Islet-Cell Metabolism during Insulin Release

EFFECTS OF GLUCOSE, CITRATE, OCTANOATE, TOLBUTAMIDE, GLUCAGON AND THEOPHYLLINE

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1. Concentrations of glucose 6-phosphate and 6-phosphogluconate were studied in islets of Langerhans isolated from rat pancreas and incubated in the presence of various agents that induce insulin release. 2. In response to rising concentrations of extracellular glucose (2–10 mM) there is a linear increase in the intracellular concentration of glucose 6-phosphate, though this is not the case for 6-phosphogluconate, the intracellular concentration of which only increases when the external glucose concentration exceeds 5 mM. 3. Tolbutamide, octanoate and citrate, all of which promote insulin secretion from isolated islets, increase the intracellular concentrations of glucose 6-phosphate and 6-phosphogluconate. The results obtained in the presence of octanoate and citrate are compatible with an inhibitory effect of citrate on islet-cell phosphofructokinase. 4. Theophylline and glucagon when incubated with islets *in vitro* promote insulin release and cause a rise in 6-phosphogluconate concentration and not in that of glucose 6-phosphate. 5. It is suggested that the further metabolism of glucose 6-phosphate through a pathway other than glycolysis is essential for insulin release. One such pathway involves its oxidation to 6-phosphogluconate, which seems to be a necessary accompaniment of insulin secretion due to glucose. The possibility that agents other than glucose promote insulin release by enhancing the oxidation of glucose 6-phosphate through this pathway is discussed.

Although it is widely supposed that glucose has to be phosphorylated in order to induce insulin release (Coore & Randle, 1964; Ashcroft & Randle, 1968*a*), the subsequent metabolism of glucose 6-phosphate in islets of Langerhans leading to secretion is the subject of speculation (Malaisse, Malaisse-Lagae & Mayhew, 1967*a*). To date, several of the known metabolic pathways for the further metabolism of glucose 6-phosphate, such as glycolysis (Brolin, Berne & Linde, 1967), the UDP-glucose pathway to glycogen (Brolin, Berne & Linde, 1969) and the pentose phosphate pathway (Matschinsky, Kauffman & Ellerman, 1968*a*), have been shown to exist in islets, but there have been few attempts to correlate the operation of these pathways with insulin release.

It has been shown previously that the pentose, ribose, as well as certain pentose derivatives, were potent stimulators of insulin release (Montague & Taylor, 1968*a*), and it therefore seemed possible that the conversion of glucose 6-phosphate into pentoses through the pentose phosphate pathway might be associated with glucose-induced insulin release. In the present work an attempt has been

made to study the conversion of glucose 6-phosphate into 6-phosphogluconate when insulin is being released in response to glucose and a number of other secretory agents.

EXPERIMENTAL

Sources of hormones and reagents for hormone assay. Rat insulin was a gift from Dr A. J. Moody of the Novo Research Institute, Copenhagen, Denmark. Assays for insulin were performed with the immunoassay kit (code IM 39) available from The Radiochemical Centre, Amersham, Bucks. Crystalline glucagon, free of insulin, was generously given by Dr Mary Root of Lilly Laboratories Ltd., Indianapolis, Ind., U.S.A.

Enzymes. All enzymes and coenzymes used in the assays for glucose 6-phosphate and 6-phosphogluconate were from the Boehringer Corp. (London) Ltd., London W.5. Collagenase (form II) was from Koch–Light Laboratories Ltd., Colnbrook, Bucks.

Organic chemicals. Sugars, sugar derivatives and the organic acids were the purest commercially available and were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6. Theophylline was obtained from British Drug Houses Ltd., Poole, Dorset, and tolbutamide from Hoechst A.-G., Frankfurt/M, Germany.

Incubation medium. In all incubations with isolated rat islets of Langerhans the medium was a bicarbonate-buffered salt solution (Gey & Gey, 1936) containing bovine serum albumin fraction V (2 mg./ml.). The albumin was purchased from Armour Pharmaceutical Co. Ltd., Eastbourne, Sussex.

Isolation of islets of Langerhans. Islets of Langerhans were prepared by the collagenase digestion of pancreatic tissue taken from male albino Wistar rats, as described in detail elsewhere (Howell & Taylor, 1968; Montague & Taylor, 1968a). The isolated islets were incubated in a medium containing 2 mM-glucose for 30 min. at 37° under O₂ + CO₂ (95:5) before use in experiments.

Insulin secretion from isolated rat islets. For the study of the effects of agents on insulin secretion, groups of ten islets were incubated in conical flasks containing 2 ml. of medium. The gas phase was O₂ + CO₂ (95:5) and the medium contained glucose or glucose and the agent under study. The islets were incubated at 37° for 30 min. with gentle shaking. At the end of this period a sample of the medium was taken for determination of its insulin content by the immunoassay method of Hales & Randle (1963). Rat insulin was used as standard for the immunoassay. In experiments to determine the effects of the sodium salts of various organic acids on insulin release iso-osmoticity of the incubation medium was maintained by omission of an amount of NaCl equivalent to that of the added acid salt.

Determination of glucose 6-phosphate and 6-phosphogluconate content of islets. The determination of glucose 6-phosphate and 6-phosphogluconate was carried out by the method described previously (Montague & Taylor, 1968a), which is based on an enzymic cycling procedure (Lowry, Passonneau, Hasselberger & Schulz, 1964).

Isolated rat islets of Langerhans were incubated in groups of ten as described above for the study of insulin secretion. After sampling of the medium for determination of the insulin released the contents of the flasks were pooled in groups of three. The incubation medium was decanted from the islets, which were rapidly cooled to -76° in a bath of ethanol and solid CO₂. The islets were then treated ultrasonically in 0.3 ml. of 6% (w/v) HClO₄ (kept at 0°).

The glucose 6-phosphate and 6-phosphogluconate concentrations in the HClO₄ extracts were determined by the enzyme cycling technique as described in detail by Matschinsky, Passonneau & Lowry (1968b). In our hands this technique gave recoveries of 94% for glucose 6-phosphate and 95% for 6-phosphogluconate when added to HClO₄ extracts of islets.

The values recorded are very similar at 30 min. and 60 min., in agreement with the observations made by Matschinsky & Ellerman (1968).

RESULTS

The intracellular concentrations of glucose 6-phosphate and 6-phosphogluconate found in islets incubated with various concentrations of glucose in the incubation medium are shown in Fig. 1, together with the rates of insulin release observed under these conditions. On raising the extracellular glucose concentration from 2 mM to 10 mM there was a linear increase in the intracellular concentration of glucose 6-phosphate. However, there was no increase in secretion or concentration

of 6-phosphogluconate until the extracellular glucose concentration had been raised to 5 mM. Above this concentration both secretion and 6-phosphogluconate concentration showed a dramatic and parallel increase.

Table 1 shows the effects of octanoate (5 mM), citrate (5 mM) and pyruvate (20 mM) on insulin secretion and on the concentrations of glucose 6-phosphate and 6-phosphogluconate in islets incubated with 2 mM-glucose. Parallel to the increased rate of insulin release observed with citrate and octanoate there was an increased intracellular accumulation of both glucose 6-phosphate and 6-phosphogluconate. Pyruvate did not stimulate insulin release or cause an intracellular accumulation of glucose 6-phosphate.

The results in Table 2 show that sodium α -oxoglutarate (20 mM) and sodium succinate (20 mM) did not stimulate the release of insulin from isolated rat islets of Langerhans.

The intracellular concentrations of glucose 6-phosphate and 6-phosphogluconate found in islets incubated with theophylline, glucagon or tolbutamide are shown in Table 3. There was an increased accumulation of 6-phosphogluconate with all these agents, paralleling their effects on insulin release. The intracellular concentrations of glucose 6-

Fig. 1. Effects of various concentrations of glucose on insulin release and the intracellular concentrations of glucose 6-phosphate and 6-phosphogluconate in rat islets of Langerhans incubated *in vitro*. Isolated rat islets of Langerhans were incubated for 30 min. at 37° in a bicarbonate-buffered medium containing albumin (2 mg./ml.). The medium contained various concentrations of glucose. Insulin released into the medium was measured immunologically (see the text). At the end of the incubation the islets were disrupted ultrasonically in HClO₄ and the concentrations of glucose 6-phosphate (○) and 6-phosphogluconate (●) in the HClO₄ extracts were determined as described in the Experimental section. Each point is the mean of ten observations, with the standard deviations represented by the vertical lines.

Table 1. *Effects of glucose, citrate, octanoate and pyruvate on insulin release and the concentrations of glucose 6-phosphate and 6-phosphogluconate in rat islets of Langerhans incubated in vitro*

Isolated rat islets of Langerhans were incubated and rates of insulin release and the intracellular concentrations of glucose 6-phosphate and 6-phosphogluconate were determined as described in Fig. 1. The medium contained either glucose alone (2 mM or 20 mM) or glucose (2 mM) with sodium citrate (5 mM), sodium octanoate (5 mM) or sodium pyruvate (20 mM). The effects of all the substances tested were compared with those of 2 mM- and 20 mM-glucose as controls in each experiment. The results are given as means $\pm$ S.D.; the numbers of observations are given in parentheses. Each observation is a duplicated determination made on a separate group of 30 islets.

Addition to the medium	Concn. of glucose (mM)	Insulin released (μ units/islet/30 min.)	Concn. of glucose 6-phosphate (pmoles/30 islets)	Concn. of 6-phosphogluconate (pmoles/30 islets)
D-Glucose	2	45 $\pm$ 9 (16)	18 $\pm$ 4 (8)	4 $\pm$ 1 (8)
D-Glucose + citrate	2	108 $\pm$ 15 (16)*	39 $\pm$ 6 (8)*	11 $\pm$ 2 (8)*
D-Glucose + octanoate	2	102 $\pm$ 15 (16)*	37 $\pm$ 6 (8)*	10 $\pm$ 2 (8)*
D-Glucose + pyruvate	2	50 $\pm$ 8 (16)	20 $\pm$ 5 (8)	—
D-Glucose	20	120 $\pm$ 15 (16)*	44 $\pm$ 6 (8)*	12 $\pm$ 3 (8)*

* Indicates values significantly different from the 2 mM-glucose control value with $P < 0.01$.

Table 2. *Effects of α -oxoglutarate and succinate on insulin release from isolated rat islets of Langerhans incubated in vitro*

The experimental conditions were as described in Fig. 1. The effects of sodium α -oxoglutarate (20 mM) and sodium succinate (20 mM) on insulin release were compared with those of 2 mM- and 20 mM-glucose as controls in each experiment. The results are given as means $\pm$ S.D.; the numbers of observations are given in parentheses.

Addition to the medium	Concn. of glucose (mM)	Insulin released (μ units/islet/30 min.)
D-Glucose	2	42 $\pm$ 8 (20)
D-Glucose + α -oxoglutarate	2	45 $\pm$ 9 (20)
D-Glucose + succinate	2	42 $\pm$ 8 (20)
D-Glucose	20	120 $\pm$ 15 (20)*

* Indicates values significantly different from the 2 mM-glucose control with $P < 0.01$.

phosphate were decreased in the presence of theophylline and glucagon and increased with tolbutamide.

DISCUSSION

Effects of glucose on islet-cell glucose 6-phosphate and 6-phosphogluconate concentrations. The demonstration of a marked accumulation of both glucose 6-phosphate and 6-phosphogluconate in rat islets in response to a rise in the extracellular glucose concentration (Fig. 1) is in agreement with the results obtained by Matschinsky & Ellerman (1968), Matschinsky *et al.* (1968a) and Idahl & Hellman (1968) for islets from obese-hyperglycaemic mice.

The marked increase in glucose 6-phosphate concentration observed in rat islets under these conditions may reflect the activity of the enzymes

responsible for glucose phosphorylation in islets. Their characteristics have been discussed in detail elsewhere (Matschinsky & Ellerman, 1968; Randle & Ashcroft, 1969).

The increase in 6-phosphogluconate concentration appears to reflect the characteristics of islet-cell glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. The activities of both enzymes in rat islets are very similar (Kissane, Lacy, Brodin & Smith, 1964). In preliminary experiments based on the assay procedure of Glock & McLean (1953) glucose 6-phosphate dehydrogenase had an apparent K_m for glucose 6-phosphate of 50 μ M, whereas that of 6-phosphogluconate dehydrogenase for 6-phosphogluconate was 200 μ M. These results are compatible with the idea that a rise in the concentration of 6-phosphogluconate accompanies an increased utilization of glucose 6-phosphate through the pentose phosphate pathway. Whereas the intracellular concentration of glucose 6-phosphate appears to be related linearly to the glucose concentration, this is not the case for that of 6-phosphogluconate, which rises only when the extracellular glucose concentration exceeds 5 mM (Fig. 1). These results suggest that there is a change in the activity of glucose 6-phosphate dehydrogenase when the extracellular glucose concentration exceeds 5 mM and may reflect an activation of the enzyme by glucose 6-phosphate or by a change in the NADPH/NADP⁺ concentration ratio.

Malaisse, Malaisse-Lagae & Wright (1967b) have reported that the release of insulin in response to glucose from rat pancreas slices shows a threshold at 5 mM-glucose. The results in Fig. 1 reveal a similar pattern for insulin release from isolated rat islets. It is significant that the rate of rise of 6-phosphogluconate concentration closely parallels

Table 3. *Effects of tolbutamide, glucagon and theophylline on insulin release and the concentrations of glucose 6-phosphate and 6-phosphogluconate in rat islets of Langerhans incubated in vitro*

The experimental conditions were as described for Fig. 1. The results are given as means $\pm$ S.D.; the numbers of observations are given in parentheses.

Addition to the medium	Concn. of glucose (mM)	Insulin release (μ units/islet/30 min.)	Concn. of glucose 6-phosphate (pmoles/30 islets)	Concn. of 6-phosphogluconate (pmoles/30 islets)
D-Glucose	5	45 $\pm$ 8 (20)	25 $\pm$ 5 (9)	4 $\pm$ 1 (8)
D-Glucose + tolbutamide (200 μ g./ml.)	5	126 $\pm$ 18 (20)*	40 $\pm$ 5 (8)*	10 $\pm$ 2 (8)*
D-Glucose + glucagon (5 μ g./ml.)	5	120 $\pm$ 18 (20)*	12 $\pm$ 3 (8)*	10 $\pm$ 2 (8)*
D-Glucose	20	120 $\pm$ 15 (20)	45 $\pm$ 6 (8)	11 $\pm$ 2 (8)
D-Glucose + theophylline (5 mM)	20	180 $\pm$ 20 (20)*	25 $\pm$ 4 (8)*	17 $\pm$ 2 (8)*

* Indicates values significantly different from the glucose control value with $P < 0.01$.

the pattern of insulin release, suggesting that the operation of the pentose phosphate pathway is closely connected with glucose-induced insulin release.

Effects of tolbutamide on islet-cell glucose 6-phosphate and 6-phosphogluconate concentrations. Tolbutamide produces a rise in glucose 6-phosphate and 6-phosphogluconate concentrations when incubated with islets (Table 3). The reactions leading to the increase in glucose 6-phosphate are unclear. It has been suggested that tolbutamide may decrease the activity of glucose 6-phosphatase in islet cells (Lazarus & Volk, 1962). However, Ashcroft & Randle (1968b) have not been able to demonstrate any effect of tolbutamide on the glucose 6-phosphatase activity of mouse islet homogenates. The demonstration of an effect of a sulphonylurea related to tolbutamide on glycogen breakdown in mouse islets (Hellman & Idahl, 1969) suggests that the rise in glucose 6-phosphate concentration observed in the presence of tolbutamide (Table 3) could, at least in part, have come from glycogen breakdown. The increase in 6-phosphogluconate concentration, which has also been reported by Idahl (1969), may follow as a consequence of the increased availability of glucose 6-phosphate within the β -cell.

Effects of theophylline and glucagon on islet glucose 6-phosphate and 6-phosphogluconate concentrations. Glucagon and theophylline are known to increase the intracellular concentration of cyclic AMP in a variety of tissues (Sutherland & Rall, 1960; Butcher, 1966). Both agents are effective in stimulating insulin release, and various workers have suggested that cyclic AMP may mediate this effect in the β -cell (Malaisse *et al.* 1967a; Sussman & Vaughan, 1967; Turtle & Kipnis, 1967). The site of action of cyclic AMP in the β -cell is unknown, but it may act directly on the secretory mechanism or indirectly as a consequence of an alteration in islet-cell metabolism. It seems unlikely that cyclic AMP mediates the effect of glucose on the release

mechanism, since imidazole does not prevent the secretory response of the islet to glucose, even though it is thought to lower the intracellular concentration of cyclic AMP (Malaisse-Lagae & King, 1968). Further, evidence is accumulating that suggests that the effect of cyclic AMP on insulin release may be mediated by alterations in the metabolism of glucose by the β -cell. Thus Malaisse *et al.* (1967a) were unable to obtain effects of glucagon and theophylline on insulin release unless glucose was also present in the incubation medium. Further, the effects of theophylline on insulin release are blocked by inhibitors of glucose metabolism such as 2-deoxyglucose and mannoheptulose.

If cyclic AMP alters the metabolism of glucose by the β -cell, then changes in the concentrations of glucose metabolites might be expected with theophylline and glucagon. Table 3 shows that there is a decreased concentration of glucose 6-phosphate and an increased concentration of 6-phosphogluconate in rat islets in the presence of both these agents. The increased concentration of 6-phosphogluconate is taken to indicate that there is an increased conversion of glucose 6-phosphate into 6-phosphogluconate under these conditions. Since the concentration of glucose 6-phosphate is decreased, the rise in 6-phosphogluconate concentration might reflect an increase in the activity of glucose 6-phosphate dehydrogenase mediated directly or indirectly by cyclic AMP.

Effects of citrate and octanoate on insulin secretion and islet-cell metabolism. In contrast with other tricarboxylic acid-cycle intermediates citrate markedly affects insulin release (Table 1). Similar effects of citrate on the release of insulin have been reported by Candela, Castrillon, Hernandez & Cortazar (1964) and Martin & Gagliardino (1967). In confirmation of the studies of Sanbar & Martin (1967), 5 mM-octanoate was also found to stimulate insulin release from isolated rat islets. It is noteworthy that both these substances increase the

concentration of glucose 6-phosphate and 6-phosphogluconate in rat islets. As has been suggested previously (Montague & Taylor, 1968b) these results are explicable if it is assumed that citrate inhibits islet-cell phosphofructokinase, as it is known to do in other tissues such as muscle (Randle, Newsholme & Garland, 1964). Octanoate might then be expected to increase the concentrations of citrate in islet cells and these could then cause a rise in glucose 6-phosphate concentration by direct inhibition of phosphofructokinase. Matschinsky, Rutherford & Ellerman (1968c) have shown that citrate is a potent inhibitor of islet-cell phosphofructokinase.

The activity of islet-cell phosphofructokinase may be important in the regulation of glycolysis in the β -cell. This is clearly a possible way in which short-chain fatty acids may regulate islet-cell metabolism when these substances induce insulin secretion. Since glycolysis is thought to be inhibited under these conditions, it cannot be regarded as providing the signal for insulin release. In addition it must be inferred that the effect of short-chain fatty acids must be to divert glucose 6-phosphate to some pathway, other than glycolysis, that leads to insulin release.

Significance of the rise in islet-cell 6-phosphogluconate concentration during insulin release. All the agents that have been used to promote insulin release in islets also cause a marked rise in 6-phosphogluconate concentration, and this takes place irrespective of the concentrations of glucose 6-phosphate. It might be argued that the increase in 6-phosphogluconate concentration was due to an inhibition of the enzyme 6-phosphogluconate dehydrogenase, but it seems unlikely that all the agents used to promote insulin release should also inhibit the enzyme. Further, the metabolism of glucose is accompanied by a similar increase in 6-phosphogluconate concentration in islet cells, and under these conditions $^{14}\text{CO}_2$ production from glucose by the pentose phosphate pathway appears to be increased (Keen, Sells & Jarrett, 1965). Particularly noteworthy is the striking similarity in the pattern of 6-phosphogluconate accumulation and the rate of insulin release in response to variation in the extracellular glucose concentration already referred to.

We would conclude that during the release of insulin in response to glucose there is a simultaneous increase in the oxidation of glucose through the pentose phosphate pathway, and this may be true of other agents that induce insulin release.

Effects of pyruvate, succinate and α -oxoglutarate on insulin release. High concentrations of pyruvate, succinate and α -oxoglutarate were without effect on insulin secretion from isolated rat islets (Tables 1 and 2). A similar absence of effect of pyruvate on

insulin release has been reported by Grodsky *et al.* (1963). Further, Coore & Randle (1964) found that pyruvate, glutamate and fumarate did not stimulate the release of insulin from rabbit pancreas slices. These results suggest that the operation of the tricarboxylic acid cycle does not provide the signal for insulin release, although they do not exclude the possibility that metabolism through the cycle may be essential for some other aspect of the secretory response.

High concentrations of pyruvate in the incubation medium did not increase the concentrations of glucose 6-phosphate in islet cells (Table 1). This suggests that gluconeogenesis from pyruvate may be limited in islets, a suggestion that finds support in the observation by Brolin, Berne, Peterson & Larsons (1968) that fructose 1,6-diphosphatase activity is absent from islets from obese-hyperglycaemic mice.

Glucose metabolism and insulin release. Glucose is metabolized by the β -cell to maintain a variety of intracellular processes necessary for release. These include ATP synthesis and may involve membrane synthesis and the intracellular translocation of insulin. However, none of these processes may themselves activate the secretory mechanism. For this purpose some type of trigger mechanism has to be invoked. This may involve the rapid metabolism of glucose by a pathway giving one or more substances that may provide the signal for release.

The very close parallel between glucose oxidation and insulin release (Ashcroft & Randle, 1968c) suggests that the catabolism of glucose is associated with the secretory process. From the evidence presented above it is unlikely that glycolysis or the tricarboxylic acid cycle provides the signal for release. Pathways involving UDP-glucose are also unlikely to provide the signal since the formation of UDP-glucose in the β -cell would be expected to lead to the synthesis of glycogen (Hellman & Idahl, 1969), but insulin release may be associated with glycogen breakdown under some conditions (Malaisse *et al.* 1967a).

Role of pentose phosphate in islets. The effects of pentoses or their derivatives on insulin secretion, now widely observed in several species (Boder, Root, Chance & Johnson, 1968; Kuzuya, Kanazawa & Kosaka, 1969; Sloviter & Petkovic, 1969), together with the present results, suggest a role for the pentose phosphate pathway in the secretory activities of the β -cell. It seemed possible that this pathway might also be associated with insulin synthesis. However, xylitol, a pentose derivative that promotes release, is without effect on incorporation of labelled amino acids into insulin (Taylor, 1969).

It seems much more likely that the increased

activity of the pathway is associated with some aspect of the release mechanism. Similar increases in the activity of the pentose phosphate pathway have been noted in other endocrine tissues that are actively secreting, such as adrenal cortex (Weaver & Landau, 1963), thyroid (Dumont, 1965) or pituitary (Krass & Labella, 1965). A possible function of the pathway in secretion is the provision of NADPH, perhaps necessary for some phase of secretion. It has already been suggested that high concentrations of NADPH may provoke insulin secretion from fish islets *in vitro* (Watkins, Cooperstein, Dixit & Lazarow, 1968).

The question arises as to whether the pentose phosphate pathway provides the signal for release of insulin or whether it is merely some essential adjunct to the secretory process. Because the nature of the processes leading to insulin release are not known, this question cannot be answered with certainty at the present time. Insulin secretion involves movement of β -granules to the cell surface and their subsequent discharge by a complex series of interactions between the cell and the β -granule membranes (Lacy, 1961). It is possible that more than one type of regulatory mechanism is involved in this sequence of events and therefore that more than one type of signal may be involved. The operation of the pentose phosphate pathway may provide one such signal, although this does not exclude the possibility of its having some other essential role in the secretory process.

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REFERENCES

- Ashcroft, S. J. H. & Randle, P. J. (1968a). *Biochem. J.* **107**, 599.
- Ashcroft, S. J. H. & Randle, P. J. (1968b). *Nature, Lond.*, **219**, 857.
- Ashcroft, S. J. H. & Randle, P. J. (1968c). *Lancet*, i, 278.
- Boder, G. B., Root, M., Chance, R. E. & Johnson, I. S. (1968). *J. Cell Biol.* **39**, 16.
- Brolin, S. E., Berne, C. & Linde, B. (1967). *Diabetes*, **16**, 21.
- Brolin, S. E., Berne, C. & Linde, B. (1969). In *The Structure and Metabolism of the Pancreatic Islets* (in the Press).
- Brolin, G. B., Berne, C., Peterson, B. & Larsons, A. (1968). *J. Histochem. Cytochem.* **16**, 654.
- Butcher, R. W. (1966). *Pharmacol. Rev.* **18**, 237.
- Candela, J. L. R., Castrillon, A. M., Hernandez, D. M. & Cortazar, T. C. (1964). *Med. exp., Basel*, **11**, 47.
- Coore, H. G. & Randle, P. J. (1964). *Biochem. J.* **93**, 66.
- Dumont, J. E. (1965). *Ann. Soc. Sci. méd. nat. Brux.* **18**, 176.
- Gey, G. O. & Gey, M. K. (1936). *Amer. J. Cancer*, **27**, 45.
- Glock, G. E. & McLean, P. (1953). *Biochem. J.* **55**, 400.
- Grodsky, G. M., Batts, A. A., Bennett, L. L., Vcella, C., McWilliams, N. B. & Smith, D. F. (1963). *Amer. J. Physiol.* **205**, 638.
- Hales, C. N. & Randle, P. J. (1963). *Biochem. J.* **88**, 137.
- Hellman, B. & Idahl, L. A. (1969). In *The Structure and Metabolism of the Pancreatic Islets* (in the Press).
- Howell, S. L. & Taylor, K. W. (1968). *Biochem. J.* **108**, 17.
- Idahl, L. A. (1969). In *The Structure and Metabolism of the Pancreatic Islets* (in the Press).
- Idahl, L. A. & Hellman, B. (1968). *Acta endocr., Copenhagen*, **59**, 479.
- Keen, H., Sells, R. & Jarrett, R. J. (1965). *Diabetologia*, **1**, 28.
- Kissane, J. M., Lacy, P. E., Brolin, S. E. & Smith, C. H. (1964). In *The Structure and Metabolism of the Pancreatic Islets*, p. 281. Ed. by Brolin, S. E., Hellman, B. & Knutson, H. Oxford: Pergamon Press Ltd.
- Krass, M. E. & Labella, F. S. (1965). *Molec. Pharmacol.* **1**, 306.
- Kuzuya, T., Kanazawa, Y. & Kosaka, K. (1969). *Endocrinology*, **84**, 200.
- Lacy, P. E. (1961). *Amer. J. Med.* **31**, 851.
- Lazarus, S. S. & Volk, B. W. (1962). *Diabetes*, **11**, (suppl.), 2.
- Lowry, O. H., Passonneau, J. V., Hasselberger, F. X. & Schulz, D. W. (1964). *J. biol. Chem.* **239**, 18.
- Malaisse, W. J., Malaisse-Lagae, F. & King, S. (1968). *Diabetologia*, **4**, 370.
- Malaisse, W. J., Malaisse-Lagae, F. & Mayhew, D. (1967a). *J. clin. Invest.* **46**, 1724.
- Malaisse, W. J., Malaisse-Lagae, F. & Wright, P. H. (1967b). *Endocrinology*, **80**, 99.
- Martin, J. M. & Gagliardino, J. J. (1967). *Nature, Lond.*, **213**, 630.
- Matschinsky, F. M. & Ellerman, J. E. (1968). *J. biol. Chem.* **243**, 2730.
- Matschinsky, F. M., Kauffman, F. C. & Ellerman, J. E. (1968a). *Diabetes*, **17**, 475.
- Matschinsky, F. M., Passonneau, J. V. & Lowry, O. H. (1968b). *J. Histochem. Cytochem.* **16**, 29.
- Matschinsky, F. M., Rutherford, C. R. & Ellerman, J. E. (1968c). *Biochem. biophys. Res. Commun.* **33**, 855.
- Montague, W. & Taylor, K. W. (1968a). *Biochem. J.* **109**, 333.
- Montague, W. & Taylor, K. W. (1968b). *Nature, Lond.*, **217**, 853.
- Randle, P. J. & Ashcroft, S. J. H. (1969). *Biochem. J.* **112**, 1r.
- Randle, P. J., Newsholme, E. A. & Garland, P. B. (1964). *Biochem. J.* **93**, 652.
- Sanbar, S. S. & Martin, J. M. (1967). *Metabolism*, **16**, 482.
- Sloviter, H. A. & Petkovic, M. R. (1969). *Nature, Lond.*, **221**, 371.
- Sussman, K. E. & Vaughan, G. D. (1967). *Diabetes*, **16**, 449.
- Sutherland, E. W. & Rall, T. W. (1960). *Pharmacol. Rev.* **12**, 265.
- Taylor, K. W. (1969). *Proc. 3rd. Int. Congr. Endocrin., Mexico; Excerpta med.* (in the Press).
- Turtle, J. R. & Kipnis, D. M. (1967). *Biochem. biophys. Res. Commun.* **28**, 797.
- Watkins, D., Cooperstein, S. J., Dixit, P. K. & Lazarow, A. (1968). *Science*, **162**, 283.
- Weaver, G. & Landau, B. R. (1963). *Endocrinology*, **73**, 640.