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COMMENTARY

ALLOXAN TOXICITY TO THE PANCREATIC B-CELL

A NEW HYPOTHESIS

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Alloxan has been used for four decades to induce diabetes mellitus in experimental animals [1, 2]. The diabetogenic action of alloxan results from a selective toxic effect on the insulin-producing pancreatic B-cell (see Ref. 3 for a review). The aims of the present report are, first, to define a new hypothesis recently put forward to account for such a selective cytotoxicity [4] and, second, to review a number of previous reports dealing with the mode of action of alloxan in order to assess whether the experimental findings collected in these prior studies are compatible with our new hypothesis.

1. *Definition of a new hypothesis*

We have recently proposed that the selective toxicity of alloxan to the pancreatic B-cell results from the coincidence of two distinct and independent features: a rapid accumulation of the drug into islet cells and their exquisite sensitivity towards peroxides [4]. This hypothesis is based on the following considerations.

1.1. *Generation of toxic oxygen-containing radicals.* The work of Heikkila and his colleagues [5–11] has led to the view that the cytotoxicity of alloxan is mediated through the generation of reactive oxygen-containing radicals in a cascade of chemical reactions.

When exposed to reducing substances (e.g. ascorbic acid) and molecular oxygen, alloxan and its reduced analogue, dialuric acid, form a reduction–oxidation cycle in which superoxide anion radicals ($O_2^{\cdot -}$) are formed during autooxidation of dialuric acid [5, 6]. Superoxide radicals can dismute spontaneously to form hydrogen peroxide, according to the following reaction:

$$2O_2^{\cdot -} + 2H^+ \rightarrow H_2O_2 + O_2.$$

This reaction can be catalyzed by superoxide dismutase. The net balance of the reactions so far considered would be such that 1 mole of O_2 is consumed and 1 mole of H_2O_2 formed for each mole of dialuric acid reconverted to alloxan. Hydroxyl radicals ($OH^{\cdot}$), which are considered as the most reactive oxygen-containing species, are then generated from the superoxide radical anion and hydrogen peroxide in the metal-ion-catalyzed Haber–Weiss reaction [6]:

$$O_2^{\cdot -} + H_2O_2 \rightarrow OH^{\cdot} + OH^- + O_2.$$

Alloxan-induced luminol luminescence in a cell-free system was recently proposed as a tool to investigate the mechanism of alloxan-dependent free-radical generation [12].

An important feature of the reactions listed earlier is their sequential ordering. It is understandable, therefore, that any system susceptible to divert or scavenge either the intermediate or final products of these reactions will tend to counteract the cytotoxic effect of alloxan. This was indeed documented on several occasions. For instance, the inhibition of glucose-stimulated insulin release, which is seen when pancreatic islets are pretreated *in vitro* with alloxan, is considerably less marked when the exposure to alloxan is performed in the presence of either superoxide dismutase, catalase or the iron-chelating agent diethylenetriaminepentaacetic acid [13]. Superoxide dismutase, catalase and diethylenetriaminepentaacetic acid also protect against the inhibition by alloxan of ^{86}Rb uptake by pancreatic islets [14, 15]. Likewise, scavengers of hydroxyl radicals, such as butanol, mannitol, benzoate and dimethylsulfoxide counteract the effect of alloxan on ^{86}Rb accumulation [14]. Moreover, the diabetogenic action of alloxan *in vivo* is prevented by superoxide dismutase covalently linked to polyethylene glycol and injected 12 hr prior to alloxan [16] and by a number of hydroxyl radical scavengers including dimethylsulfoxide [7], amygdalin [8], dimethylurea [9] and thiourea (but not hydroxyurea and urea), methanol, ethanol, *n*-propanol and *n*-butanol [10, 11]. The four aliphatic alcohols in this series were shown not to react *in vitro* with $O_2^{\cdot -}$ or H_2O_2, whereas their reactivity with $OH^{\cdot}$ increased with increasing chain length and paralleled their relative potency in protecting against the diabetogenic action of alloxan [10].

It is noteworthy that pretreatment of pancreatic islets by other autooxidizing compounds, such as dihydroxyfumarate, causes a dose-related inhibition of glucose-stimulated insulin release [17]. Superoxide dismutase, catalase, diethylenetriaminepentaacetic acid, dimethylurea or glucose (but not galactose) all protect against such an inhibition when present together with dihydroxyfumarate in the incubation medium [17, 18].

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Taken as a whole, these findings support the view that the cytotoxic action of alloxan results from the sequential generation of such reactive species as $O_2^{\cdot-}$, H_2O_2 and $OH^{\cdot}$, with emphasis on the toxicity of the hydroxyl radical. It should be noted, however, that, in the studies so far reviewed, it was not established whether the protective agent had to penetrate into the B-cell or, at least, into its cell boundary in order to protect against alloxan.

1.2. *Alloxan uptake by pancreatic islet cells.* Early studies on radioactive alloxan uptake by toadfish islets have suggested that the drug does not accumulate in islet cells [19]. However, later reports clearly indicated that alloxan is taken up by islet cells. Hammarström and his colleagues [20, 21] first observed that radioactivity accumulates in islet cells examined by microautoradiography in specimens of pancreas removed 15–240 min after intravenous administration of [2-^{14}C]alloxan to mice. Thereafter, Weaver *et al.* [22] demonstrated that [2-^{14}C]alloxan (but not alloxanic acid) rapidly accumulates in rat islets, maximal uptake being reached after 2 (at 37°) to 5 (at 23°) min exposure to the drug and amounting to 48–70% of the urea space (after correction for the extracellular space of distribution of [^{3}H]sucrose). After 5 min incubation at 37°, about half of the intracellular radioactive material comigrates with alloxanic acid, indicating intracellular decomposition of alloxan [22]. We observed that, during 5 min incubation at 23°, [2-^{14}C]alloxan accumulates in islet cells to almost the same extent as D-[U-^{14}C]glucose [4].

We believe that the rapidity of alloxan accumulation in islet cells is an important feature in the expression of its diabetogenic action. This belief is based not so much on the knowledge that alloxan is rapidly inactivated in and/or removed from the blood stream after intravenous administration [23], but on experimental data reviewed later in this report and indicating that agents which prevent alloxan uptake by islet cells protect against its deleterious effects. In this perspective, it is essential to note that alloxan also rapidly accumulates in hepatocytes (but not in muscle) suggesting that its cellular uptake is not sufficient to fully account for its specific toxicity towards islet cells [4]. Incidentally, our finding that alloxan is rapidly taken up by liver cells may account for the accumulation of radioactivity in the intestine, below the entrance of the common bile duct, as observed in mice killed 60 min after injection of [2-^{14}C]alloxan [21].

The view that the rapid accumulation of alloxan in islet cells is required for expression of its diabetogenic action may also explain why B-cells are much more sensitive to alloxan than other endocrine cells within the islets. Indeed, we recently observed that, during 5 min incubation at 23°, the apparent space of distribution of [2-^{14}C]alloxan is much larger, relative to the corresponding [^{14}C]urea space, in single or coupled B-cells than in single non-B islet cells [24]. A comparable difference was observed when the space of distribution of [U-^{14}C]3-*O*-methyl-D-glucose, which inhibits alloxan uptake by islet cells [22], was measured in the purified populations of B and non-B islet cells [24]. In this respect, it should be noted that we were unable to detect any major difference between these two types of cells when measuring their glutathione peroxidase activity [4].

In conclusion, alloxan rapidly accumulates in the B-cell. Such a rapid uptake is apparently required for expression of the cytotoxic action of alloxan. However, the rapid uptake of the drug is not sufficient to fully account for the selective toxicity of alloxan to the B-cell.

1.3. *The sensitivity of islet cells to peroxides.* Within the limits of the experimental data so far reviewed, a number of hypotheses could be put forward to account for the selective cytotoxicity of alloxan to the pancreatic B-cell. For instance, islet cells could differ from other cells in which alloxan also rapidly accumulates (e.g. liver cells) by an unusually high efficiency in either reducing alloxan to dialuric acid or catalyzing the iron-dependent formation of $OH^{\cdot}$ from $O_2^{\cdot-}$ and H_2O_2 [16]. Alternatively, islet cells may be less efficient than other cells in diverting $O_2^{\cdot-}$ and/or H_2O_2 from the pathway eventually leading to $OH^{\cdot}$ production. The latter hypothesis was indirectly approached by examining the susceptibility of pancreatic islets and other tissues to an exogenous peroxide [4].

In order to assess the sensitivity of intact islets and other tissues to peroxides, we have exposed them *in vitro* to increasing concentrations of *tert*-butyl hydroperoxide, the substrate currently used in the assay of glutathione peroxidase. The selection of this peroxide was motivated by the consideration that its entry into different cells was unlikely to display the same tissue specificity as that characterizing the uptake of alloxan. Among five different tissues examined for such a purpose, the islets were the most sensitive to peroxide, as judged from the lowest concentration of *tert*-butyl hydroperoxide required to significantly affect [U-^{14}C]glucose oxidation [4]. Such a concentration was at least twice higher in muscle, 10 times higher in the exocrine pancreas and 25 times higher in kidney and liver than in islets. These findings indicate that islet cells are poorly protected against peroxides. Since the generation of H_2O_2 participates in the cascade of reactions involved in the cytotoxicity of alloxan, it is likely that the exquisite sensitivity of islet cells to peroxides accounts, in part at least, for their susceptibility to alloxan. The latter view is supported by the finding that islets removed from guinea pigs are about 5 times less sensitive than rat islets to *tert*-butyl hydroperoxide [25], this situation coinciding with a lesser sensitivity of guinea pigs, as distinct from rats, to the diabetogenic action of alloxan [26].

We now wish to consider the factor(s) possibly responsible for the exquisite sensitivity of islet cells to peroxides. In 1979, Grankvist *et al.* [14] noted that "nothing is known about the activity in B-cells of those enzymes which may determine the liability of cells to engage in toxic autooxidation processes, e.g. catalase and superoxide dismutase". Being engaged at that time in a study of enzymes involved in the control of the thiol:disulfide balance in pancreatic islets [27, 28], we soon became aware that the enzymatic pattern of islet cells could indeed account for their sensitivity to peroxides. Thus, comparing five different tissues (islet, muscle, exocrine pancreas, kidney and liver), we observed a tight

correlation between the activity of glutathione peroxidase and the minimal concentration of *tert*-butyl hydroperoxide required to alter glucose oxidation, the lowest values for both variables being found in pancreatic islets and the highest values in liver [4]. A comparable, albeit not identical, hierarchy in the activity of glutathione peroxidase was independently reported by Grankvist *et al.* [29]. These authors also measured the activity of catalase and both CuZn- and Mn-superoxide dismutases. When the tissues were ranked from highest to lowest with regard to the activity of each investigated enzymes, the average rank values, which were used as crude indices of the enzymatic protection against toxic oxygen-containing species, followed an order not vastly different from that reached in our study of glutathione peroxidase, namely 1.5 for liver, 2.3 for kidney, 4.0 for the exocrine pancreas, 5.5 for islets and 8.0 for muscle [29]. Nevertheless, the authors concluded that differences in enzyme activity between islets and other tissues were not large enough to explain the greater susceptibility of islet cells to alloxan.

We believe that, in order to account for such a susceptibility, both the capacity of islet cells to rapidly take up alloxan and their sensitivity to peroxides should be taken into account. Indeed, when these two features are considered, it becomes evident that: (i) muscle could be protected against alloxan, despite high sensitivity to peroxides, because of poor or slow penetration of alloxan into muscle cells; (ii) liver could be little affected by alloxan, despite rapid accumulation of the drug in hepatocytes, thanks to its resistance to peroxides; and (iii) islet B-cells would be highly vulnerable to alloxan due to coinciding rapid uptake of the drug and exquisite sensitivity to peroxides.

1.4. *Protective action of carbohydrate and non-carbohydrate nutrients against alloxan.* It is well established that glucose protects against the cytotoxic action of alloxan. For instance when pancreatic islets are exposed *in vitro* to alloxan, the consequent inhibition of glucose-stimulated insulin release is much less marked or totally suppressed if glucose is present at a sufficiently high concentration together with alloxan in the incubation medium. More information on the protective effect of glucose and other hexoses is presented later in this report (see Section 2.5). It is generally thought that noncarbohydrate nutrients fail to protect against alloxan. For instance, Scheynius and Täljedal [30] reported that neither L-alanine nor L-leucine (4 mmoles/kg body weight) administered *in vivo* protect against alloxan. Likewise, Borg [31] reported that, in contrast to the results obtained with D-glucose, preincubation of islets with L-leucine prior to exposure to alloxan failed to protect the islets against the inhibitory effect of alloxan on either L-leucine or D-glucose oxidation.

We have recently reached a different conclusion and demonstrated that several noncarbohydrate nutrients are indeed able to provide a significant protection against the inhibitory effect of alloxan upon glucose-induced insulin release [32]. L-Glutamine, 2-ketoisocaproate, L-leucine and its nonmetabolized analog 2-aminobicyclo(2,2,1)heptane-2-carboxylic acid (BCH) all exerted a significant protective effect. The protective action of BCH was considerably increased by L-glutamine. In a series of prior publications, we have shown that the insulinotropic capacity of 2-ketoisocaproate [33], L-leucine [34] and BCH [35], used alone or in combination with L-glutamine [36–38], depends on the capacity of these nutrients to stimulate catabolic fluxes in the islet cells. For instance, the secretory response to BCH was attributed to activation of glutamate dehydrogenase and subsequent facilitation of 2-ketoglutarate oxidative catabolism [35]. The finding that these nutrients also provide protection against alloxan affords, in our opinion, a decisive support to the view that the sensitivity of islet cells towards alloxan is modulated by the generation rate of reducing equivalents and, hence, the ability to prevent the accumulation of oxygen-containing cytotoxic radicals.

More precisely, we have proposed [32] that nutrient secretagogues, by augmenting the generation rate of reducing equivalents (especially NADPH), allow for the rapid recirculation of glutathione in the following two reactions catalyzed by glutathione peroxidase and glutathione reductase, respectively:

$$H_2O_2 + 2GSH \rightarrow GSSG + 2H_2O$$
$$GSSG + 2NADPH \rightarrow 2GSH + 2NADP^+.$$

The latter view is compatible with the knowledge that islets exposed to alloxan (or to *tert*-butyl hydroperoxide) display an abnormally low GSH/GSSG ratio [4].

1.5. *Alteration of the protective action of* D-*glucose.* We have observed that the protective action of D-glucose, and that of 2-ketoisocaproate, against the alloxan-induced inhibition of glucose-stimulated insulin release are impaired when the exposure of islets to alloxan is performed in a medium containing, in addition to the nutrient under consideration, NH_4Cl or menadione [39]. The effects of the latter two agents on islet function appear to be mainly attributable to a lowering of the islet content of reduced pyridine nucleotides (NADH and NADPH) [40, 41]. Hence, these findings support the view that the capacity of nutrients to protect against alloxan tightly depends on their capacity to augment the generation rate of reducing equivalents.

1.6. *Validity of* in vitro *studies as a model for studying the diabetogenic action of alloxan.* In most of the experiments so far reviewed, the effect of alloxan was tested *in vitro* in pancreatic islets. It could be argued that the biochemical and functional effects of alloxan *in vitro* are not necessarily relevant to the cytotoxic action of the drug *in vivo*. This objection does not seem pertinent since microscope photometric analysis of trypan blue uptake by suspensions of pancreatic islet cells demonstrated that B-cells *in vitro* are killed by alloxan and, hence, represent a valid model for studying the diabetogenic action of the drug [42]. Thus, prior exposure to freshly dissolved alloxan (but not to alkali-treated alloxan), causes a time-dependent decrease of frequency of unstained nuclei in the islet cells incubated in the presence of trypan blue. Glucose (5 and 20 mM) affords a dose-related protection against this effect of alloxan.

2. Review of prior studies on the mode of action of alloxan

The new hypothesis defined in the first part of this report may account for or, at least, is compatible with a number of prior observations dealing with the mode of action of alloxan upon pancreatic islet cells.

2.1 *Effect of alloxan on the plasma membrane.* The most commonly held view concerning the mode of action of alloxan is that the drug damages the B-cell membrane by acting at or near a site involved in glucose-stimulated insulin release. Thus, in the most recent review on the cytotoxicity of alloxan, published in 1981, Cooperstein and Watkins [43] concluded "that the bulk of evidence supports the plasma membrane as the primary site of action of alloxan and that any intracellular effects are probably secondary and not critical to its cytotoxicity".

The latter opinion is based, in part, on the work conducted by Cooperstein and Watkins [19, 43] on toadfish islets and indicates that alloxan increases, in this system, the permeability of the cell membrane to mannitol, an effect counteracted by several sugars and by NADPH [44, 45]. In rat pancreatic islets, however, McDaniel and his colleagues failed to observe any effect of alloxan on the permeability of rat pancreatic islets to sucrose, D-mannitol or L-glucose [46]. These authors also failed to detect any measurable effect of alloxan on the carrier-mediated transport of D-glucose or 3-*O*-methylglucose into islet cells [46]. Likewise, Borg [31] failed to detect any significant effect of alloxan on leucine transport into mouse islets.

None of these negative findings detracts from the view that alloxan eventually leads to alteration in the properties of islet cell plasma membrane. For instance, Orci *et al.* [47] reported that freeze-fracture of rat islets treated *in vitro* with alloxan shows a decrease in the number of intramembranous particles of islet cell plasma membrane and that this decrease is prevented at a high concentration of glucose. Alloxan also impairs functional events located at the plasma membrane. For instance, alloxan inhibits ^{86}Rb pumping into islet cells, an effect antagonized by D-glucose or 3-*O*-methyl-D-glucose but not by L-glucose or D-mannoheptulose, which however suppresses the protective action of D-glucose [48]. According to Idahl *et al.* [48], the inhibition of ion pumping is not attributable to either a decrease in the islet cell ATP content or alteration of the ouabain-sensitive ATPase. However, in this study, the turnover of ATP was not measured and the ouabain-sensitive ATPase activity failed to reach statistical significance, whether in control or alloxan-treated islets. Another example of an effect of alloxan presumably operative at the level of the plasma membrane consists in a rapid but transient increase in the ^{86}Rb fractional outflow rate from perfused rat islets [49].

Although these observations document that alloxan indeed alters the structural organization and may affect the functional behaviour of islet cell plasma membrane, they do not demonstrate that the primary site of action of alloxan is located at the cell membrane. It is quite conceivable that the morphological and functional changes in the B-cell membrane are secondary to biochemical events initiated inside the islet cells. For instance, the increase in ^{86}Rb outflow provoked by alloxan and ascribed by Henquin *et al.* [49] to depolarization of the plasma membrane [50] could also be due to a change in the islet cell redox state, which, according to a current view, exerts a tight control on K^+ conductance in the islet cells [51–53].

2.2 *Alloxan and adenylate cyclase.* An alteration of adenylate cyclase has been considered as involved in the response to alloxan. Indeed, exposure of islets to alloxan suppresses the increase in their cyclic AMP content as normally evoked by glucose at high concentrations [54]. When the islets were exposed to alloxan in the presence of glucose (or 3-*O*-methylglucose), the capacity of glucose to increase the cyclic AMP content of the islets was restored [54]. The capacity of nutrient secretagogues (e.g. D-glucose) to augment the cyclic AMP content of islets is currently ascribed to activation of adenylate cyclase by endogenous calmodulin [55, 56] and, as such, would be secondary to the accumulation of Ca^{2+} in the islet cells, an accumulation which itself closely depends on the stimulation and integrity of catabolic events, including the induction of a more reduced state in (cytosolic) redox couples [57, 58]. Hence, the fact that alloxan prevents the glucose-induced increase in the islet cyclic AMP content is compatible with an alteration of the redox state and a decreased metabolic response (see Section 2.3) in alloxan-treated islets. Moreover, according to Borg [59], the basal and fluoride-stimulated activity of adenylate cyclase in islet homogenates is not different in control and alloxan-treated islets, respectively.

A somewhat more puzzling observation is that theophylline or caffeine, and possibly dibutyryl-cyclic AMP (but not cyclic AMP itself) cause protection against the inhibitory effect of alloxan on either glucose-stimulated insulin release [60, 61] or ^{86}Rb uptake [14]. The most likely explanation for this finding is that methylxanthines interfere with the uptake of alloxan by the islets. Indeed, the uptake of alloxan by rat islets incubated at 37° is decreased by 58% in the presence of 20 mM caffeine [22]. This inhibitory effect is not quite surprising since methylxanthines also inhibit the transport of D-glucose [62] and, as suggested by indirect evidence, that of *N*-acetylglucosamine [63] in the islet cells.

The modest protective effect of dibutyryl-cyclic AMP, which is better seen in the presence of a low concentration of glucose (5.6 mM) than in the absence of glucose [61], should be considered in the light of several observations which suggest that those agents which increase the islet content in cyclic AMP (e.g. theophylline) stimulate the catabolism of endogenous nutrients such as glutamine [64] and fatty acids [65], leading to a slight elevation of the ATP/ADP ratio [65], a modest increase in the output of lactic acid [66] and a minor decrease in the ^{86}Rb fractional outflow rate [67] in islets deprived of exogenous nutrient.

2.3. *Alteration of glucose metabolism.* It is well documented that prior exposure of pancreatic islets to alloxan impairs glucose metabolism in the islet cells [4, 68, 69]. Gunnarson and Hellerström [68] first reported that, in islets removed from mice killed

10 min after intravenous injection of alloxan, glucose (or mannose, but not pyruvate) is oxidized at a decreased rate both at low (3.3 mM) or high (16.7 mM) glucose concentration. In these islets, glucose also fails to increase O_2 uptake (despite apparently normal basal respiration) and does not stimulate insulin release. Likewise, in isolated mouse islets exposed *in vitro* to alloxan, the endogenous respiration rate is normal, but glucose slightly decreases (instead of increasing) O_2 uptake and is both poorly utilized (^3H_2O production from D-[5-^{3}H]glucose) and poorly oxidized [69]. Glucose utilization, however, appears more resistant than glucose oxidation to the inhibitory action of alloxan [69]. These findings were interpreted as reflecting an interaction of alloxan with intracellular sites involved in the oxidative metabolism of the B-cell. A primary action of alloxan upon mitochondria was even postulated by Boquist and Nelson, as recently reviewed [70].

The knowledge that glucose metabolism is impaired in alloxan-treated islets is obviously not sufficient to identify the primary site of action of alloxan in islet cells. However, this finding is of considerable interest in interpreting various other abnormalities of islet function encountered after exposure to alloxan.

2.4. *Alloxan and proinsulin biosynthesis.* Since the integrity of glucose metabolism is required for allowing the sugar to stimulate proinsulin biosynthesis [71], it is not surprising that alloxan inhibits glucose-stimulated proinsulin biosynthesis. This was demonstrated in islets removed from mice killed 10 min after injection of alloxan [72] or in rat islets exposed *in vitro* to alloxan [73]. It was recently reported that, within 5 min of exposure to alloxan, the drug causes DNA strand breaks, increases poly(ADP–ribose) synthetase activity and, within 20 min, lowers the islet NAD content, the three events being considered as causally related and possibly involved in the abnormality of proinsulin biosynthesis [74, 75]. These findings again indicate that alloxan exerts early cellular effects beyond the plasma membrane, in this case at the nuclear level.

2.5. *The protective action of* D-*glucose, its isomer, anomers and epimers.* Glucose is known to protect the B-cell against the cytotoxic action of alloxan. This was demonstrated using a number of indices of alloxan toxicity. For instance, glucose when injected *in vivo* together with or shortly before (but not shortly after) alloxan protects against the diabetogenic action of the drug [30, 76, 77]. Likewise, *in vitro*, when islets are exposed to glucose prior to or during the period of exposure to alloxan, the inhibitory effect of the drug is decreased or abolished, whatever the functional variable under consideration: glucose oxidation [69], glucose-induced cyclic AMP accumulation [54], glucose-stimulated proinsulin biosynthesis [73] or insulin release [78], trypan blue uptake [42] or ^{86}Rb pumping [48].

It is unlikely that the protective effect of glucose reflects a decreased uptake of alloxan by the islet cells. On the contrary, according to Weaver *et al.* [22], several carbohydrates (e.g. D-glucose, D-mannose and D-glyceraldehyde) known to protect, to variable extents, against alloxan may increase the uptake of [2-^{14}C]alloxan by rat pancreatic islets. In a study performed with purified single or coupled B-cells, we observed no effect of D-glucose on the uptake of [2-^{14}C]alloxan [24].

As already mentioned in this report (see Sections 1.4 and 1.5), we believe that the protective action of D-glucose can be accounted for by the effect of the sugar to increase the generation rate of reducing equivalents, especially NADPH, in the islet cells. Several observations in the literature are compatible with such a view.

First, it should be realized that the protective action of D-glucose does not correspond to a none-or-all mechanism. For instance, the diabetogenic action of alloxan *in vivo* is dose-related in the 10–80 mg/kg range [76]. At a fixed dose of alloxan (e.g. 40 mg/kg), the protective action of glucose, as judged from either the plasma glucose concentration reached 24 hr after injection of alloxan or the water intake, is dose-related in the 0.5–4.0 mmoles/kg range [76, 77]. Likewise, *in vitro*, the inhibition of glucose-stimulated insulin release observed after 5 min exposure to alloxan, is dose-related at alloxan concentrations ranging from 0.12 to 0.65 mM, maximal inhibition being reached at the latter or higher concentrations [79]. At a given concentration of alloxan, the protective effect of glucose increases as the glucose concentration present in the medium during exposure to alloxan is increased (e.g. in the 5.6–27.8 mM range) [78]. Although these quantitative aspects of glucose and alloxan interaction in the B-cell do not rule out other modes of action, they are compatible with the view that glucose (by increasing the generation rate of reducing equivalents) and alloxan (by increasing the utilization rate of such equivalents) act antagonistically and in a dose-related fashion upon the pancreatic B-cell.

Second, distinct hexoses differ in their capacity to protect against alloxan. As judged from the diabetogenic action of alloxan *in vivo*, D-glucose is more effective than D-mannose, whereas neither D-fructose nor D-galactose exert any detectable protective effect [30, 76]. Likewise, *in vitro*, D-glucose is more efficient than D-mannose, whereas D-fructose, D-galactose or L-glucose only cause minor to unsignificant protection against the alloxan-induced inhibition of glucose-stimulated insulin release [78]. These converging observations illustrate that the respective capacity of different hexoses to protect against alloxan is strictly superimposable to their capacity to stimulate glycolysis or insulin release in pancreatic islets [80]. Such a parallelism is compatible with the view that the protective action of hexoses depends on their capacity to be metabolized in the islet cells.

Third, both *in vivo* [76, 81] and *in vitro* [73, 82–84], the α-anomer of D-glucose provides greater protection against alloxan than the β-anomer. This anomeric specificity was documented *in vitro* using either glucose-stimulated insulin release [73, 82, 83], glucose oxidation [84] or glucose-stimulated proinsulin biosynthesis [73] as the alloxan-sensitive variable. These findings are in good agreement with the hypothesis presented in this report, since the α-anomer of D-glucose is more efficiently metabolized than the β-anomer, as indicated by higher rates of glycolysis and glucose oxidation in islets exposed to

α- as distinct from β-D-glucose [85]. This difference in metabolic rates is thought to be attributable to the α-stereospecificity of both phosphoglucose isomerase [85] and phosphoglucomutase [86]. We have recently observed that the α-anomer of D-mannose is also better able than its β-anomer to protect against alloxan [87]. This situation again coincides with higher rates of glycolysis and hexose oxidation in the islets exposed to α- as distinct from β-D-mannose [87].

Last, it was repeatedly documented that mannoheptulose, which itself exerts no protective action against alloxan [48, 76, 77], suppresses the protective action of glucose, or that of mannose [77], on the alloxan-induced alteration of islet function, whether *in vitro* [48, 78] or *in vivo* [76, 77]. Since mannoheptulose inhibits glucose phosphorylation in the islets [88], the effect of the heptose is compatible with the view that glucose needs to be metabolized in the islet cells in order to exert its protective action against alloxan.

In conclusion, there is a strict parellelism between the capacity of several sugars to stimulate (or inhibit) catabolic fluxes or insulin release in pancreatic islets and their capacity to protect against the deleterious effect of alloxan, again suggesting a key role for the metabolism of these sugars in the expression of their protective capacity.

2.6. *The protective action of* 3-O-*methyl*-D-*glucose.* In most previous studies, the commonly held view concerning the protective action of D-glucose was that alloxan acts specifically on a receptor or transport system involved in the process of glucose-stimulated insulin release. This view was apparently supported by the finding that the nonmetabolized analogue of D-glucose, 3-*O*-methyl-D-glucose mimics the effect of D-glucose in preventing both the *in vitro* alloxan-induced inhibition of glucose metabolism [49], ^{86}Rb uptake [48] and glucose-stimulated cyclic AMP accumulation [54] or insulin release [49, 78] and the *in vivo* diabetogenic action of alloxan [76, 77]. The similarity between the effects of 3-*O*-methyl-D-glucose and D-glucose, respectively, is indeed suggestive of a competition between alloxan and 3-*O*-methyl-D-glucose for a hypothetical gluco-receptor system presumed to exist in islet tissue, even more so as the α-anomer of 3-*O*-methylglucose was reported to protect more efficiently than the β-anomer against the diabetogenic action of alloxan [89].

The observation by Weaver *et al.* [22] that 3-*O*-methyl-D-glucose inhibits [2-^{14}C]alloxan uptake by pancreatic islets provides, in our opinion, a more simple and realistic explanation for the protective action of the glucose analogue, if our contention is correct that the accumulation of alloxan within the B-cell is required for expression of its diabetogenic potency.

The latter explanation may appear incompatible with the finding that mannoheptulose antagonizes the protective action of 3-*O*-methyl-D-glucose against alloxan [76, 77]. However, it should be first noted that mannoheptulose is less efficient in this respect than in abolishing the protective action of metabolized hexoses [76, 77]. Second, the inhibitory effect of mannoheptulose on the protective action of 3-*O*-methyl-D-glucose having been so far tested, to our knowledge, solely *in vivo*, it may reflect interference of the heptose with the protective action of endogenous circulating glucose. It was indeed shown that, when animals are injected simultaneously with alloxan and mannoheptulose, the amount of alloxan required to provoke a given degree of hyperglycemia is decreased in a dose-related fashion as the amount of mannoheptulose injected is increased [76]. Thus, mannoheptulose seems to sensitize the B-cell to alloxan cytotoxicity, presumably by removing the protection of endogenous circulating glucose.

The α-stereospecificity of the protective action of 3-*O*-methyl-D-glucose, if confirmed, raises the idea that the transport system for 3-*O*-methyl-D-glucose may display anomeric specificity, whereas such does not appear to be the case for the D-glucose transport system [82]. Incidentally, other agents than 3-*O*-methyl-D-glucose may protect against alloxan by preventing its uptake by the islet cells. Such is apparently the case for cytochalasin B [22]. Cytochalasin D, which unlike cytochalasin B does not inhibit glucose transport into islet cells, fails to protect against the inhibitory action of alloxan on glucose-stimulated insulin release [90]. A qualified analogy between alloxan and glucose transport across the B-cell plasma membrane should be considered in the light of the structural similarity between alloxan and D-glucose, as judged by stereospecific criteria [91].

2.7. *Specificity of the inhibitory action of alloxan towards distinct secretagogues.* The fact that exposure of pancreatic islets to alloxan impairs their capacity to release insulin in response to stimulation with D-glucose could theoretically result from either alteration of the insulin-releasing machinery at a distal site in the sequence of metabolic, ionic and motile events eventually leading to extrusion of secretory granules or to a specific defect of the earliest step in such a sequence, namely at the level of D-glucose recognition as a stimulus for insulin release. It is relevant, therefore, to consider whether the decreased secretory activity of alloxan-treated islets is restricted to the process of glucose-stimulated insulin release.

Prior exposure of the islets to alloxan inhibits insulin release evoked by a variety of nutrient secretagogues such as D-glucose, D-glyceraldehyde, 2-ketoisocaproate and L-leucine [31, 49]. Since the insulinotropic capacity of these nutrients invariably coincides with an increase in the islet cell NADH/NAD^+ and/or NADPH/$NADP^+$ ratio (see Section 1.4), it is conceivable that the inhibitory action of alloxan on the secretory response to these nutrient secretagogues reflects alteration of their metabolism. For instance, it was recently observed that leucine oxidation is indeed impaired in islets pretreated with alloxan [31]. Moreover, alloxan, by altering the redox state of the islets, may prevent the stimulation of insulin release which is otherwise expected from stimulation of islet catabolism by nutrient secretagogues.

According to Tomita [92, 93], prior exposure of the islets to alloxan (at low but not at high glucose concentration) inhibits leucine-stimulated insulin output but fails to affect the secretory response to

arginine, provided that the latter response is measured at a low concentration of D-glucose (3.3 mM). This finding is in good agreement with our model for the cytotoxic action of alloxan. Indeed, in contrast to leucine-induced insulin release, the process of arginine-induced insulin release is obviously not dependent on stimulation of islet metabolism, arginine failing to cause either a phosphate flush [94] or a decrease in ^{86}Rb outflow from perifused islets [95].

Alloxan may also cause a delayed if not immediate impairment of tolbutamide-induced insulin release [49, 78]. This finding does not detract from our hypothesis concerning the mode of action of alloxan since the effect of tolbutamide upon insulin release, after exposure to alloxan, was tested in the presence of D-glucose (3.0 or 5.6 mM) and since alloxan inhibits glucose metabolism even when the sugar is used at such low concentrations [49, 69] and, by doing so, is likely to modulate the magnitude of the secretory response to hypoglycemic sulfonylureas. Indeed, the effect of alloxan upon the profile of the secretory response to tolbutamide, as documented by Tomita *et al.* [78], is quite similar to that seen when the effect of tolbutamide in control islets (not exposed to alloxan) is tested at decreasing concentrations of extracellular glucose in the 5.0–1.7 mM range [96].

Taken as a whole, these findings suggest that, shortly after exposure to alloxan, the secretory response of the islets is more severely affected when nutrient secretagogues, as distinct from nonmetabolized insulinotropic agents, are used in order to stimulate insulin release.

2.8. *Stimulation of insulin release by alloxan.* According to Weaver *et al.* [91], alloxan in the absence of glucose causes a transient release of insulin peaking during the third minute of exposure to the drug and rapidly declining thereafter. This alloxan-induced transient release of insulin is abolished in the absence of extracellular Ca^{2+} and is associated with stimulation of ^{45}Ca uptake by the islets [91]. In the presence of 3-*O*-methyl-D-glucose, alloxan fails to provoke such a transient release of insulin. The latter finding could suggest that the transient stimulation of insulin release by alloxan is dependent, like the cytotoxic action of the drug, on the accumulation of alloxan inside the islet cells. If so, the explanation for such a transient stimulation of insulin release, which Henquin *et al.* [49] failed to observe, could well be different from the postulated action of alloxan on the same receptor as that claimed to mediate the secretory response to D-glucose [91].

3. *Conclusions*

The present review, which is far from being exhaustive, indicates that the results of prior studies on the mode of action of alloxan are compatible with the view that the selective toxicity of the drug to the pancreatic B-cell is essentially attributable to the coincidence of a rapid accumulation of the drug in B-cells and their exquisite sensitivity to peroxides, as suggested by our recent experimental findings [4, 24, 25, 32, 39, 87].

Beyond its relevance to the mode of action of alloxan, the experimental data reviewed in the present report also suggest that, at variance with a view repeatedly expressed [97, 98], studies on the mode of action of alloxan are unlikely to contribute to the identification of a hypothetical membrane glucose receptor site. In my opinion, such a glucoreceptor should be considered, in the absence of factual evidence [99, 100], as a myth which unfortunately pervaded for two decades the conception of glucose recognition by the pancreatic B-cell.

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