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Effects of Alloxan on Insulin Release by Isolated Mouse Pancreatic Islets[1] (38225)

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Studies of the *in vitro* effects of alloxan on pancreatic secretory activity have been complicated by the instability of the alloxan molecule at physiological temperatures and pH (1). The diabetogenic activity of alloxan solutions is known to be completely destroyed by 15 min of incubation at 37° (2).

In spite of the instability of the alloxan molecule, *in vitro* studies on the pancreatic effects of the chemical have been conducted. Howell and Taylor (3) have reported experiments in which they administered alloxan to rabbits then isolated the animals pancreata for *in vitro* experiments. Gunnarsson and Hellerstrom (4) have employed a similar approach in their experiments with mouse pancreata. Under these experimental conditions alloxan has been found to increase the release of insulin from isolated tissues.

Lazarow and his co-workers have utilized a different approach. They have taken advantage of the fact that the half-life of alloxan solutions is markedly prolonged if the solutions are maintained at 0° (5). These workers have studied the effects of alloxan on the permeability of toad fish pancreatic islets maintained at 0° throughout the entire experimental period (6–8). They have reported that alloxan increases the permeability of *beta* cell membranes to extracellular D-mannitol as well as intracellular proteins.

By combining the basic principles of the 2 techniques mentioned above, i.e., exposing isolated islets to alloxan at 0° then incubating them at 37°, we have been able to study the effects of the diabetogen on insulin release by isolated mouse pancreatic islets. Under the conditions of our experiment, an *in vitro* concentration of 1×10^{-3} *M* alloxan causes a significant release of insulin from isolated islets. Further, increased insulin release requires approximately 4.5 hr to occur.

Materials and Methods. Random bred, male albino mice (20–25 g) were used throughout these experiments. The animals were obtained from the Laboratory Supply Company (Indianapolis) and were acclimated to laboratory conditions for 1 week prior to use. Mice had free access to food and water from the time of acquisition to the time of sacrifice by cervical dislocation.

Pancreatic islets were isolated at 3–4° by the collagenase method (9). Following isolation, groups of three similarly sized islets were placed in incubation tubes containing Krebs–Ringer bicarbonate buffer supplemented with 3 mg/ml bovine serum albumin. This buffer formula contains 60 mg/100 ml of D-glucose, a concentration which causes only minimal insulin secretion from isolated islets. Therefore, little insulin release was expected from control islets.

Alloxan monohydrate was dissolved in cold buffer at concentrations ranging from 1×10^{-4}–1×10^{-2} *M*. The islets were exposed to these concentrations of alloxan for 30 min at 0°. At the conclusion of the preincubation exposure period, an aliquot of the medium was analyzed for immunoreactive

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insulin (IRI) by the method of Hales and Randle (10). The islets were then rinsed with fresh buffer and transferred to alloxan-free buffer at 37°. Incubation at 37° with a gas phase of 95% oxygen, 5% carbon dioxide, was carried out for up to 6.5 hr.

Aliquots of the incubation medium were taken at various intervals for later analysis of IRI content. At the completion of the incubation period all tubes were inspected to insure that three islets were present.

Insulin release was expressed as μU-IRI/islet. The results were statistically analyzed by analysis of variance.

Results. Insulin release during the pre-incubation, alloxan exposure period was minimal. Both control and alloxan exposed islets released approximately 2–3 μU of IRI during this initial 30 min period. We have never detected a significant difference between the various treatment groups after completion of this exposure period.

The results shown in Fig. 1 summarize the effects of various concentrations of alloxan on IRI release during 3 hr of incubation at 37°. It can be seen from these results that exposure of the islets to the 1×10^{-2} *M* concentration of alloxan produced a significant increase in IRI release. The increase was initially evident after 1.5 hr of incubation and continued through the remainder of the experiment ($P < 0.01$). After 3 hr of incubation the alloxan exposed islets had released 5 times more insulin than had the control islets.

Exposure of the islets to the lower concentrations of alloxan did not result in IRI release during the three hours of incubation. This was a disappointing observation since earlier workers have reported *in vitro* effects of alloxan on the premeability of toad fish islets after exposure to only 2.5×10^{-4} *M* (6–8).

For this reason, a second experiment with the 1×10^{-3} *M* concentration of alloxan was conducted. In this experiment the islets were exposed to alloxan as described but incubation at 37° was carried out for 6.5 rather than 3 hr. The results of this experiment are shown in Fig. 2.

By employing the longer incubation period we were able to demonstrate an effect of the lower concentration of alloxan on IRI release. The increased release from the exposed islets became evident at the 4.5 hr interval and continued throughout the remainder of the experiment ($P < 0.01$). After 6.5 hr of incubation the mean IRI release by the exposed islets was 198 μU/islet as compared to only 23 μU/islet for controls.

The long latent period for the onset of IRI release from alloxan exposed islets was surprising. Earlier workers have reported increases in the permeability of toad fish islets within 1 hr of exposure to alloxan

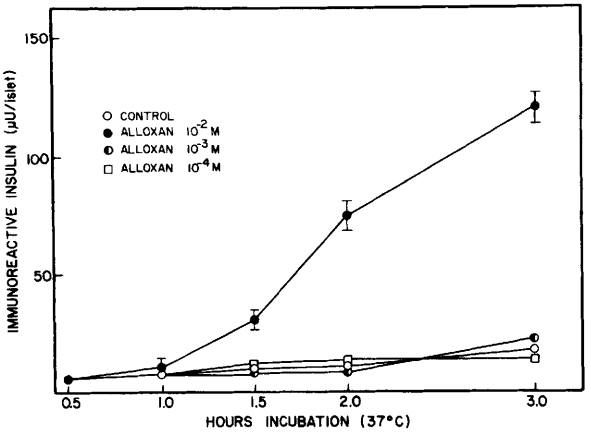

FIG. 1. Effect of alloxan on insulin release from isolated mouse pancreatic islets. Each point represents the mean IRI release from 6 sets of islets, each containing 3 islets per set. Vertical lines represent standard errors of the means.

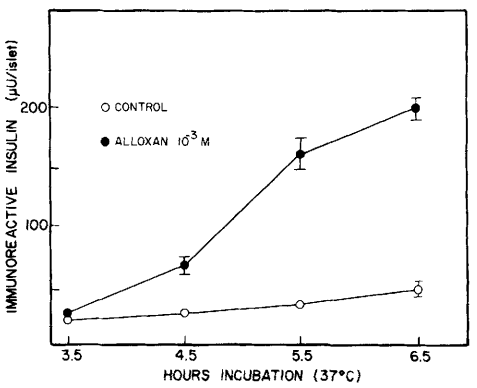

FIG. 2. Effect of $1 \times 10^{-3} M$ alloxan on insulin release by isolated mouse pancreatic islets. Each point represents the mean IRI release from 4 sets of islets, each containing 3 islets per set. Vertical lines represent standard errors of the means.

(6–8). Since those earlier experiments were conducted at 0° we decided to study the effect of alloxan on isolated mouse islets at the lower temperature.

In this experiment the islets were exposed to $1 \times 10^{-2} M$ alloxan for 30 min at 0°. After the exposure the islets were rinsed with cold buffer and transferred to fresh, alloxan-free buffer and maintained at 0° for 3 additional hours. Aliquots of the medium were taken for IRI analysis at 30 min intervals. The results of the experiment are summarized in Table I.

It can be seen from the data shown in Table I that the $1 \times 10^{-2} M$ concentration of alloxan failed to induce IRI release when the islets were maintained at 0°. In the earlier experiment (Fig. 1) this concentration of alloxan resulted in a mean IRI release of 121 μU/islet after 3 hr of incubation at 37°. After 3 hr at 0°, alloxan exposed islets released only 5.3 μU/islet. Also, there was no difference in magnitude of release of IRI between exposed and control islets.

TABLE I. Effect of Alloxan ($10^{-2} M$) on Insulin Release[a] From Isolated Mouse Pancreatic Islets at 0°.

Time (hours)	Alloxan ($10^{-2} M$) ($n = 6$)	Control ($n = 6$)
0.5	2.5 ± 0.2	2.3 ± 0.2
1.0	3.0 ± 0.2	3.5 ± 0.5
1.5	4.7 ± 1.1	3.6 ± 0.4
2.0	5.0 ± 0.5	4.1 ± 0.2
3.0	5.3 ± 0.6	4.7 ± 0.3

[a] μU-Immunoreactive insulin/islet.

Discussion. The results of these experiments demonstrate that *in vitro* exposure of isolated mouse pancreatic islets to alloxan results in increased IRI release. The mechanism of this release is unknown. It has been suggested, however, that the release is due to an uncontrolled leakage of the hormone through damaged *beta* cell membranes (3, 11).

These results also demonstrate some basic differences between experiments conducted with mouse islets and those in which toad fish islets are employed. Earlier reports have shown that the onset of alloxan-induced increases in *beta* cell membrane permeability is rapid in the toad fish islet, appearing within 1 hr. In the mouse islet, however, changes in IRI release are not evident until several hours after alloxan exposure. Further, the duration of this latent period is related to the concentration of alloxan to which the islets have been exposed. After exposure of mouse islets to $10^{-2} M$ alloxan, changes in IRI release take 1.5 hr to develop while exposure to $10^{-3} M$ results in increased IRI release after 4.5 hr.

Also, the temperature requirements for the production of the alloxan effects are different for mouse islets than they are for toad fish islets. Exposure of toad fish islets to alloxan results in increased *beta* cell membrane permeability even though the entire experiment is conducted at 0° (6–8). Mouse islets, however, do not exhibit increased IRI release in response to alloxan if they are maintained at 0°. The exact temperature requirements for demonstration of the alloxan effect in mouse islets has not been determined.

In spite of the fact that IRI release cannot be demonstrated at 0°, it seems likely that alloxan binds to and possibly damages the *beta* cell membrane at the lower temperature. This conclusion is based on the fact that alloxan was present only during

the preincubation exposure periods. The islets were rinsed with fresh, alloxan-free buffer prior to their transfer to the final incubation medium. It is unlikely that alloxan contamination of the incubation medium would occur under these conditions. If such contamination did occur, its significance would be questionable. The diabetogenic activity of alloxan is completely lost after 15 min of incubation at 37° (2).

It is interesting that the *in vitro* response of isolated mouse pancreatic islets is similar, in a chronological sense, to the development of hypoglycemia after the administration of diabetogenic doses of the chemical. In our laboratories the hypoglycemic phase of the response to alloxan begins approximately four hours after the intravenous injection of 75 mg/kg. This observation has led us to suspect that the *in vitro* response of the isolated islets corresponds to the pancreatic events which initiate the hypoglycemic phase of the *in vivo* response to diabetogenic doses of the chemical.

Summary. Exposure of isolated mouse pancreatic islets to 1×10^{-2} and 1×10^{-3} *M* alloxan at 0° results in increased insulin release when the islets are later incubated at 37°. Significant insulin release does not occur if the islets are maintained at 0°. The latent period for insulin release is related to alloxan concentration. Exposure to 1×10^{-2} *M* alloxan requires 1.5 hr incubation at 37° while exposure to 1×10^{-3} *M* requires 4.5 hr. It is believed that the insulin release observed *in vitro* corresponds to the *in vivo* insulin release that causes the hypoglycemic response to alloxan.

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