615–3622
- Folch, J., Lees, M. & Sloane-Stanley, G. H. (1957) *J. Biol. Chem.* **226**, 497–509
- Garland, P. B. & Randle, P. J. (1963) *Nature (London)* **199**, 381–382
- Gepts, W., Christophe, J. & Mayer, J. (1960) *Diabetes* **63**–69
- Gey, G. O. & Gey, M. K. (1936) *Am. J. Cancer* **27**, 45–49
- Hawthorne, J. N. (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Hawthorne, J. N. & Dawson, R. M. C., eds.), pp. 423–440, Elsevier, Amsterdam
- Hedekov, C. J., Hertz, L. & Nissen, C. (1972) *Biochim. Biophys. Acta* **261**, 388–397
- Hellerström, C. (1967) *Endocrinology* **81**, 105–112
- Hellerström, C. & Gunnarsson, R. (1970) *Acta Diabetol. Lat.* **7**, 127–151
- Hellman, B. (1961) *Acta Endocrinol. Copenhagen* **36**, 596–602
- Hellman, B. & Idahl, L.-Å. (1969) *Endocrinology* **84**, 1–8
- Hellman, B. & Idahl, L.-Å. (1972) *Diabetes* **21**, 999–1002
- Hellman, B., Hellerström, C., Larsson, S. & Brolin, S. E. (1961) *Z. Zellforsch.* **55**, 235–246
- Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J., Simon, E. & Täljedal, I.-B. (1972) *Mol. Pharmacol.* **8**, 1–7
- Hellman, B., Idahl, L.-Å., Lernmark, Å., Sehlin, J. & Täljedal, I.-B. (1974) *Biochem. J.* **138**, 33–45
- Hinegardner, R. T. (1971) *Anal. Biochem.* **39**, 197–201
- Hokin, L. E. (1968) *Int. Rev. Cytol.* **23**, 187–208
- Howell, S. L. & Taylor, K. W. (1968) *Biochem. J.* **108**, 17–24
- Keen, H., Field, J. B. & Pastan, I. H. (1963) *Metab. Clin. Exp.* **12**, 143–147
- Kissane, J. M. & Robins, E. (1958) *J. Biol. Chem.* **233**, 184–188
- Lacy, P. E. (1962) *Diabetes* **11**, 509–513
- Lernmark, Å. & Fex, G. (1972) *FEBS Lett.* **25**, 287–291
- Lin, B. J. & Haist, R. E. (1969) *Can. J. Physiol. Pharmacol.* **47**, 791–801
- Lin, B. J. & Haist, R. E. (1973) *Endocrinology* **92**, 735–742
- Matschinsky, F. M. & Ellerman, J. E. (1968) *J. Biol. Chem.* **243**, 2730–2736
- Milner, R. D. G. & Hales, C. N. (1967) *Diabetologia* **3**, 47–49
- Milner, R. D. G. & Hales, C. N. (1969) *Biochem. J.* **113**, 473–479
- O'Leary, J. L. (1930) *Anat. Rec.* **45**, 27–58
- Orci, L., Malaisse-Lagae, F., Ravazzola, M., Amherdt, M. & Renold, A. E. (1973) *Science* **181**, 561–562
- Simon, E. & Kraicer, P. F. (1966) *Isr. J. Med. Sci.* **2**, 785–799
- Snedecor, G. W. (1956) *Statistical Methods*, Iowa State University Press, Ames
- Strickland, K. P. (1973) in *Form and Function of Phospholipids* (Ansell, G. B., Hawthorne, J. N. & Dawson, R. M. C., eds.), pp. 9–42, Elsevier, Amsterdam
- Umbreit, W. W., Burris, R. H. & Stauffer, J. F. (1964) *Manometric Techniques*, 4th edn., p. 132, Burgess Publishing, Minneapolis
- Westman, J., Andersson, A., Hellerström, C. & Petersson, B. (1970) *Acta Diabetol. Lat.* **7**, 557–589