Alloxan Reversibly Impairs Glucagon Release and Glucose Oxidation by Pancreatic A₂-Cells

Claes-Göran ÖSTENSON

Department of Histology, Biomedicum, University of Uppsala, Box 571, S-751 23 Uppsala, Sweden

(Received 9 October 1979)

Alloxan is known as a selective B-cell cytotoxic substance, and there is so far little evidence for a direct toxic effect on the other islet cell types. To elucidate further whether such effects occur, the actions of alloxan on glucagon release and glucose oxidation were studied in isolated normal or A₂-cell-rich pancreatic islets of the guinea pig. The A₂-cell-rich islets were obtained from animals injected with streptozotocin 1–2 weeks before islet isolation. After exposure to alloxan (2 or 5 mM) *in vitro* for 30 min at 4°C, the islets were incubated in media containing either 1.7 mM-glucose or 16.7 mM-glucose plus 30 m.i.u. of bovine insulin/ml. In both types of islet, alloxan abolished the ability of glucose and insulin both to decrease glucagon release and to increase the rate of glucose oxidation. A high concentration of glucose (28 mM) during exposure to alloxan protected against these injurious effects. Tissue culture of alloxan-treated islets for 24 h in 5.5 mM-glucose restored neither the suppressive effect of glucose on glucagon release nor the inhibition of glucose oxidation of the A₂-cells. However, culture for 1 week completely normalized both the glucagon-secretory response and glucose oxidation by both kinds of islets. It is therefore concluded that alloxan affects the secretory mechanism of not only the B-cell but also of the islet A₂-cell, although this latter cell type is not primarily destroyed by the drug. The data furthermore support the concept of a relationship between glucose metabolism and the glucose-mediated glucagon release of the A₂-cell.

It has been previously shown that exposure of pancreatic B-cells to the diabetogenic drug alloxan rapidly impairs both their insulin production (Rerup, 1970; Gunnarsson & Hellerström, 1973; Tomita *et al.*, 1974; Gunnarsson, 1975; Dulin & Soret, 1977) and glucose metabolism (Gunnarsson & Hellerström, 1973; Jain *et al.*, 1978; Borg *et al.*, 1979). This action of alloxan may reflect an irreversible injury, which subsequently leads to the well-known degeneration and death of the B-cell (Rerup, 1970; Grankvist *et al.*, 1977). A possible effect of alloxan on other islet cell types, in particular the pancreatic A₂-cell, has been suggested by recent reports demonstrating that alloxan also affects glucagon release (Pagliara *et al.*, 1977; Goto *et al.*, 1978). Since the secretion of this hormone has been shown to be regulated by the insulin-sensitive glucose metabolism of the A₂-cell (Östenson, 1979) it is difficult to decide whether alloxan acts directly on the A₂-cell or via an inhibited insulin release of the B-cell. To investigate this question further, both normal and A₂-cell-rich guinea pig islets were used to study the effects, *in vitro*, of alloxan on glucagon release and glucose oxidation of the A₂-cell.

Experimental

Chemicals

Streptozotocin (lot 60, 273–2) was kindly donated by Dr. W. E. Dulin, The Upjohn Co., Kalamazoo, MI, U.S.A. Collagenase (CLS, 126 units/mg) was supplied by Worthington Biochemical Corp., Freehold, NJ, U.S.A. Tissue-culture medium RPMI 1640 was from Flow Laboratories, Irvine, Ayrshire, Scotland, U.K. Hanks' solution and calf serum were obtained from Statens Bakteriologiska Laboratorium, Stockholm, Sweden, and penicillin and streptomycin from Glaxo Laboratories, Greenford, Middx., U.K. Alloxan monohydrate (mol.wt. 160.1) and antimycin A were purchased from Sigma Chemical Co., St. Louis, MO, U.S.A. Bovine plasma albumin (fraction V) was obtained from Armour Pharmaceutical Co., Eastbourne, Sussex, U.K. D-[U-¹⁴C]Glucose (336 Ci/mol) was from The Radiochemical Centre, Amersham, Bucks., U.K. Bovine insulin (10 times crystallized), porcine ¹²⁵I-labelled glucagon, rabbit anti-(porcine glucagon) serum K 4023 and porcine glucagon standard were supplied by Novo A/S,

Bagsvaerd, Denmark. Hyamine, PPO (2,5-diphenyloxazole) and dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] were purchased from Packard Instruments Co., Downers Grove, IL, U.S.A. Other chemicals used were of analytical grade.

Methods

Animals and preparation of isolated islets. A total of 128 male guinea pigs, each weighing approx. 200g, was used. In order to obtain A₂-cell-rich pancreatic islets (Petersson *et al.*, 1970), 98 of these animals were given a single intraperitoneal injection of streptozotocin (375 mg/kg body wt.). The remaining guinea pigs were not injected and served as control animals. The effects of the streptozotocin injection were assessed both by measurements of body weight and blood glucose concentration (Beckman Glucose Analyser 2; Beckman Instruments, Fullerton, CA, U.S.A.) and by tests for the presence of urinary glucose (Clinistix, Ames Co., Slough, Berks., U.K.). After 1–2 weeks the animals were killed by decapitation and their pancreatic glands were removed. In each streptozotocin-treated animal a small piece of the pancreatic gland was fixed in Bouin solution, and the remainder used for isolation of pancreatic islets by collagenase digestion (Howell & Taylor, 1968). Islets were isolated in a similar way from the non-injected guinea pigs. The isolation procedure was carried out aseptically and the islets were subsequently maintained, free-floating, in tissue culture (Andersson *et al.*, 1973; Andersson, 1978) for 24–48 h. For this purpose, islets were transferred to plastic Petri dishes containing 4.5 ml of tissue-culture medium RPMI 1640, 0.5 ml of calf serum, antibiotics (100 i.u. of penicillin G/ml plus 0.1 mg of streptomycin/ml) and 5.5 mM-glucose.

Morphological study. The Bouin-fixed pancreatic pieces were embedded in paraffin, cut into thin sections (7 μ m) and stained with aldehyde fuchsin trichrome (Gomori, 1950) for subsequent study by light microscopy.

Exposure to alloxan. Cultured normal and A₂-cell-rich islets were exposed, *in vitro*, to alloxan at 4°C as described by Borg *et al.* (1979). For this purpose, islets were treated for 30 min in 2 ml of Krebs–Ringer phosphate buffer (incubation medium I) (Krebs, 1933) containing 1.7 mM-glucose and 2 or 5 mM-alloxan monohydrate. The alloxan was dissolved in ice-cold buffer and the pH adjusted from approximately 6.5 to 7.4 within 1–2 min before use. The islets were then washed twice in cold Krebs–Ringer phosphate buffer containing 1.7 mM-glucose. Control islets were treated similarly, except that alloxan was omitted from the buffer solutions.

In experiments designed to study whether glucose is able to protect the A₂-cells against the action of

alloxan, islets were treated as above, but the Krebs–Ringer phosphate buffer was now supplemented with 28 mM-glucose and 5 mM-alloxan monohydrate. Other islets, treated with alloxan in Krebs–Ringer phosphate buffer with 1.7 mM-glucose, were handled aseptically and subsequently maintained in tissue culture, for 1 or 7 days in RPMI 1640 containing 10% calf serum and 5.5 mM-glucose. Control islets, treated with Krebs–Ringer phosphate buffer only, were cultured in a similar way. The culture medium was changed on the third and fifth day.

Glucagon release. Islets were exposed to either alloxan or Krebs–Ringer phosphate buffer only and then incubated, in groups of 10, in a shaking water bath (100 strokes/min) at 37°C for 120 min in 250 μ l of a bicarbonate buffered solution (incubation medium II) (Gey & Gey, 1936). Incubation medium II contained bovine plasma albumin (2 mg/ml) together with D-glucose (1.7 mM) or D-glucose (16.7 mM) + bovine insulin (30 m-i.u./ml), as given in the Tables. The insulin was initially dissolved in 30 mM-HCl at a concentration of 1 mg/ml and was subsequently diluted 800-fold with the appropriate buffer solution. After incubation, samples of the medium were immediately frozen for assay of glucagon (Heding, 1971), using porcine ¹²⁵I-labelled glucagon, porcine glucagon standard and rabbit anti-(porcine glucagon) serum K 4023. The dry weights of the groups of islets were determined as described by Hellerström (1967), using a Cahn Electrobalance (model 4100; Cahn Instrument, Paramount, CA, U.S.A.) sensitive to 0.1 μ g.

Glucose oxidation. Groups of 10 isolated islets were incubated in small glass vials (Keen *et al.*, 1963), containing 100 μ l of a bicarbonate-buffered solution (Gey & Gey, 1936) supplemented with D-[U-¹⁴C]glucose (1.2 Ci/mol) and non-radioactive carrier glucose to give a final concentration of either 1.7 mM or 16.7 mM. Bovine insulin (30 m-i.u./ml) was added to the high-glucose medium. The vials, suspended in glass scintillation bottles tightly capped with rubber membranes, were gassed with O₂/CO₂ (19:1). The bottles were shaken in a water bath (100 strokes/min) at 37°C, and islet metabolism was stopped after a 90-min incubation by injection of 100 μ l of 0.05 mM-antimycin A in 70% (v/v) ethanol into the vials (Edwards *et al.*, 1972). This was immediately followed by an injection of 250 μ l of Hyamine into the outer vessel. CO₂ was liberated from the incubation medium by injection of 100 μ l of 0.4 mM-sodium phosphate buffer, pH 6.0, into the incubation vial. The bottles were incubated for a further 2 h at 37°C to allow the CO₂ to be absorbed into the Hyamine. The radioactivity was assayed in a liquid-scintillation spectrometer (Packard Tri-Carb, model 3255) after dissolving the Hyamine in

10ml of toluene containing 5 g of PPO/l and 0.05 g of dimethyl-POPOP/l. Blank incubations, without islets, were treated in the same way. The weight of the incubated islets was determined as previously described (Hellerström, 1967).

Expression of results. The rate of glucagon release was expressed as pg of glucagon/h per μ g islet dry wt. Glucose oxidation was calculated as pmol of glucose converted to ¹⁴CO₂/h per μ g islet dry wt. The results were expressed as the mean $\pm$ S.E.M. of the number of experiments given in parentheses, each of which represented the average of one to four observations from the same animal. Statistical significances were evaluated with the aid of Student's *t* test (Snedecor, 1956).

Results

Animals

All streptozotocin-injected animals displayed glucosuria and a marked weight loss, their mean weight at death being 179 ± 2 g (*n* = 98) compared with 209 ± 2 g before the injection (*n* = 98; *P* < 0.001). The serum glucose concentration was 9.0 ± 0.2 mM in the streptozotocin-treated group (*n* = 73) and 8.6 ± 0.3 mM in the normal animals (*n* = 20; not significant). Light microscopy of pancreatic sections revealed that in all streptozotocin-treated animals the islets were predominantly composed of A₂-cells with only a few remaining B-cells.

Glucagon release

Table 1 shows that normal islets, which had not been exposed to alloxan, decreased their rate of glucagon release when incubated with glucose (16.7 mM) and insulin (30 m-i.u./ml). An even stronger inhibition of the glucagon release was seen

when the experiment was repeated using A₂-cell-rich islets. When either of these two groups of islets was first incubated with alloxan (2 or 5 mM), the inhibitory effect of the high-glucose and insulin challenge on the glucagon release was, however, completely abolished. As seen at the bottom of Table 1, islets which were treated with alloxan in the presence of a high glucose concentration (28 mM) still showed a decreased glucagon release in response to glucose plus insulin, thus demonstrating a protective influence of glucose against the effect of alloxan. Moreover, pretreatment of A₂-cell-rich islets with high glucose concentrations (28 mM) seemed to enhance the glucagon release during the subsequent incubation in 1.7 mM-glucose (*P* < 0.05 versus islets pretreated in 1.7 mM-glucose alone).

The duration of the alloxan effects on the regulation of glucagon release is shown in Table 2. After 1 day of tissue culture the alloxan-treated islets were still resistant to the suppressive effects of glucose and insulin on glucagon release. However, islets maintained in culture for 7 days after being exposed to alloxan displayed a normal suppression of glucagon release by glucose plus insulin, at the end of the culture period.

Glucose oxidation

The effects of alloxan on the rates of glucose oxidation of normal and A₂-cell-rich islets are given in Table 3. Both A₂-cell-rich and normal islets that were not treated with alloxan increased their rate of glucose oxidation more than 3-fold in response to a high glucose concentration. In agreement with the observations on glucagon release, this effect of glucose disappeared after treatment with alloxan. A protective effect of glucose on this action of alloxan on islet metabolism was observed when islets were incubated with both alloxan and 28 mM-glucose. In

Table 1. Glucagon release from alloxan-treated normal and A₂-cell-rich islets

Normal or A₂-cell-rich islets were incubated for 30 min at 4°C in medium I containing the additions given in the Table. They were subsequently incubated for 2 h at 37°C in medium II containing either glucose or glucose + insulin in order to study the rate of glucagon secretion. Results are expressed as mean values $\pm$ S.E.M. (pg of glucagon/h per μ g islet dry wt.). Numbers of experiments are shown in parentheses. **P* < 0.05 versus 1.7 mM-glucose; ***P* < 0.01 versus 1.7 mM-glucose; ****P* < 0.001 versus 1.7 mM-glucose.

Additions to incubation medium I		Glucagon release			
		Normal islets		A ₂ -cell-rich islets	
		16.7 mM-Glucose		16.7 mM-Glucose	
Glucose (mM)	Alloxan (mM)	1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml	1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml
1.7	—	39.2 $\pm$ 3.2 (10)	27.9 $\pm$ 3.1 (9)*	65.1 $\pm$ 3.9 (16)	38.1 $\pm$ 3.2 (17)***
1.7	2	37.1 $\pm$ 1.7 (6)	39.7 $\pm$ 3.8 (7)	66.0 $\pm$ 9.7 (7)	69.2 $\pm$ 10.1 (7)
1.7	5	27.2 $\pm$ 2.0 (7)	29.4 $\pm$ 2.3 (9)	74.1 $\pm$ 6.2 (5)	70.5 $\pm$ 8.8 (5)
28	—	41.3 $\pm$ 3.4 (7)	29.1 $\pm$ 0.9 (6)**	80.7 $\pm$ 6.5 (11)	46.9 $\pm$ 3.8 (12)***
28	5	40.9 $\pm$ 2.5 (7)	29.1 $\pm$ 1.8 (6)**	82.6 $\pm$ 6.9 (11)	46.5 $\pm$ 2.0 (12)***

Table 2. *Glucagon release from alloxan-treated normal and A₂-cell-rich islets maintained in tissue culture*

Normal or A₂-cell-rich islets were incubated for 30 min at 4°C in medium I containing 1.7 mM-glucose with or without 5 mM-alloxan. After a culture period of 1 or 7 days after the alloxan treatment, the islets were incubated with either glucose or glucose + insulin for studies of their glucagon secretion. Results are expressed as mean values ± S.E.M. (pg of glucagon/h per µg islet dry wt.). Numbers of experiments are shown in parentheses. ***P* < 0.01 versus 1.7 mM-glucose; ****P* < 0.001 versus 1.7 mM-glucose.

		Glucagon release			
		Normal islets		A ₂ -cell-rich islets	
Culture period (days)	Alloxan (mM)	16.7 mM-Glucose		16.7 mM-Glucose	
		1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml	1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml
1	—	34.5 ± 1.3 (5)	24.4 ± 1.6 (7)**	86.8 ± 5.9 (5)	47.3 ± 3.8 (5)***
1	5	38.4 ± 3.3 (7)	38.2 ± 2.6 (6)	84.9 ± 10.1 (5)	82.8 ± 5.3 (5)
7	—	38.2 ± 1.8 (5)	24.9 ± 1.5 (5)***	82.0 ± 4.9 (6)	34.8 ± 4.2 (5)***
7	5	40.4 ± 3.6 (5)	26.3 ± 1.7 (5)**	67.6 ± 4.7 (6)	41.6 ± 2.9 (6)***

Table 3. *Glucose oxidation by alloxan-treated normal and A₂-cell-rich islets*

Normal or A₂-cell-rich islets were incubated for 30 min at 4°C in medium I containing the additions given in the Table. Islets were subsequently incubated for 90 min at 37°C in medium II containing either glucose or glucose + insulin in order to determine the rate of ¹⁴CO₂ production from [U-¹⁴C]glucose. Results are given as mean values ± S.E.M. (pmol of glucose oxidized/h per µg islet dry wt.). Numbers of experiments are shown in parentheses. ***P* < 0.01 versus 1.7 mM-glucose; ****P* < 0.001 versus 1.7 mM-glucose.

		Glucose oxidation			
Addition to incubation medium I		Normal islets		A ₂ -cell-rich islets	
Glucose (mM)	Alloxan (mM)	16.7 mM-Glucose		16.7 mM-Glucose	
		1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml	1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml
1.7	—	4.6 ± 0.7 (12)	15.7 ± 2.5 (10)***	4.0 ± 0.4 (11)	12.6 ± 1.7 (9)***
1.7	5	5.6 ± 0.7 (12)	6.2 ± 0.9 (12)	3.9 ± 0.4 (13)	4.0 ± 0.6 (10)
28	—	6.2 ± 0.6 (7)	18.0 ± 2.4 (6)***	2.5 ± 0.4 (5)	13.6 ± 1.1 (5)***
28	5	4.7 ± 0.6 (7)	18.3 ± 1.8 (7)***	3.1 ± 0.3 (5)	18.4 ± 3.6 (6)**

Table 4. *Glucose oxidation by alloxan-treated normal and A₂-cell rich islets maintained in tissue culture*

Normal or A₂-cell-rich islets were incubated for 30 min at 4°C in medium I containing 1.7 mM-glucose with or without 5 mM-alloxan. After a culture period of 1 or 7 days after the alloxan treatment, islets were incubated with either glucose or glucose + insulin in order to determine the rate of ¹⁴CO₂ production from [U-¹⁴C]glucose. Results are given as mean values ± S.E.M. (pmol of glucose oxidized/h per µg islet dry wt.). Numbers of experiments are shown in parentheses. ***P* < 0.01 versus 1.7 mM-glucose; ****P* < 0.001 versus 1.7 mM-glucose; †*P* < 0.05 versus corresponding group of normal islets; †††*P* < 0.001 versus corresponding group of normal islets.

		Glucose oxidation			
		Normal islets		A ₂ -cell-rich islets	
Culture period (days)	Alloxan (mM)	16.7 mM-Glucose		16.7 mM-Glucose	
		1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml	1.7 mM-Glucose	+ 30 m-i.u. of insulin/ml
1	—	7.2 ± 0.5 (5)	15.7 ± 1.4 (6)***	4.3 ± 0.9 (6)†	13.7 ± 2.2 (5)**
1	5	7.8 ± 0.4 (6)	14.9 ± 1.5 (7)**	3.6 ± 0.6 (6)†††	5.7 ± 0.8 (6)†††
7	—	6.3 ± 0.6 (6)	12.7 ± 0.8 (5)***	3.6 ± 0.6 (6)†	15.3 ± 1.7 (5)***
7	5	5.5 ± 0.7 (7)	13.7 ± 1.1 (7)***	5.3 ± 0.8 (6)	17.9 ± 2.0 (7)***

these experimental conditions, both normal and A₂-cell-rich islets showed a 4–6-fold stimulation of glucose-oxidation rate when challenged by high extracellular concentrations of glucose plus insulin.

Table 4 shows that tissue culture of normal islets for 1 day after alloxan exposure was sufficient to restore the oxidative response to glucose. After a similar culture period, however, A₂-cell-rich islets

still failed to increase their rate of glucose oxidation in response to glucose. Nevertheless, after 7 days in culture both islet groups showed an increased rate of glucose oxidation when exposed to high glucose concentration plus insulin despite the preceding treatment with alloxan.

Discussion

We have previously shown that the A₂-cell is sensitive to insulin and that the suppressive effect of insulin on glucagon release may be mediated via an increased intracellular ATP concentration generated by stimulated glucose metabolism (Östenson, 1979). The insulin-dependence of the A₂-cell makes it difficult, however, to evaluate whether any interaction between alloxan and the A₂-cell is direct or mediated via alterations of the extracellular insulin concentration. In order to overcome this problem, the A₂-cell-rich islet preparation previously described by Petersson *et al.* (1970) and Howell *et al.* (1971) was used for the present studies. These islets are made up of more than 60% of A₂-cells and therefore appear suited for studies of alloxan effects on both the glucagon release and the metabolism of this cell type (Petersson *et al.*, 1970; Edwards *et al.*, 1972). Glucose oxidation has previously been shown to be a sensitive criterion for alloxan toxicity of the B-cell (Borg *et al.*, 1979).

The use of streptozotocin for destruction of the B-cells of the experimental animals raises the question as to what extent this drug also affects the A₂-cell. Several studies have failed to demonstrate morphological signs of direct damage of the A₂-cell by streptozotocin (Petersson *et al.*, 1970; Howell *et al.*, 1971; Andersson *et al.*, 1973). Also, there have been no detectable functional alterations of these cells other than those explained by the loss of the surrounding B-cells and resulting deficiency of insulin (Petersson *et al.*, 1970; Andersson *et al.*, 1973; Östenson, 1979). It is noteworthy in this context that streptozotocin-treated guinea pigs show blood glucose concentrations similar to, or slightly lower than, those found in normal animals (Petersson *et al.*, 1970; Östenson, 1979). These combined observations indicate that isolated islets of guinea pigs treated with streptozotocin may constitute a valid preparation for studies of the effects of alloxan on the A₂-cell.

The present data confirm and extend previous observations indicating a strong inhibition of glucagon release when A₂-cell-rich islets are exposed to high concentrations of both glucose and insulin. A similar, but less pronounced, inhibition was also noted in normal islets, but previous data indicate that this effect may be seen even in the absence of exogenous insulin (Östenson, 1979). The latter

observation suggests that the secretion of insulin from the predominant B-cells in the normal islets may lead to a rapid saturation of insulin-recognition sites on A₂-cells during static incubations.

Furthermore, it was found that alloxan completely blocked the inhibition of glucagon release by glucose plus insulin, and that this action of alloxan could be prevented by glucose. These observations confirm previous reports showing effects of alloxan on glucagon release from the perfused rat pancreas (Pagliara *et al.*, 1977; Goto *et al.*, 1978). In addition, it has been previously shown that alloxan blocks glucose-stimulated insulin release, and that this action of alloxan is readily prevented by glucose (Gunnarsson & Hellerström, 1973; Tomita *et al.*, 1974; Pagliara *et al.*, 1977). It appears therefore that alloxan affects the mechanism of glucose regulation of hormone release of the A₂-cell in basically the same way as that of the B-cell. The particularly pronounced action of the drug on the A₂-cell-rich islets indicates a direct interaction with the A₂-cell, presumably resulting in a reduced ability of these cells to recognize changes in extracellular glucose concentrations. The failure of an increased glucose concentration to enhance the rate of glucose oxidation in islets treated with alloxan further supports this hypothesis. Thus the impaired regulation of glucagon release may reflect a primary disturbance of the glucose metabolism of the A₂-cell.

Previous studies of the effects of alloxan *in vivo* have consistently failed to demonstrate a lasting toxic action of this drug on the A₂-cell (Rerup, 1970; Dulin & Soret, 1977). The present observations on islets maintained in tissue culture confirm the transient nature of the effects of alloxan on both the glucagon release and glucose oxidation of the A₂-cell. It should be noted in this context that, in the guinea pig, a lasting destruction of the B-cells cannot be achieved with alloxan (Rerup, 1970; Dulin & Soret, 1977). Our data nevertheless indicate that the glucose oxidation of the B-cells must have been acutely affected by alloxan, since exposure of isolated normal islets to alloxan blocked the stimulating action of glucose on the rate of glucose oxidation. However, the glucose stimulation was restored after 1 day in culture, once again emphasizing the reversibility of alloxan action on the B-cells in the guinea pig.

In total, our data indicate that the toxic effect of alloxan is less selective than hitherto believed, although cell types other than the pancreatic B-cell obviously escape permanent damage. This action is not unexpected, in view of the high reactivity of alloxan with a number of cellular constituents, including amino acids, thiols, metals and proteins (Webb, 1966). The common molecular mechanism by which alloxan affects the A₂- and B-cells of the pancreatic islet is, however, still obscure but may

involve a site related to the glucose metabolism of these cells.

The skilled technical assistance of Anna-Britta Andersson, Ing-Britt Hallgren, Ewa Hansson and Astrid Nordin is gratefully acknowledged. This work was supported by grants from the Swedish Medical Research Council (12X-109 and 12X-2297), the Swedish Diabetes Association, the Medical Faculty of the University of Uppsala and Stiftelsen Clas Groschinskys Minnesfond, Stockholm, Sweden.

References

- Andersson, A. (1978) *Diabetologia* **14**, 397–404
- Andersson, A., Petersson, B., Westman, J., Edwards, J. C., Lundqvist, G. & Hellerström, C. (1973) *J. Cell Biol.* **57**, 241–247
- Borg, L. A. H., Eide, S. J., Andersson, A. & Hellerström, C. (1979) *Biochem. J.* **182**, 797–802
- Dulin, W. E. & Soret, M. G. (1977) in *The Diabetic Pancreas* (Volk, B. W. & Wellman, K. F., eds.), pp. 425–465, Plenum Press, New York
- Edwards, J. C., Hellerström, C., Petersson, B. & Taylor, K. W. (1972) *Diabetologia* **8**, 93–98
- Gey, G. O. & Gey, M. K. (1936) *Am. J. Cancer* **27**, 45–76
- Gomori, G. (1950) *Am. J. Clin. Pathol.* **20**, 661–664
- Goto, Y., Seino, Y., Taminato, T., Inoue, Y., Kadowaki, S., Mori, K. & Imura, H. (1978) *Endocrinology* **102**, 1496–1500
- Grankvist, K., Lernmark, Å. & Täljedal, I.-B. (1977) *Biochem. J.* **162**, 19–24
- Gunnarsson, R. (1975) *Mol. Pharmacol.* **11**, 759–765
- Gunnarsson, R. & Hellerström, C. (1973) *Horm. Metab. Res.* **5**, 404–409
- Heding, L. G. (1971) *Diabetologia* **7**, 10–19
- Hellerström, C. (1967) *Endocrinology* **81**, 105–112
- Howell, S. L. & Taylor, K. W. (1968) *Biochem. J.* **108**, 17–24
- Howell, S. L., Edwards, J. C. & Whitfield, M. (1971) *Horm. Metab. Res.* **3**, 37–43
- Jain, K., Asina, S. & Logothetopoulos, J. (1978) *Biochem. J.* **176**, 31–37
- Keen, H., Field, J. B. & Pastan, I. (1963) *Metab. Clin. Exp.* **12**, 143–147
- Krebs, H. A. (1933) *Hoppe-Seyler's Z. Physiol. Chem.* **217**, 191–227
- Östenson, C.-G. (1979) *Diabetologia* **17**, 325–330
- Pagliara, A. S., Stillings, S., Zawulich, W. S., Williams, A. D. & Matschinsky, F. M. (1977) *Diabetes* **26**, 973–979
- Petersson, B., Hellerström, C. & Gunnarsson, R. (1970) *Horm. Metab. Res.* **2**, 313–317
- Rerup, C. C. (1970) *Pharmacol. Rev.* **22**, 485–518
- Snedecor, G. W. (1956) in *Statistical Methods*, 5th edn., pp. 35–65, Iowa State College Press, Ames
- Tomita, T., Lacy, P. E., Matschinsky, F. M. & McDaniel, M. L. (1974) *Diabetes* **23**, 517–534
- Webb, J. L. (1966) *Enzyme and Metabolic Inhibitors*, vol. 3, pp. 408–417, Academic Press, New York and London