HYPERGLYCEMIA FOLLOWING \textit{\textit{p}}-HYDROXYMERCURIBENZOATE ADMINISTRATION TO MICE

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Previous studies of the pancreatic islets of alloxan-treated mice disclosed that the earliest structurally detectable lesions were localized in B-cell mitochondria. Since this might be due to a direct or indirect action of alloxan upon these organelles, possibly of pathogenetic significance for the B-cytotoxic action of the drug, a search was made for compounds, with a known inhibitory action on mitochondrial function \textit{in vitro}, which affect the blood glucose concentration and/or islet morphology in mice \textit{in vivo}.

Among the compounds thus tested, \textit{p}-hydroxymercuribenzoate (PMB) was found to induce transient hyperglycemia of short duration and some structural alterations in B-cells. In addition, alterations in serum inorganic phosphate (Pi) concentration and acid-base balance were found in mice treated with PMB. Differences were found between fed and starved mice as to the response to PMB. These actions of PMB are presented in this work. The closely related \textit{p}-chloromercuribenzoic acid and chloromercuribenzenzene-\textit{p}-sulphonic acid have been used in investigations of insulin release from isolated mouse islets and PMB has been found not to increase the \textit{in vitro} permeability of toadfish islets.

MATERIALS AND METHODS

\textit{Animals and treatment} - Non-diabetic male adult C57BL-KsJ-+/+ mice from a local stock were used. They were kept under standard laboratory conditions at a constant temperature of 22 \textdegree{}C. The animals had free access to water and a standard

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laboratory ration containing 1.0% calcium and 0.75% phosphorus. The vitamin D₃ content was approximately 700 IU per 100 g diet. Both fed and starved mice were used. Starved mice were deprived of food for 24 h before experimentation and were then allowed to eat again after blood sampling at the 4-h observation time.

Based upon the kind of treatment given, the following experimental groups were differentiated: (1) saline (controls)-fed; (2) saline (controls)-starved; (3) PMB-fed; (4) PMB-starved.

PMB dissolved in saline was given in groups (3) and (4) at a dose of 5 × 10⁻³ mol/kg b.w. PMB was supplied by Sigma Chemical Co., St. Louis/Mo., U.S.A. All injections were given i.p. under light ether anaesthesia.

**Blood glucose determination** - Blood was obtained by cutting the tip of the tail, and blood glucose was assayed by the glucose oxidase method, using Glox* (AB KABI, Stockholm, Sweden) at the following predetermined intervals: before (0-time), and 1, 2 and 4 h, and 1, 2, 3, 4 and 7 days after the injections.

**Determination of serum Pi, pH, pCO₂, pO₂ and standard bicarbonate** - The serum concentration of Pi, pH, pCO₂, pO₂ and standard bicarbonate was determined 10 min after treatment (corresponding to the interval used between pretreatment sampling and alloxan administration in previous studies) in groups (1) - (4). Serum Pi was also assayed 2 h following treatment. Blood for these analyses was obtained by heart puncture at 22 °C. Purely arterial blood could not be obtained in sufficient amount by this method. With the aim of avoiding venous admixture, only blood with a minimum pO₂ of 6.0 kPa was used for further analyses. Blood was sampled in every group about 08 °h.

Serum Pi was determined with the aid of a Technicon Autoanalyzer (Technicon Instr. Co., Ardsley/N.Y., U.S.A.). pH, pCO₂, pO₂ and standard bicarbonate were assayed with standard technique using a Radiometer ABL-I (Radiometer, Copenhagen, Denmark). This determination was carried out immediately after blood sampling.

The Wilcoxon test was used for statistical analyses of data.

**Light and electron microscopy** - Two animals from each group were killed 10 min, 2 h, and 1 day following PMB administration. Immediately after killing, specimens were taken from the pancreas for light and electron microscopic study. Specimens for light microscopy were fixed in Bouin’s fluid and stained with standard techniques for islet cells.

Some specimens for electron microscopy were fixed in 2.5% glutaraldehyde in 0.34 M Veronal acetate buffer, pH 7.4, followed by postfixation in osmium tetroxide in the same buffer. Embedding was carried out in Epon 812 and thick sections stained with toluidine blue were used for identification of the islets. The thin sections were stained with uranyl acetate and lead citrate.

Other specimens for electron microscopy were fixed for 2 h in 3% glutaraldehyde and 2% potassium pyroantimonate adjusted to pH 0.4 with 0.1 M acetic acid, followed by fixation for 1 h in 1% osmium tetroxide and 2% potassium pyroantimonate. The sections were left either unstained or stained with uranyl acetate and lead citrate. All electron microscopic sections were examined with a Siemens Elmiskop 1 A or 101.