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THIOL-GROUP REACTIVITY, HYDROPHILICITY AND STABILITY OF ALLOXAN, ITS REDUCTION PRODUCTS AND ITS *N*-METHYL DERIVATIVES AND A COMPARISON WITH NINHYDRIN

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Abstract—The diabetogenic agent, alloxan, is a hydrophilic and chemically unstable compound. The logarithm of the octanol/water partition coefficient of alloxan was found to be −1.86; its half-life at pH 7.4 and 37° in phosphate buffer was 1.5 min. The partition coefficients and half-lives of the alloxan reduction products, alloxantin and dialuric acid, were very similar to those of the parent compound; *N*-methylalloxan and *N,N'*-dimethylalloxan were less hydrophilic but more unstable. Ninhydrin was found also to be hydrophilic although this compound, in contrast to alloxan and its derivatives, was quite stable in aqueous solution. Alloxan and its *N*-methyl derivatives were reduced by thiols and in the presence of glutathione and cysteine, rapid redox cycling occurred, with formation of 'active oxygen' species; no such reaction was observed, however, with ninhydrin. Comparatively slow redox cycling was recorded with alloxan derivatives and dithiothreitol although rapid cycling occurred with ninhydrin and this dithiol. Such differences may explain why ninhydrin does not share with alloxan a selective toxic effect upon the pancreatic B-cell.

Alloxan and ninhydrin are *vic*-triketones and have many chemical properties in common [1–5]. However, while both agents inhibit pancreatic B-cell glucokinase [6] and through this mechanism cause inhibition of glucose-induced insulin secretion [7, 8] only alloxan is known to induce a selective necrosis of the pancreatic B-cell [3,8,9 for review] and hence causes diabetes. Identification of differences in the physical and chemical properties of alloxan and related compounds compared with those of ninhydrin could therefore be important in understanding the selective pancreatic B-cell toxicity of alloxan.

Alloxan is an unstable chemical, yet it reaches the pancreatic B-cells in sufficient quantity to exert its toxic effects; more unstable chemicals may, however, decompose before reaching the pancreatic B-cells and thus cause no harm. It has been suggested [10] that alloxan is selectively taken up by the pancreatic B-cells, so that its relative aqueous/lipid solubility may be important. The damage to B-cells is believed to be initiated by 'active oxygen' species [11] which are formed through redox cycling between alloxan and thiols [12]; reactivity with thiols and rapid redox cycling may therefore be crucial to its toxic action.

In the present study, we have determined the partition coefficient, stability, thiol group reactivity and rate of redox cycling of alloxan, its reduction products alloxantin and dialuric acid, and its *N*-methyl and *N,N'*-dimethyl derivatives and compared these properties with those of ninhydrin.

MATERIALS AND METHODS

Alloxan monohydrate, L-cysteine, L-dithiothreitol (DTT), diethylenetriaminepentaacetic acid (DTPA) and superoxide dismutase (from bovine erythrocytes) were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.) and alloxantin from Serva (Heidelberg, Germany). 1-Octanol was from Aldrich (Steinheim, Germany). Ninhydrin, glutathione (GSH) and all other reagents of analytical grade were from Merck (Darmstadt, Germany). Catalase (from bovine liver) was from Boehringer (Mannheim, Germany). Dialuric acid was synthesized by the method of Biltz and Damm [13], *N*-methylalloxan and *N,N'*-dimethylalloxan according to the method of Biltz [14]. 1,3-Dihydro-1,3-dioxo-*spiro*[2*H*-indene-2,2'-thiazolidine]-4'-carboxylic acid, the condensation product of ninhydrin and cysteine, was prepared as described by Prota and Ponsiglione [15]. 2-Hydroxyindane-1,3-dione was obtained by reduction of ninhydrin with stannous chloride by the method of Ruhemann [16].

Spectral changes during the interaction between alloxan, *N*-methylalloxan, *N,N'*-dimethylalloxan, dialuric acid, or alloxantin and GSH, cysteine or DTT were monitored on a Perkin-Elmer 551S UV/VIS spectrophotometer. Reactions were conducted at 25°, 30° or 37° in 50 mM potassium phosphate buffer at pH 7.4 or in Hepes-buffered (10 mM) Krebs–Ringer bicarbonate buffer [17]. Recordings of the spectral changes were started immediately after adding the test materials to the buffer solution containing the thiols. Buffer solutions were supplemented with 50 μM DTPA where indicated.

Oxygen uptake by alloxan and derivatives and by ninhydrin in the presence of thiols was measured using a Yellow Springs Instruments Model 53 oxygen monitor. The buffer employed was 50 mM potassium

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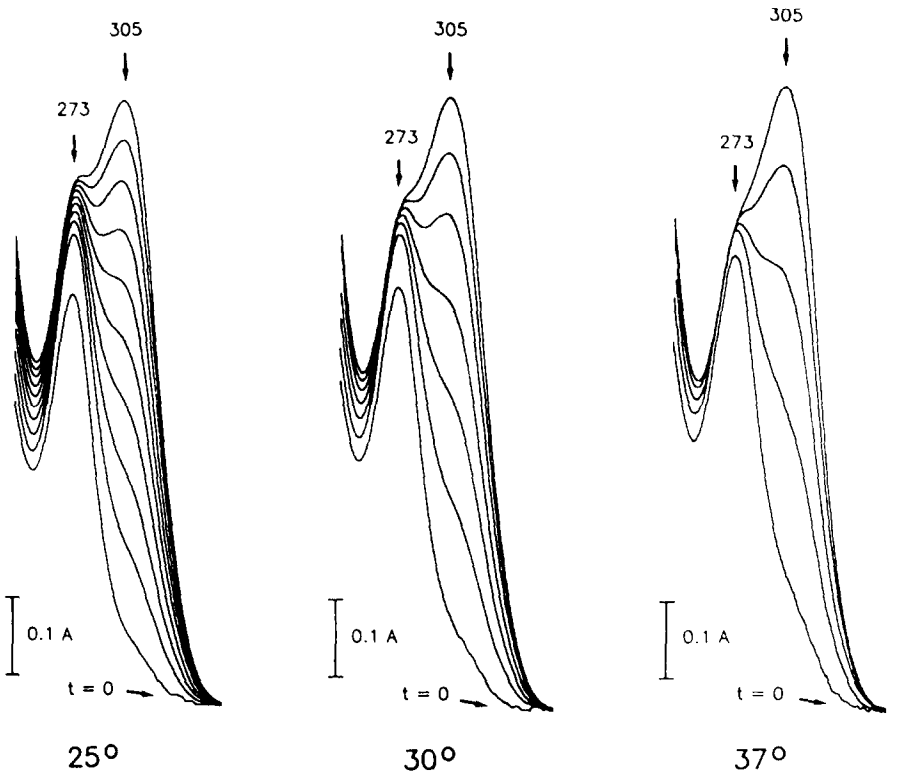

Fig. 1. Spectral changes during the interaction of alloxan (50 μM) and GSH (1 mM) in 50 mM phosphate buffer supplemented with 50 μM DTPA at pH 7.4 and 25°, 30° or 37°. Spectra between 370 and 240 nm were recorded at 2.5 min intervals starting immediately after adding alloxan to GSH. Absorption maxima at 273 and 305 nm are indicated.

phosphate at pH 7.4 and the temperature was maintained at 25°. Recordings of oxygen uptake were started immediately after addition of the test materials to the buffered thiol solutions; in experiments in which the effects of superoxide dismutase and catalase were investigated these were added before the test substances.

Concentrations of dialuric acid and of alloxan, *N*-methylalloxan, *N,N'*-dimethylalloxan and alloxantin after prior conversion to dialuric acid or the respective methyl derivatives by addition of 1 mM DTT were determined through measurement of absorbance at 273 nm using ε_{273} 16,000 M^{-1} cm^{-1} [18]. Concentrations of ninhydrin were determined by measurement of absorbance at 232 nm [19, 20].

Partition coefficients for alloxan, *N*-methylalloxan, *N,N'*-dimethylalloxan, dialuric acid and alloxantin were measured in the octanol/water system [21]. The water phase in which the test agents were dissolved in a final concentration of 1 mM was either a 10 mM HCl solution or a 50 mM potassium phosphate buffer with a pH of 7.4. Two millilitre portions of each phase were shaken on a mechanical shaker for 2 hr at room temperature (22°). Separation of the phases was achieved through centrifugation for 30 min at 5000 rpm. Concentrations of all test agents were determined in both phases against appropriate standards and blank values. Partition coefficients of alloxan and ninhydrin in a hexane/water system were determined identically. Partition coefficients were calculated as described [22] according to the formula

$$P = \frac{C_{org}}{C_{aq}}.$$

Stabilities of alloxan, *N*-methylalloxan, *N,N'*-dimethylalloxan, dialuric acid and alloxantin were determined after incubation for various time periods in 50 mM phosphate buffer with or without 50 μM DTPA at 25° or 37° and at pH 7.4. For half-life determinations at 25°, test agents were incubated for various times as appropriate between 0 and 10 min and at 37° between 0 and 5 min. Incubation was terminated through addition of 1 mM DTT. Liver homogenate and liver cytosol for control experiments on stability of alloxan in the presence of biological material were obtained as described previously [6].

RESULTS

Spectral changes during the interaction between thiols and alloxan derivatives and ninhydrin

Addition of alloxan (50 μM) to a solution of GSH (1 mM) at pH 7.4 showed immediately a spectrum with an absorption peak at 273 nm. Gradually thereafter, a shoulder, which slowly changed into a

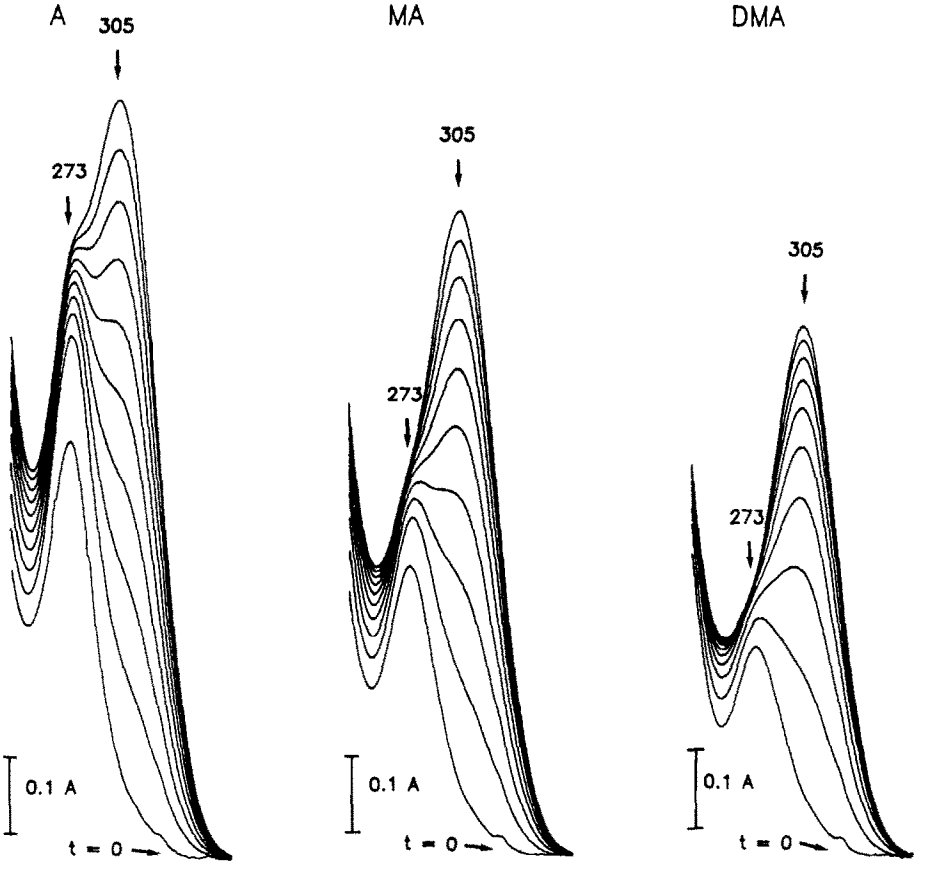

Fig. 2. Spectral changes during the interaction of alloxan (A) (50 μM), *N*-methylalloxan (MA) (50 μM), or *N,N'*-dimethylalloxan (DMA) (50 μM) and GSH (1 mM) in 50 mM phosphate buffer supplemented with 50 μM DTPA at pH 7.4 and 25°. Spectra between 370 and 240 nm were recorded at 2.5 min intervals starting immediately after adding alloxan to GSH. Absorption maxima at 273 and 305 nm are indicated.

peak, appeared at 305 nm (Fig. 1). The rate of formation of the latter peak at 37° was double that at 25° (Fig. 1). Addition of cysteine (1 mM) to a mixture of alloxan (50 μM) plus glutathione (1 mM) suppressed the generation of the absorption peak at 305 nm (curve not shown). Completely identical spectra to those shown in Fig. 1 for alloxan were also recorded with dialuric acid (50 μM), alloxantin (25 μM) and alloxan (25 μM) plus dialuric acid (25 μM) in the presence of 1 mM GSH (curves not shown). The rate of formation of the absorption peak at 305 nm, and the rate of decrease in absorption at 273 nm, was faster with the N-methylated alloxan derivatives than with the parent compound. The rates decreased in the order *N,N'*-dimethylalloxan > *N*-methylalloxan > alloxan (Fig. 2).

Addition of alloxan, dialuric acid, *N*-methylalloxan, *N,N'*-dimethylalloxan (all at 50 μM) or alloxantin (25 μM) and alloxan (25 μM) plus dialuric acid (25 μM) to solutions of cysteine (1 mM) or DTT (1 mM) gave only the absorption peak at 273 nm (curves not shown).

Ninhydrin showed a strong absorption band in the ultraviolet at 232 nm (the exact value was 231.5 nm),

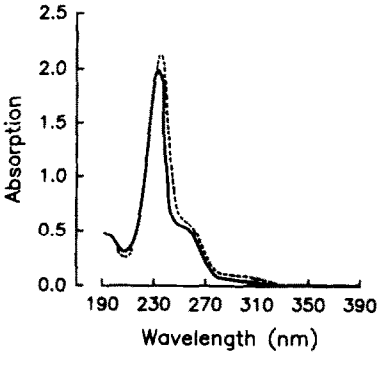

Fig. 3. Spectra of ninhydrin (——) and of 1,3-dihydro-1,3-dioxo-*spiro*[2*H*-indene-2,2'-thiazolidine]-4'-carboxylic acid (– – –).

with a shoulder at around 253 nm (Fig. 3). For ninhydrin ε_{232} was determined and found to be 39,500 M^{-1} cm^{-1}. This value is consistent with that reported by MacFayden [19] but somewhat higher

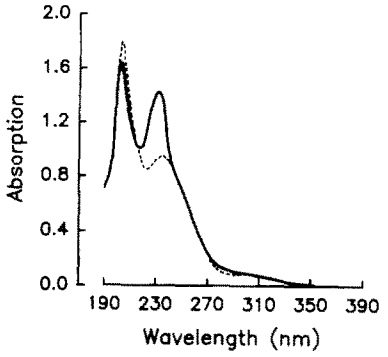

Fig. 4. Spectral changes during the interaction of ninhydrin (50 μM) and cysteine (1 mM) in 50 mM phosphate buffer supplemented with 50 μM DTPA at pH 7.4 and 25°. Spectra between 190 and 390 nm were recorded at 2.5 min intervals starting immediately after adding ninhydrin to cysteine. The first (———) and fifth (– – –) scans are indicated.

than that of Chaplin [20]. The spectrum of ninhydrin (50 μM) remained unchanged in the presence of GSH (1 mM). In contrast, in the presence of cysteine both the shoulder and peak decreased in intensity, the latter shifting to a slightly higher wavelength. At the same time, absorption at 200–205 nm increased (Fig. 4). This spectrum was quite different to that of 1,3-dihydro-1,3-dioxo-*spiro* [2*H*-indene-2,2′-thiazolidine]-4-carboxylic acid (Fig. 3).

The spectrum of the product of the reaction between ninhydrin and DTT was strongly dependent upon pH. In 2 N HCl, the solution was colourless, with maximum absorption at 229 nm, $\varepsilon = 37{,}900\ M^{-1}\ cm^{-1}$. At pH 7.4, a strong peak in the ultraviolet at 259 nm ($\varepsilon = 27{,}750$) was accompanied by a weaker absorption in the visible (maximum absorption 482 nm, $\varepsilon = 1690$) and the solution was pink in colour. In 2 N NaOH, the solution turned blue, with the peak in the visible shifting to 497 nm ($\varepsilon = 1570$). A peak at 352 nm ($\varepsilon = 3300$) was also recorded in alkaline solution. These spectra are very similar to those recorded for the un-ionized form of 2-hydroxyindane-1,3-dione and its mono- and di-anions, respectively [23]. The identity of the reaction product as the dione was confirmed by comparison of the spectra with those of an authentic sample.

Oxygen uptake during the interaction between thiols and alloxan derivatives and ninhydrin

Oxygen was consumed by solutions of alloxan and GSH (Table 1). Oxygen utilization was strongly inhibited by superoxide dismutase and, to a lesser extent, by catalase. The rate of oxygen uptake was higher with the N-methylated derivatives, decreasing in the order *N,N′*-dimethylalloxan > *N*-methylalloxan > alloxan. Superoxide dismutase had less effect upon oxygen uptake by the N-methylated compounds than that by the parent compound, although the effect of catalase was similar with all three compounds.

Comparable results were obtained with alloxan and its derivatives in the presence of cysteine. With DTT, however, very little oxygen uptake was recorded either with alloxan or the N-methylated compounds (Table 1).

Results with ninhydrin were the exact converse of those with alloxan. Little or no oxygen uptake was recorded with this substance in the presence of GSH or cysteine but very rapid oxygen consumption occurred with DTT (Table 1). In the latter case, oxygen uptake was decreased by 43% by catalase and almost completely abolished by superoxide dismutase.

Partition coefficients of alloxan derivatives and ninhydrin

Partition coefficients for alloxan, dialuric acid, alloxantin, *N*-methylalloxan and *N,N′*-dimethylalloxan were determined in an octanol/water system. The log values of the partition coefficients at pH 2.0 were −1.86 for alloxan, −1.85 for dialuric acid and −1.82 for alloxantin. Methylation decreased the partition coefficients, the log values being −1.04 for *N*-methylalloxan and −0.57 for *N,N′*-dimethylalloxan. Octanol/water partition coefficients could not be determined with ninhydrin because it may react with octanol, as the triketone reacts easily with primary alcohols in aqueous solution to give the corresponding hemiketals [24]. In contrast, alloxan forms hemiketals only under strictly anhydrous conditions [14] and therefore cannot react in this way under the conditions of the partition coefficient experiments. The log values of the partition coefficients in a hexane/water system were for alloxan −1.65 at pH 2.0 and for ninhydrin −1.19 at pH 2.0 and −1.33 at pH 7.4. All values presented are means of 8–10 determinations. Due to the instability of alloxan, dialuric acid, alloxantin, *N*-methylalloxan and *N,N*-dimethylalloxan the partition coefficients for these agents had to be determined at a pH value of 2. For ninhydrin, partition coefficients could be determined both at pH 2 and pH 7.4 and were found to be independent of the pH value.

Stabilities of alloxan derivatives and ninhydrin

The half-lives of alloxan (50 μM), dialuric acid (50 μM), alloxantin (25 μM), or alloxan (25 μM) plus dialuric acid (25 μM) in phosphate buffer without addition of DTPA at pH 7.4 did not differ significantly. At 25°, the half-lives ranged between 5.0 and 5.5 min, and at 37° between 1.4 and 1.6 min (Table 2). In control experiments it was confirmed that the half-life of alloxan was not affected by addition of liver homogenate or liver cytosol. However, methylation of alloxan significantly ($P < 0.01$) decreased the half-life in the order alloxan < *N*-methylalloxan < *N,N′*-dimethylalloxan (Table 2). Decomposition of alloxan was found to be 20% faster in Krebs–Ringer bicarbonate than in phosphate buffer, both at 25° and 37° in the presence and absence of DTPA.

Incorporation of DTPA in the buffer significantly increased the half-life of dialuric acid although no effect upon the decomposition of the other compounds was observed (Table 2).

A solution of ninhydrin (50 μM) in phosphate buffer at pH 7.4 remained unchanged for more than 3 hr at 37°.

Table 1. Oxygen uptake by alloxan, *N*-methylalloxan, *N,N'*-dimethylalloxan and ninhydrin in the presence of thiols

Compound	Thiol	Initial rate of oxygen uptake (μmol/L/min)		
		Control	+ SOD	+ CAT
Alloxan	GSH	8.5 ± 0.4	0.7 ± 0.2 (−92%)	4.0 ± 0.3 (−53%)
N-Methylalloxan	GSH	13.7 ± 0.3	5.5 ± 0.1 (−60%)	6.8 ± 0.1 (−50%)
N,N'-Dimethylalloxan	GSH	16.5 ± 0.4	11.6 ± 0.8 (−30%)	8.0 ± 0.8 (−52%)
Ninhydrin	GSH	0.7 ± 0.2	ND	ND
Alloxan	Cysteine	7.2 ± 0.3	3.3 ± 0.3 (−54%)	3.9 ± 0.2 (−45%)
N-Methylalloxan	Cysteine	13.8 ± 0.6	6.2 ± 0.6 (−55%)	7.4 ± 0.5 (−46%)
N,N'-Dimethylalloxan	Cysteine	18.3 ± 0.8	13.0 ± 0.5 (−29%)	9.6 ± 0.1 (−48%)
Ninhydrin	Cysteine	0	ND	ND
Alloxan	DTT	1.9 ± 0.1	0.0 (−100%)	0.8 ± 0.1 (−58%)
N-Methylalloxan	DTT	2.1 ± 0.1	0.4 ± 0.1 (−81%)	1.1 ± 0.2 (−48%)
N,N'-Dimethylalloxan	DTT	2.0 ± 0.1	0.3 ± 0.1 (−85%)	1.0 ± 0.1 (−50%)
Ninhydrin	DTT	125.9 ± 2.9	1.7 ± 0.2 (−99%)	71.5 ± 4.2 (−43%)

The concentration of thiol was 1 mM in all cases; the concentration of all test agents was 50 μM. Superoxide dismutase (SOD) and catalase (CAT) were added as indicated, both at a concentration of 10 μg/mL. Results shown are the means ± SE of at least four determinations.
ND, not determined.

Table 2. Stability of alloxan, *N*-methylalloxan, *N,N'*-dimethylalloxan, dialuric acid and alloxantin in 50 mM phosphate buffer (pH 7.4)

Agent	Half-life (min)			
	25°	25° + DTPA (50 μM)	37°	37° + DTPA (50 μM)
Alloxan (50 μM)	5.0 ± 0.4	5.1 ± 0.3	1.5 ± 0.0	1.6 ± 0.0
Dialuric acid (50 μM)	5.5 ± 0.1	7.0 ± 0.1	1.6 ± 0.0	2.2 ± 0.1
Alloxantin (25 μM)	5.2 ± 0.1	5.7 ± 0.1	1.4 ± 0.1	1.5 ± 0.1
Alloxan (25 μM) + dialuric acid (25 μM)	5.3 ± 0.2	5.7 ± 0.2	1.5 ± 0.0	1.7 ± 0.0
N-Methylalloxan (50 μM)	3.0 ± 0.1	3.3 ± 0.1	0.9 ± 0.0	0.9 ± 0.1
N,N'-Dimethylalloxan (50 μM)	2.0 ± 0.1	2.0 ± 0.1	0.4 ± 0.0	0.4 ± 0.0

Results shown are the means ± SE of four determinations. The half-life values for dialuric acid were significantly lower ($P < 0.01$) in the absence than in the presence of DTPA.

DISCUSSION

Alloxan is reduced by cysteine to dialuric acid [25, 26], the latter compound having a characteristic absorption band at 273 nm [18]. The similar spectra seen with the N-methylated derivatives in the presence of cysteine indicate that these are likewise reduced to the corresponding dialuric acids. These compounds are also formed by reduction with DTT, again as evidenced by their UV spectra.

Glutathione similarly reduces alloxan to dialuric acid but a secondary reaction leads to formation of a conjugate, "Compound 305", of unknown structure [25]. The observation that the addition of cysteine to a mixture of alloxan plus glutathione suppressed the generation of the absorption peak at 305 nm supports the contention that glutathione forms an adduct with alloxan but not with dialuric acid [27]. In the presence of GSH, the 273 nm peak of the dialuric acid gradually decreases to be replaced by the 305 nm peak of the conjugate [12]. As shown in the present study, *N*-methylalloxan and *N,N'*-dimethylalloxan behave similarly, although the glutathione conjugates of these compounds are formed more rapidly than that of the parent compound, possibly reflecting a higher rate of redox cycling (see below).

Ninhydrin forms a reduction product, 2-hydroxyindane-1,3-dione, which is analogous to dialuric acid [16]. This compound was formed by reaction of ninhydrin with DTT but, in contrast to alloxan, ninhydrin was not reduced by cysteine or GSH. No reaction was recorded between the latter thiol and ninhydrin and while reaction between cysteine and ninhydrin was observed, the spectrum of the product bore no resemblance to that of 2-hydroxyindane-1,3-dione. Furthermore, the product of this reaction was not the condensation product described by Prota and Ponsiglione [15] and its structure remains unknown.

Dialuric acid readily auto-oxidizes at neutral pH, and in the presence of GSH or cysteine a redox cycle is established with consumption of oxygen and formation of the oxidized thiol. Oxygen uptake was found to be inhibited by superoxide dismutase and catalase, indicating the formation of superoxide radical and hydrogen peroxide in the reaction [12].

The results of the present experiments on oxygen uptake by alloxan in the presence of cysteine or GSH were very similar to those previously reported. The rates of oxygen uptake by *N*-methylalloxan and *N*,*N*′-dimethylalloxan in the presence of these thiols were higher than that by alloxan, suggesting that the auto-oxidation rates of N-methylated dialuric acids are higher than that of dialuric acid itself. The smaller inhibitory effect of superoxide dismutase on oxygen uptake by the N-methylated alloxans indicates that superoxide-driven radical chain reactions, as described for dialuric acid [12] are less important in the auto-oxidation of the N-methylated derivatives. With all the alloxan derivatives, the rate of oxygen uptake was decreased by approximately 50% by catalase, consistent with hydrogen peroxide destruction by the reaction:

$$2H_2O_2 \rightarrow 2H_2O + O_2.$$

Dialuric acid does not form a conjugate with GSH, so formation of "Compound 305" in the presence of an excess of thiol is dependent upon regeneration of alloxan through redox cycling, a proportion of the alloxan being conjugated at each cycle. The rate of production of conjugate would therefore increase with the rate of redox cycling, in accord with the rapid formation of "Compound 305" observed with the N-methylated alloxans.

Alloxan and its methyl derivatives were readily reduced by DTT. The very slow redox cycling observed in the presence of this thiol indicates that auto-oxidation of the dialuric acids is inhibited; DTT has been shown to inhibit effectively other auto-oxidation reactions [28].

Like dialuric acid, 2-hydroxyindane-1,3-dione readily auto-oxidizes [29, 30] and redox cycling is to be expected with agents capable of reducing ninhydrin. Of the thiols tested, only DTT reduced ninhydrin to the dione and only this compound promoted significant redox cycling with ninhydrin. The rate of oxygen uptake by ninhydrin was very high, indicating rapid oxidation of 2-hydroxyindane-1,3-dione; this is consistent with earlier reports of the lability of this compound [16]. The marked inhibition of oxygen uptake by superoxide dismutase suggests that the auto-oxidation is strongly dependent upon chain reactions involving the superoxide radical; the inhibitory effect of catalase is indicative of hydrogen peroxide formation during the reaction.

The logarithm of the partition coefficient for alloxan in an octanol/water system approached −2, indicating that this is a very hydrophilic compound [22]. Alloxantin and dialuric acid behaved like alloxan. Partition coefficients increased with increasing N-methylation, although *N*-methylalloxan and *N*,*N*′-dimethylalloxan are still hydrophilic. The logarithm of the partition coefficient for alloxan in a hexane/water system was around −1.7 also supporting the conclusion that alloxan is very hydrophilic. The logarithm of the partition coefficient for ninhydrin in a hexane/water system being in the range of −1.2 to −1.3, while somewhat lower than that of alloxan, indicates that ninhydrin is also very hydrophilic.

The half-life of alloxan at pH 7.4 and 37° was 1.5 min, consistent with the results of Patterson *et al.* [25] and Miwa and Okuda [31]. The half-life of alloxan in Krebs–Ringer bicarbonate buffer was found to be 20% shorter than in phosphate buffer thus confirming the observation by Miwa and Okuda [31]. *N*,*N*′-Dimethylalloxan is reported to be a very unstable compound [27, 32] and this was confirmed in the present experiments, its half-life being only 0.4 min. The *N*-methyl derivative was of intermediate stability, with a half-life of 0.9 min. The half-lives of all the alloxan derivatives were more than three times higher at 25° than at 37°. The half-life of dialuric acid was significantly increased in the presence of the chelating agent, DTPA, possibly reflecting inhibition of metal-catalysed auto-oxidation [33]. The behaviour of alloxantin was identical to that of alloxan and dialuric acid and to an equimolar mixture of these compounds, consistent with the rapid dissociation of alloxantin at physiological pH [34]. The behaviour of ninhydrin was again unlike that of the alloxan derivatives, this compound being perfectly stable for hours at 37° and pH 7.4.

The similarities and differences between alloxan and ninhydrin identified in the present study permit some comment on possible reasons for the disparate toxicological effects of these substances. Clearly, the absence of a diabetogenic effect of ninhydrin does not reflect rapid decomposition in solution; this compound was much more stable than alloxan, which, despite its short half-life, is able to reach the pancreatic B-cells in toxic amount. Similarly, the hydrophilicity of both ninhydrin and alloxan suggests that gross differences in lipid/aqueous partitioning *in vivo* are not responsible.

In contrast, the observed differences between alloxan and ninhydrin with regard to reactivity toward monothiols may be important. It has been suggested [12] that redox cycling in the presence of cysteine or glutathione may be involved in the specific toxic effect of alloxan on the pancreatic B-cells. The failure of ninhydrin to undergo redox cycling in the presence of these thiols may therefore be significant. The dithiol DTT, unlike the monothiols, reduced both alloxan and ninhydrin. This observation is of interest in view of the fact that both these compounds inhibit glucokinase by a process which involves reaction with two adjacent SH groups in the sugar-binding site of the enzyme [35].

The similar chemical properties of alloxan, alloxantin and dialuric acid are consistent with the redox interconvertibility of these substances and the observation that all three compounds are diabetogenic [36, 37]. The N-methylated alloxans likewise underwent redox cycling with monothiols. The rate of this reaction was higher than with the parent compound, and, other factors being equal, it would thus be expected that the N-methylated derivatives would be of higher diabetogenic activity.

N-Methylalloxan like alloxan itself is diabetogenic with comparable potency, while *N,N'*-dimethylalloxan does not cause diabetes in animals [36–38]. From the present *in vitro* studies, *N,N'*-dimethylalloxan would have been expected to be the most toxic member of the group. Thus, its inactivity *in vivo* may reflect decomposition of this very unstable compound.

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