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Inhibition by Alloxan of Mitochondrial Anion Transport

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According to our Pi-pH hypothesis for the development of alloxan diabetes, alloxan passes the plasma membrane of the B-cells and acts intracellularly (4). The primary intracellular action of alloxan is believed to be inhibition of a sulfhydryl dependent carrier for inorganic phosphate (Pi), which leads to deranged cellular function, deficient energy production, and finally necrosis. The primary action of alloxan upon mitochondria is supported by our qualitative (1) and quantitative (16) ultrastructural studies on the endocrine pancreas of alloxan-treated mice, and by investigation of the in vitro effects of alloxan on glucose metabolism in mouse B-cells (8).

Alloxan is very unstable and exists at physiological pH mainly in the anion form (19). One of the most well-known properties of alloxan is its SH-reactivity. Thus, it is able to oxidize cysteine to cystine and reduced glutathione (GSH) to oxidized glutathione (GSSG), associated with a reduction of alloxan to dialurate which is non-diabetogenic unless it is reoxidized to alloxan.

Lazarow (14) observed that compounds containing SH-groups protect against alloxan diabetes, and proposed that the diabetogenecity of alloxan is due to inactivation of SH-groups. Free SH-groups are necessary for insulin synthesis, and are normally supplied in the B-cells by cysteine and glutathione or other substrates (17). According to Lazarow (15), one reason for the selectivity of alloxan for the B-cells may be a lower concentration of SH compounds in these cells than in other cells. In support of Lazarow's hypothesis, Falkmer (11) reported a reduction of the GSH content in isolated islet tissue and erythrocytes of Cottus scorpius about 40 min after the administration of alloxan. Havu (13) who studied different monothiol (iodoacetic acid and allyl isothiocyanate) and dithiol ($CoCl_2$, $CdCl_2$ and $NaAsO_2$) reagents, found that all these compounds were taken up by the islet parenchyma of Cottus scorpius and were diabetogenic. However, using a modification of the ortho-phthalaldehyde method, Havu (13) observed that the GSH concentration of the B-cell rich islet parenchyma of Cottus scorpius

was higher than that of the adjacent acinar pancreatic tissue. In later works from Lazarow's group (10) it has been suggested that alloxan does not enter the parenchymal cells of toad fish islets, but acts on SH-groups in the plasma membrane of the B-cells (9).

Interestingly with respect to alloxan diabetes and the SH-reactivity of alloxan, most compounds inhibiting Pi transport in mitochondria are SH-group reagents, which are able to react with the SH-groups of the Pi carrier. Examples of such compounds are the maleimide derivatives N-ethyl-maleimide (NEM) and N-(N-acetyl-4-sulfamoylphenyl) maleimide, and the organic mercurials mersalyl, p-chloromercuriphenylsulfonate and p-hydroxymercuribenzoate (PMB). In preceding studies we have observed that PMB treatment caused a decreased serum insulin concentration in fed and starved mice, and altered mitochondrial volume and pyroantimonate precipitation in the B-cells, transient hyperglycemia, and protection against alloxan toxicity in fed mice (2,3).

Different SH-reagents may have different effects upon mitochondrial Pi transport, and in addition to action on the translocation of Pi, some SH-reagents affect, at least under some conditions, also the mitochondrial carriers for dicarboxylate, tricarboxylate, ATP/ADP, glutamate and pyruvate.

The permeability properties of the SH-reagents with regard to the inner mitochondrial membrane play a prominent role for the reactivity with the SH-groups of the carriers. Some SH-reagents (penetrants) enter the matrix space of the mitochondria, whereas others do not penetrate the inner membrane (non-penetrants). Consequently, the mitochondrial effects of the former category of SH-reagents differ from those of the latter category. The reactivity of the SH-reagents with the ionic carrier is also influenced by the pH and temperature, by the concentration of the reagents, and by the time of incubation.

The organic mercurial compounds react in a reversible manner with simple thiols and with exposed SH-groups of cysteine residues within proteins. They do not readily penetrate the inner mitochondrial membrane, and do not inhibit ATP/ADP and glutamate transport, but inhibit the translocation of pyruvate and proline, and at higher concentrations than those used to inhibit the Pi carrier they also affect di- and tricarboxylate transport (12).

The basis of inhibition of the Pi carrier by the maleimide derivatives is an irreversible reaction of the SH-groups of the carrier with the activated double bond within the maleimide molecule. NEM penetrates the mitochondrial inner membrane. It also inhibits the glutamate carrier, and under some conditions, the ATP/ADP carrier. However, in contrast to the organic mercurials, it does not inhibit Pi transport through the dicarboxylate carrier (12).

In order to test the Pi-pH hypothesis (4) we have studied the action of

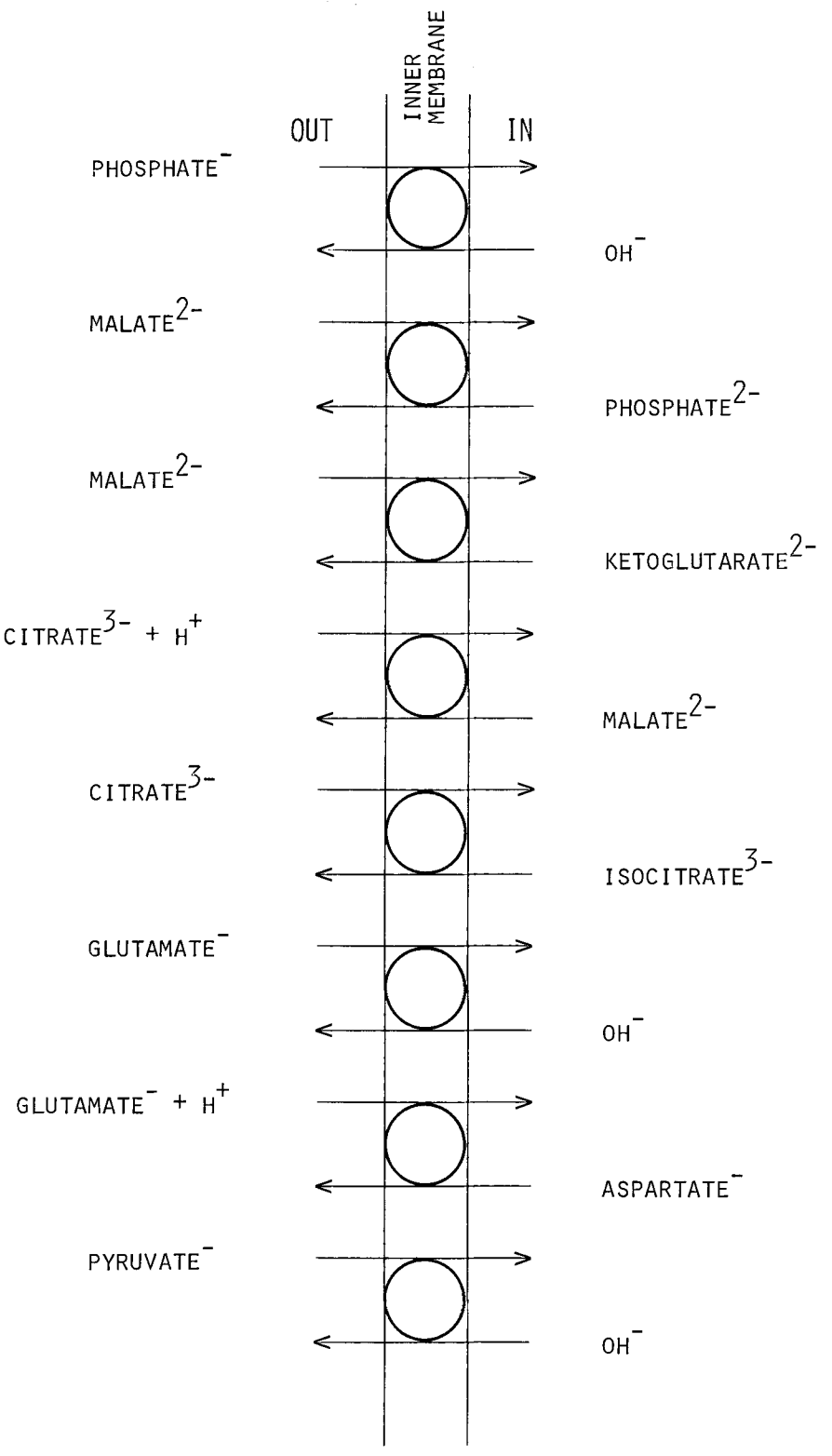
alloxan on Pi transport in isolated mouse (C57BL) mitochondria using the technique of passive osmotic swelling in iso-osmotic ammonium phosphate solutions (5). We have studied mitochondria from liver (5,6), kidney and heart (7), and are now making attempts to investigate those from the endocrine pancreas. In addition, study has been carried out on liver mitochondria of the effect of alloxan on the carriers for dicarboxylate, tricarboxylate, glutamate and pyruvate.

The carriers to which attention has been paid are schematically represented in the Figure, which is based upon information in the literature about anionic transport in isolated mitochondria from rat liver. It should be noted that the mitochondrial carriers of the mouse probably are similar to, although not necessarily identical to those of the rat. The effects of alloxan on mitochondrial anionic transport have been compared with those of the well-known SH-reacting inhibitors mersalyl, NEM and PMB, as well as with those of N-butylmalonate and 1, 2, 3-benzene tricarboxylate which inhibits dicarboxylate and tricarboxylate transport, respectively.

The studies carried out so far indicate that alloxan causes a retardation of Pi transport in mitochondria from liver and kidney, whereas Pi transport in heart mitochondria possibly is stimulated by alloxan (5,6,7). These findings are consistent with the finding that alloxan in sufficient concentration damages not only B-cells, but also some other kinds of cells, including liver and kidney (tubules). In contrast, to the best of our knowledge there is no report that alloxan also may destroy myocardial cells, which may agree with the lack of inhibition by alloxan of Pi transport in isolated heart mitochondria.

Our studies of liver mitochondria have shown that the action on Pi transport is dependent on the concentration of alloxan, the time of exposure to alloxan, and the pH (5,7). Thus, using 10 mM alloxan maximum inhibition is found at 2 min preincubation, and with 2.5 mM alloxan maximum inhibition occurs at 10 min preincubation. The inhibition is stronger at or below pH 7.4, than above pH 7.4.

Alloxan has also been found to decrease the transport of dicarboxylate ($malate^{2-}/phosphate^{2-}$), tricarboxylate ($citrate^{3-}+H^{+}/malate^{2-}$), pyruvate ($pyruvate^{-}/OH^{-}$) and glutamate ($glutamate^{-}/OH^{-}$). Whereas there is only one possibility for alloxan action on the Pi ($phosphate^{-}/OH^{-}$) carrier, there are, as seen from the Figure, different possibilities for action of alloxan on dicarboxylate, tricarboxylate and glutamate transport, and there are data in the literature suggesting also a carrier-free uptake of pyruvate. So far, we do not exactly know the nature of the inhibition by alloxan of the latter transport mechanisms. However, within the brackets above those carriers have been specified which our data indicate to be the "targets" for alloxan action. As to the Figure it should also be noted that the entrance of one


INNER MEMBRANE
OUT
IN
PHOSPHATE⁻
OH⁻
MALATE²⁻
PHOSPHATE²⁻
MALATE²⁻
KETOGLUTARATE²⁻
CITRATE³⁻ + H⁺
MALATE²⁻
CITRATE³⁻
ISOCITRATE³⁻
GLUTAMATE⁻
OH⁻
GLUTAMATE⁻ + H⁺
ASPARTATE⁻
PYRUVATE⁻
OH⁻

molecule of glutamate is accompanied by the entrance of one H^+, whereas the exit of aspartate occurs without H^+ movement (18).

The effects of alloxan on the carriers differ in some respects from those of the other inhibitors tested (5,7). This is not unexpected with regard to the discussion above and to the fact that alloxan is a potent SH-reagent causing B-cell necrosis and diabetes. Nothing is so far known about the permeability of alloxan through the inner mitochondrial membrane.

Summing up, our data support the Pi-pH hypothesis. It is obvious that inhibition of the mitochondrial carriers for Pi, dicarboxylate, tricarboxylate, glutamate and pyruvate leads to profound alterations in cellular function. As to cellular survival, the most essential effect of alloxan on mitochondrial ionic transport is the inhibition of the Pi carrier. Pi transport is associated also with the translocation of dicarboxylate, tricarboxylate and calcium ions. As to pyruvate it should be noted that its dehydrogenase is located in the mitochondrial matrix, and that the availability of this dehydrogenase is of great importance in the overall carbohydrate metabolism (18). Glutamate transport is, as far as the glutamate$^-$/aspartate$^-$ exchange is concerned, of importance in the translocation of reducing equivalents, since NADH does not penetrate the inner mitochondrial membrane.

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