Biochem. J. (1979) **182**, 797–802
Printed in Great Britain

Effects *in vitro* of Alloxan on the Glucose Metabolism of Mouse Pancreatic B-Cells

By L. A. Håkan BORG, Susan J. EIDE, Arne ANDERSSON and Claes HELLERSTRÖM
Department of Histology, University of Uppsala, Box 571, S-751 23 Uppsala, Sweden

(*Received 26 February* 1979)

To facilitate detailed studies of the B-cytotoxic action of alloxan we developed a model using isolated pancreatic islets of normal mice. An essential feature of this model is the low temperature employed during exposure to alloxan, which minimizes the degradation of the drug. The islets were incubated with alloxan for 30min at 4°C and subsequently various aspects of their metabolism were studied. The O_2 consumption was measured by the Cartesian-diver technique. Islets exposed to 2mM-alloxan and control islets had the same endogenous respiration, whereas the O_2 uptake of the alloxan-treated islets was inhibited and that of the control islets stimulated when they were incubated with 28mM-glucose as an exogenous substrate. The islet glucose oxidation was estimated by measurement of the formation of $^{14}CO_2$ from [U-^{14}C]glucose at 37°C. Compared with the controls, alloxan-treated islets showed a decrease in the glucose-oxidation rate in a dose-dependent manner. Pretreatment of the islets with 28mM-glucose for 30min at 37°C completely protected against this effect, whereas preincubations at glucose concentrations below 16.7mM failed to exert any protective effect. The glucose utilization was estimated as the formation of ^3H_2O from [5-^{3}H]glucose. Alloxan (2mM) failed to affect islet glucose-utilization rate in the presence of either 2.8 or 28mM-glucose. In contrast, islets exposed to 5 or 10mM-alloxan exhibited lowered glucose utilization. It is concluded that *in vitro* alloxan has an acute inhibitory effect on the islet glucose metabolism, and that this action can be prevented by previous exposure to a high glucose concentration. The results are consistent with the idea that the B-cytotoxicity of alloxan reflects an interaction with intracellular sites involved in the oxidative metabolism of the B-cell.

The rapid and remarkably selective destruction of the pancreatic B-cell by alloxan (Dunn & McLetchie, 1943; Dunn *et al.*, 1943) has stimulated an intense search for the primary molecular action of this drug. The early observation that glucose protects the B-cell from alloxan (Sen & Bhattacharya, 1952; Bhattacharya, 1954) and that this effect is blocked by mannoheptulose (Scheynius & Täljedal, 1971) led to the hypothesis that glucose might interact with alloxan at the B-cell plasma membrane, possibly involving the hexose-transport system. However, other studies have failed to demonstrate any effect of alloxan on the rate of D-glucose or 3-*O*-methyl-D-glucose entry into the B-cell (McDaniel *et al.*, 1975), and, furthermore, D-glucose does not impede the entry of alloxan itself into this cell (Weaver *et al.*, 1978). Indeed, notwithstanding the protective action of high glucose concentrations against the noxious effects of alloxan, D-glucose in fact facilitates the uptake of alloxan by the B-cell (Weaver *et al.*, 1978). Against this background it appeared that structures other than the plasma membrane might be the cellular targets of alloxan and, consequently, a study of possible intracellular interactions such as might affect mitochondrial function seemed of interest. Previous reports that alloxan given to mice *in vivo* inhibits glucose oxidation and insulin release by islets subsequently isolated from these animals (Gunnarsson & Hellerström, 1973) indicate the validity of this approach. In the present study the O_2 uptake and the glucose metabolism were measured in isolated mouse islets exposed acutely to alloxan in a carefully controlled system *in vitro*. The results indicate that the drug rapidly and reproducibly interferes with the oxidative metabolism of the islets and that this effect is efficiently prevented by glucose.

Experimental

Chemicals

Collagenase (EC 3.4.24.3; type CLS) was obtained from Worthington Biochemical Corp., Freehold, NJ, U.S.A., and bovine albumin (fraction V) from Miles Laboratories, Slough, Bucks., U.K. Alloxan (2,4,5,6-tetraoxypyrimidine) monohydrate (mol.wt. 160.1) and antimycin A were supplied by Sigma Chemical Co., St. Louis, MO, U.S.A. D-[U-^{14}C]-Glucose (281mCi/mmol), D-[5-^{3}H]glucose (19Ci/mmol) and ^3H_2O (5Ci/mmol) were purchased from

The Radiochemical Centre, Amersham, Bucks., U.K. Hyamine hydroxide 10-X (1 M in methanol), PPO (2,5-diphenyloxazole), dimethyl-POPOP [1,4-bis-(4-methyl-5-phenyloxazol-2-yl)benzene] and Insta-Gel were from Packard Instruments, Downers Grove, IL, U.S.A. D-Glucose was obtained from Mallinckrodt Chemical Works, St. Louis, MO, U.S.A., and other chemicals of analytical grade were from E. Merck, Darmstadt, West Germany.

Animals and islet isolation

Adult male NMRI mice (Anticimex AB, Sollentuna, Sweden) were starved overnight before the experiments. Pancreatic islets were isolated by a collagenase method (Howell & Taylor, 1968), washed in Hanks' solution (Hanks & Wallace, 1949) and subsequently collected with a braking pipette (Holter, 1943).

Alloxan exposure

The exposure of islets to alloxan was carried out at 4°C. Isolated islets were transferred to small Petri dishes containing 1.8ml of a Krebs–Ringer phosphate buffer (KRP) solution [118mM-NaCl, 4.8mM-KCl, 1.0mM-$CaCl_2$, 1.2mM-$MgSO_4$, 1.2mM-KH_2PO_4, 15mM-Na_2HPO_4 (Krebs, 1933)] supplemented with 2.8mM-glucose, and with the pH adjusted to 7.4. The volume of the medium was brought to 2.0ml by addition of 0.2ml of an alloxan solution prepared freshly at 4°C and containing 10, 20, 50 or 100mM-alloxan monohydrate in KRP solution supplemented with 2.8mM-glucose. The dishes were swirled briefly to mix the solutions rapidly. Thus the final alloxan concentration was 1, 2, 5 or 10mM. The islets were exposed to alloxan for 30min and then washed twice in chilled alloxan-free KRP solution containing 2.8mM-glucose. Control islets were treated similarly, except that alloxan was either omitted or had been inactivated by incubation at 37°C for 30min before the experiments at 4°C. A careful check was made to ensure that pH changes due to alloxan did not influence the results. For this purpose islets were exposed to alloxan with and without a correction for the slight decrease in pH of the incubation medium. Subsequently the O_2 uptake, glucose oxidation and glucose utilization of the islets were measured and no differences between the two sets of observations were found.

In some experiments designed to study the protection by glucose against the action of alloxan, the isolated islets were preincubated at 37°C for 30min in a bicarbonate buffer [120mM-NaCl, 5.0mM-KCl, 1.5mM-$CaCl_2$, 1.0mM-$MgCl_2$, 0.3mM-$MgSO_4$, 0.2mM-KH_2PO_4, 0.8mM-Na_2HPO_4, 27mM-$NaHCO_3$ (Gey & Gey, 1936)] containing 3mg of albumin/ml and glucose as given below. The islets were subsequently treated with alloxan (2mM) as described above, but in each case with the same glucose concentration as during the preincubation procedure.

O_2 uptake

Determination of the islet O_2 uptake was performed by the Cartesian-diver method as described by Hellerström (1967). Five to fifteen islets previously exposed to alloxan as described above or a corresponding number of control islets were incubated in 1μl of KRP solution, pH7.4. The CO_2 evolved during islet respiration was trapped in 1μl of a KOH solution (0.37M). The gas phase in each diver consisted of about 8μl of ambient air. After measurement of the O_2 uptake in the absence of glucose (endogenous respiration) for 40–60min, glucose was added to the islet incubation medium from a small side drop (0.5μl) in the diver, so that the final glucose concentration was 28mM. The change in O_2 uptake caused by the addition of glucose was then followed for a further 60min. With this experimental design each islet sample served as its own control, and the effect of glucose on the respiratory rate was therefore also expressed as a percentage of the endogenous rate. After incubation the islets were recovered from the divers and their dry weights determined with the aid of a Cahn Electrobalance, model 4100, sensitive to 0.1μg (Cahn/Ventron Corp., Paramount, CA, U.S.A.). The average dry weight per islet was 0.54μg. This value was based on weighings of 15 groups of islets, each comprising 7–12 islets.

Glucose oxidation

Groups of ten islets were incubated in glass vials (diameter 12mm, height 25mm) similar to those described by Keen *et al.* (1963). The vials were prepared in duplicate and contained 100μl of a bicarbonate-buffered incubation medium (Gey & Gey, 1936) with D-[U-^{14}C]glucose plus non-radioactive carrier glucose to a final concentration of 28mM and a specific radioactivity of 1.2mCi/mmol. The vials, suspended in standard 20ml glass scintillation flasks, were gassed with O_2/CO_2 (19:1) and sealed airtight. The flasks were then shaken continuously (100 strokes/min) in a water thermostat at 37°C until the islet metabolism was stopped after 15, 30, 60, 90 or 120min by the injection of 100μl of 0.05mM-antimycin A [dissolved in 70% (v/v) ethanol] into the centre vial. This was immediately followed by an injection of 250μl of Hyamine 10-X into the outer flask. CO_2 was liberated from the incubation medium by a subsequent injection into the centre vial of 100μl of 0.4M-Na_2HPO_4 adjusted to pH6.0. After a further incubation for 2h at 37°C to allow the liberated CO_2 to be trapped by the Hyamine 10-X, 10ml of a scintillation fluid (5g of PPO and 0.05g of dimethyl-POPOP per litre of toluene) was added to each flask

and the radioactivity determined in a liquid-scintillation spectrometer (Packard Tri-Carb, model 3255). Blank incubations without islets were treated in the same way as those with islets.

Glucose utilization

The utilization of glucose by the isolated islets was determined as the formation of ^3H_2O from D-[5-^{3}H]-glucose mainly as described by Ashcroft *et al.* (1972). Duplicate batches, each containing ten islets, were incubated in 15μl of a bicarbonate-buffered medium [118mM-NaCl, 4.8mM-KCl, 2.5mM-$CaCl_2$, 1.2mM-$MgSO_4$, 1.2mM-KH_2PO_4, 25mM-$NaHCO_3$ (Krebs & Henseleit, 1932)] placed in small glass vials (diameter 5mm, height 15mm). The medium contained 2.8 or 28mM-glucose including D-[5-^{3}H]-glucose yielding a specific radioactivity of 59 and 6mCi/mmol respectively. The vials were suspended inside glass scintillation flasks containing 0.5ml of redistilled water, which were tightly sealed with rubber membranes. After 60min incubation at 37°C with continuous shaking (100 strokes/min) in a gas phase of O_2/CO_2 (19 : 1) the metabolism was stopped by an injection through the rubber membrane of 100μl of 0.5M-HCl into the incubation vial. The sealed scintillation flasks were subsequently incubated at room temperature overnight to allow the ^3H_2O formed by the islets to equilibrate with the water in the outer flask. The radioactivity of the water was then measured by liquid-scintillation counting. For this purpose the ^3H_2O was diluted with redistilled water to 3.0ml and mixed with 3.5ml of Insta-Gel. Vials without islets were always included in order to allow for a correction for volatile impurities in the D-[5-^{3}H]-glucose. The recovery of ^{3}H from known amounts of ^3H_2O was 65% under these experimental conditions.

Calculations and expression of results

Differences in quenching between samples during liquid-scintillation counting were corrected for by the sample channels-ratio method (Baillie, 1960; Bruno & Christian, 1961).

All results are expressed as mean values±S.E.M. Linear regression was calculated by the method of least squares, and the standard errors of estimate (S.E.E.) for regression lines are given. Differences between mean values and regression lines were evaluated by Student's *t* test.

Results

The endogenous respiration of both the alloxan-treated and the control islets remained constant throughout the first hour of incubation, and no differences in O_2 uptake were recorded between these two experimental groups (Table 1). Addition of glucose to the incubation medium stimulated significantly the O_2 uptake of the control islets, but failed to stimulate the alloxan-treated islets; the respiratory rate of the latter was in fact depressed.

The time course of glucose oxidation by control islets and by islets treated with alloxan at concentrations of 2mM or 10mM is given in Fig. 1. The rate of oxidation remained approximately constant throughout the 2h of observation both for islets exposed to 2mM-alloxan and for controls. However, a statistical comparison between the lines fitting these data indicated a significantly lower oxidation rate by the alloxan-treated islets. It was also evident that glucose oxidation became completely inhibited in islets that had been exposed to 10mM-alloxan.

The dose–response relationship was evaluated in more detail by measurements of glucose oxidation during 60min incubations after exposure to various alloxan concentrations (Table 2). Whereas 1mM-alloxan had only a negligible effect, there was a 40% decrease in the rate of oxidation when the islets had been exposed to 2mM-alloxan. After exposure to 5mM- and 10mM-alloxan the inhibition increased dramatically becoming at the latter concentration essentially complete, as stated above.

To evaluate whether the decrease in islet glucose oxidation caused by alloxan could be prevented by glucose itself, islets were preincubated at 37°C with various glucose concentrations before exposure in the

Table 1. *Effect of alloxan on the respiration of mouse pancreatic islets*

Isolated islets were incubated at 4°C for 30min in a medium containing 2.8mM-glucose and 2mM-alloxan. Subsequently the rate of O_2 uptake was estimated at 37°C with the aid of Cartesian divers (Linderstrøm-Lang, 1937). After measurement of the O_2 uptake in the absence of glucose (endogenous respiration), glucose was added to 28mM and the change in O_2 uptake determined. The percentage changes were calculated for each individual experiment. Results are given as means±S.E.M. with the numbers of observations in parentheses. *P* indicates the probability that there is no difference between endogenous respiration and glucose-stimulated respiration and was estimated by a paired *t* test.

Islets	Endogenous respiration (pmol of O_2/h per μg)	Respiration at 28mM-glucose (pmol of O_2/h per μg)	*P*	Percentage change
Controls	321±18 (7)	415±31 (7)	<0.001	+28.7±4.8 (9)
Alloxan-treated	335±22 (8)	299±22 (8)	<0.01	−10.1±1.9 (9)

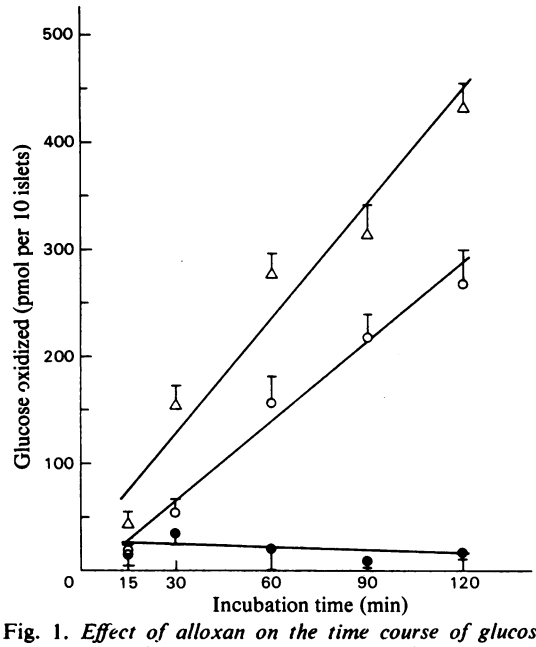

Fig. 1. *Effect of alloxan on the time course of glucose oxidation by mouse pancreatic islets*

Isolated islets were incubated at 4°C for 30min in a medium containing 2.8mM-glucose and either lacking alloxan (△) or containing 2mM-(○) or 10mM-(●) alloxan. Subsequently the glucose oxidation was estimated at 37°C by measuring the formation of $^{14}CO_2$ from 28mM-D-[U-^{14}C]glucose (1.2mCi/mmol). Each point is the mean±S.E.M. On the basis of individual observations a linear regression was calculated for each experimental group. The standard error of the estimate (S.E.E.) for the fitted lines and their slopes±S.E.M. and intercepts of the ordinate±S.E.M. are as follows. Control islets (62 observations): S.E.E., 80.1; slope, 3.5±0.3; intercept, 19.3±19.3. Islets exposed to 2mM-alloxan (32 observations): S.E.E., 56.8; slope, 2.4±0.3 ($P<0.05$); intercept, −8.9±19.7 ($P>0.05$). Islets exposed to 10mM-alloxan (31 observations): S.E.E., 38.1; slope, −0.1±0.2 ($P<0.001$); intercept, 26.5±12.8 ($P>0.05$). The P values for the differences between control islets and islets exposed to alloxan were estimated by Student's t test.

cold to 2mM-alloxan. Table 3 shows that islets that had not been exposed to alloxan oxidized glucose at the same rate, irrespective of the glucose concentration of the preincubation medium. However, alloxan-treated islets showed decreased glucose oxidation up to a glucose concentration in the preincubation medium of 16.7mM. Preincubation at a higher glucose concentration, i.e. 28mM, appeared to offer complete protection against the alloxan effect.

Glucose utilization in isolated mouse islets was studied at 2.8 and 28mM-glucose after exposure of

Table 2. *Effects of various alloxan concentrations on glucose oxidation by mouse pancreatic islets*

Isolated islets were incubated at 4°C for 30min in a medium containing 2.8mM-glucose and alloxan as indicated. Subsequently the glucose-oxidation rate was measured at 37°C from the $^{14}CO_2$ formed from 28mM-D-[U-^{14}C]glucose (1.2mCi/mmol). Results are given as means±S.E.M. with the numbers of observations in parentheses. Differences from the control value were evaluated by Student's t test. ***$P<0.001$.

Alloxan concn. (mM)	Glucose oxidation (pmol/h per 10 islets)
0 (Controls)	269.3±13.3 (38)
1	222.2±30.2 (12)
2	157.1±23.9*** (8)
5	64.1±16.0*** (11)
10	21.7±20.3*** (8)

the islets to various concentrations of alloxan (Table 4). Alloxan at a concentration of 2mM failed to affect the rate of glucose utilization in the presence of either 2.8 or 28mM-glucose. By contrast, treatment with 5mM-alloxan caused a marked inhibition, which was even more conspicuous after exposure of the islets to 10mM-alloxan.

Discussion

The extreme lability of alloxan in solution at physiological pH and temperature is a complicating factor in experiments with this drug. However, the half-life at pH 7.4 can be extended from about 1min at 37°C (Patterson *et al.*, 1949) to 3h at 4°C (Rerup, 1970). The present static incubation of islets in a chilled alloxan solution therefore served both to preserve the activity of the drug and to allow for its complete penetration into the islets during the exposure period. It is noteworthy in this context that alloxan effects on islet metabolism were recorded, despite several washings of the islets, while they were still maintained at 4°C. Presumably alloxan binds firmly to certain islet constituents during incubation and can exert its toxic action after the washing procedure, when the islet metabolism is re-activated by incubation at 37°C. Alternatively, if alloxan can be washed out, it may have already injured the islets during incubation at 4°C. In the latter case the toxic action would be independent of the metabolic activity of the B-cell.

The observation that islet respiration was affected by alloxan only at the glucose-stimulated part, whereas endogenous respiration proceeded at a constant rate, suggests that major derangements of mitochondrial function had not occurred during the acute exposure to the drug. In support of this notion, Idahl *et al.* (1977) failed to demonstrate any effect on

Table 3. *Protection by glucose against the effect of alloxan on glucose oxidation by mouse pancreatic islets*
Isolated islets were preincubated at 37°C for 30min with different glucose concentrations as indicated. During the subsequent incubation at 4°C for 30min in a medium containing the same glucose concentration the islets were exposed to 2mM-alloxan. Subsequently the glucose-oxidation rate was determined at 37°C from the $^{14}CO_2$ formed from 28mM-D-[U-^{14}C]glucose (1.2mCi/mmol). Results are given as means ± S.E.M. with the numbers of observations in parentheses. *P* indicates the probability for chance differences between alloxan-treated islets and controls for each experimental condition and was evaluated by Student's *t* test.

Glucose concn. during preincubation (mM)	Alloxan concn. (mM)	Glucose oxidation (pmol/h per 10 islets)	*P*
2.8	0	291.5 ± 18.7 (8)	
2.8	2.0	171.0 ± 16.4 (8)	<0.001
6.1	0	298.6 ± 18.2 (8)	
6.1	2.0	202.0 ± 18.6 (8)	<0.01
10.0	0	279.6 ± 18.6 (7)	
10.0	2.0	190.0 ± 11.6 (6)	<0.01
16.7	0	292.4 ± 23.7 (8)	
16.7	2.0	198.0 ± 15.4 (7)	<0.05
28.0	0	296.0 ± 27.4 (7)	
28.0	2.0	308.9 ± 27.4 (7)	>0.05

Table 4. *Effects of various alloxan concentrations on glucose utilization by mouse pancreatic islets*
Isolated islets were incubated at 4°C for 30min in a medium containing 2.8mM-glucose and alloxan as indicated. Subsequently the glucose-utilization rate was determined at 37°C from the formation of ^3H_2O from 2.8mM- or 28mM-D-[5-^{3}H]glucose (59 and 6mCi/mmol respectively). Results are given as means ± S.E.M. with the numbers of observations in parentheses. Differences from the control value for each glucose concentration were evaluated by Student's *t* test. ***P*<0.01; ****P*<0.001.

Alloxan concn. (mM)	Glucose concn. (mM)	Glucose utilization (pmol/h per 10 islets)
0 (Controls)	2.8	61.1 ± 8.7 (10)
2	2.8	72.5 ± 15.4 (4)
10	2.8	11.9 ± 3.9** (6)
0 (Controls)	28.0	594.0 ± 32.3 (46)
2	28.0	535.5 ± 77.1 (11)
5	28.0	267.4 ± 40.2*** (17)
10	28.0	166.9 ± 13.9*** (15)

the islet ATP content after incubation of islets for 20min with 2mM-alloxan in the presence of 2mM-glucose. The failure of glucose to stimulate respiration in alloxan-treated islets nevertheless shows that certain aspects of islet metabolism were disturbed and that this disturbance might be related to the oxidation of exogenous fuels.

The results discussed above were confirmed and extended in the studies of the utilization of glucose and its conversion to CO_2 by the isolated islets. The finding that an alloxan concentration of 2mM was required before a significant inhibition of the glucose oxidation occurred agrees with a report that indicated that rubidium ion pumping in pancreatic B-cells was inhibited at the same concentration (Idahl *et al.*, 1977). This is nevertheless a considerably higher concentration of alloxan than that required to affect the insulin response to glucose in a perifusion system, as evidenced by the finding by Tomita *et al.* (1974) that a significant inhibition of insulin release was caused by only 5mg of alloxan/100ml (0.3mM) in the perifusion fluid. Although discrepancies of this kind may suggest a dissociation between insulin release and islet oxidative metabolism with regard to sensitivity to alloxan, it must be kept in mind that the technique for measuring glucose oxidation in static incubations is extremely crude compared with that for measuring insulin release in a perifusion system.

Our results show that glucose was able to protect fully against the metabolic derangements caused by alloxan. However, the required concentration was somewhat above that previously reported to protect against the inhibition caused by alloxan at similar concentrations on insulin biosynthesis (Jain & Logothetopoulos, 1976), insulin release (Tomita *et al.*, 1974), islet rubidium ion pumping (Idahl *et al.*, 1977) or islet cell membrane potential (Dean & Matthews, 1972). Presumably, the longer exposure to a potent alloxan solution in our experiments was of significance in this context. The precise mechanism of glucose protection is still obscure. The finding of a facilitated alloxan uptake by the B-cell in the presence of a high glucose concentration (Weaver *et al.*, 1978) suggests that the critical interaction between glucose and the drug is at an intracellular site. The finding that an acute alloxan effect on glucose oxidation can be prevented by glucose supports this notion.

The present data on both O_2 consumption and glucose metabolism make it possible to calculate in some detail the oxidative metabolism of both the normal and alloxan-treated islets. On the basis of an

average islet dry weight of 0.54 μg, the rate of glucose oxidation can be calculated to be 50 pmol/h per μg dry wt. of islet in the presence of 28 mM-glucose. Theoretically this corresponds to an O_2 consumption of 300 pmol/h per μg, which indicates that the endogenous respiration in the presence of glucose, i.e. the difference between the total O_2 consumption and that accounted for by glucose (115 pmol/h per μg), was much smaller than when the islets respired in the total absence of glucose. Furthermore, the total glucose utilization at 28 mM-glucose was 110 pmol/h per μg, which indicates that of all the glucose metabolized by the islets only 45% was oxidized to CO_2. This value agrees favourably with previous reports that 45–50% of the glucose utilized by isolated islets is converted to lactate (Pace *et al.*, 1975; Sener & Malaisse, 1976). After treatment with 2 mM-alloxan the islet glucose-utilization rate was 99 pmol/h per μg, and the glucose-oxidation rate 29 pmol/h per μg, indicating that the oxidation now accounted for only 29% of the total glucose utilized and suggesting that the alloxan effect was confined to the oxidation of glucose. In support of this, previous observations indicate that the primary site of toxic alloxan action is neither on the glucose transport across the plasma membrane (McDaniel *et al.*, 1975) nor on anaerobic glycolysis (Gunnarsson & Hellerström, 1973). Furthermore, observations by Zawalich *et al.* (1979) indicate a rather weak effect of alloxan (1.25 mM) on glucose utilization by isolated rat islets. It should be noted, however, that in the present study alloxan concentrations of the order of 5–10 mM exerted more general effects, causing a complete block of glucose oxidation and a marked inhibition of glucose utilization.

Altogether, the present study suggests that acute exposure of mouse islets to alloxan (2 mM) interferes with the islet oxidation of exogenous glucose while leaving both total utilization and endogenous respiration intact. These observations together with the previous reports referred to above suggest that a primary action of alloxan is on the mitochondrial handling of exogenous substrates. In favour of this assumption recent results show that the oxidation of leucine is also strongly inhibited in alloxan-treated mouse islets (L. A. H. Borg, unpublished work) and that the earliest degenerative ultrastructural B-cell changes observed after alloxan treatment *in vivo* are localized in the mitochondria (Williamson & Lacy, 1959; Wellmann *et al.*, 1967; Boquist, 1977). In order to elucidate further if a mitochondrial derangement is the critical step in the interaction between alloxan and the B-cell, studies on isolated B-cell mitochondria need to be performed.

The expert technical assistance of Mrs. Heléne Pettersson and Mrs. Ing-Britt Hallgren and the financial support from the Swedish Diabetes Association, the Swedish Society for Medical Research, the University of Uppsala and the Swedish Medical Research Council (12X-109) are gratefully acknowledged.

References

Ashcroft, S. J. H., Weerasinghe, L. C. C., Bassett, J. M. & Randle, P. J. (1972) *Biochem. J.* **126**, 525–532
Baillie, L. A. (1960) *Int. J. Appl. Radiat. Isot.* **8**, 1–7
Bhattacharya, G. (1954) *Science* **120**, 841–843
Boquist, L. (1977) *Acta Pathol. Microbiol. Scand. Sect. A* **85**, 219–229
Bruno, G. A. & Christian, J. E. (1961) *Anal. Chem.* **33**, 650–651
Dean, P. M. & Matthews, E. K. (1972) *Diabetologia* **8**, 173–178
Dunn, J. S. & McLetchie, N. G. B. (1943) *Lancet* **ii**, 384–387
Dunn, J. S., Sheehan, H. L. & McLetchie, N. G. B. (1943) *Lancet* **i**, 484–487
Gey, G. O. & Gey, M. K. (1936) *Am. J. Cancer* **27**, 45–76
Gunnarsson, R. & Hellerström, C. (1973) *Horm. Metab. Res.* **5**, 404–409
Hanks, J. H. & Wallace, R. E. (1949) *Proc. Soc. Exp. Biol. Med.* **71**, 196–200
Hellerström, C. (1967) *Endocrinology* **81**, 105–112
Holter, H. (1943) *C. R. Trav. Lab. Carlsberg, Ser. Chim.* **24**, 399–478
Howell, S. L. & Taylor, K. W. (1968) *Biochem. J.* **108**, 17–24
Idahl, L.-Å., Lernmark, Å., Sehlin, J. & Täljedal, I.-B. (1977) *Biochem. J.* **162**, 9–18
Jain, K. & Logothetopoulos, J. (1976) *Biochim. Biophys. Acta* **435**, 145–151
Keen, H., Field, J. B. & Pastan, I. H. (1963) *Metab. Clin. Exp.* **12**, 143–147
Krebs, H. A. (1933) *Hoppe-Seyler's Z. Physiol. Chem.* **217**, 191–227
Krebs, H. A. & Henseleit, K. (1932) *Hoppe-Seyler's Z. Physiol. Chem.* **210**, 33–66
Linderstrøm-Lang, K. (1937) *Nature (London)* **140**, 108
McDaniel, M. L., Anderson, S., Fink, J., Roth, C. & Lacy, P. E. (1975) *Endocrinology* **97**, 68–75
Pace, C. S., Ellerman, J., Hover, B. A., Stillings, S. N. & Matschinsky, F. M. (1975) *Diabetes* **24**, 476–488
Patterson, J. W., Lazarow, A. & Levey, S. (1949) *J. Biol. Chem.* **177**, 187–196
Rerup, C. C. (1970) *Pharmacol. Rev.* **22**, 485–518
Scheynius, A. & Täljedal, I. B. (1971) *Diabetologia* **7**, 252–255
Sen, P. B. & Bhattacharya, G. (1952) *Indian J. Physiol. Allied Sci.* **6**, 112–114
Sener, A. & Malaisse, W. J. (1976) *Biochem. Med.* **15**, 34–41
Tomita, T., Lacy, P. E., Matschinsky, F. M. & McDaniel, M. L. (1974) *Diabetes* **23**, 517–524
Weaver, D. C., McDaniel, M. L. & Lacy, P. E. (1978) *Endocrinology* **102**, 1847–1855
Wellmann, K. F., Volk, B. W. & Lazarus, S. S. (1967) *Diabetes* **16**, 242–251
Williamson, J. R. & Lacy, P. E. (1959) *A.M.A. Arch. Pathol.* **67**, 102–109
Zawalich, W. S., Karl, R. C. & Matschinsky, F. M. (1979) *Diabetologia* **16**, 115–120