Induction of Alloxan Diabetes in Obese-Hyperglycemic Mice (Genotype obob)[1]

In 1962 SOLOMON and MAYER reported that administration of alloxan to obese-hyperglycemic mice failed to cause visible damage to the pancreatic B-cells; the only recorded effects of this treatment was a decreased blood sugar level. So far it has not been conclusively shown that obese-hyperglycemic mice are sensitive to the diabetogenic action of alloxan. By contrast, another B-cytotoxic substance, streptozotocin, has been found to cause serious injury to the B-cells of these animals[2,3]. In the present study we wish to report that alloxan also is efficient as a diabetogenic agent in obese-hyperglycemic mice and that its action is mediated by a destruction of the pancreatic B-cells.

Methods. Altogether 16 male obese-hyperglycemic mice, about 6 months old, were used. The animals were starved over-night before the experiment. Alloxan (Eastman Organic Chemicals, Rochester, New York, USA) was given as a 5% (w/v) solution in physiological saline through a tail vein in a dose of 150 mg/kg body weight. Care was taken not to dissolve the alloxan until just before the injection. Control animals received a corresponding volume of saline. Immediately before and at intervals after the injections, blood samples were obtained by puncture of the orbital vein plexus with a thin-walled Pasteur pipette. Serum glucose determinations were performed as described by HJELM and DE VERDIER[4], and serum immuno-reactive insulin levels as according to HALES and RANDLE[5] using crystalline mouse insulin as a standard. All surviving animals were killed by decapitation 48 h after injections. The presence of sugar and ketone bodies in the urine was tested with the aid of Clinistix® and Ketostix®. Histological examination of the pancreas was performed after fixation of the excised gland in Zenker-formal solution and staining of 4 μm or 7 μm thick sections in chrome-hematoxylin ponceau fuchsin[6] or aldehyde-fuchsin ponceau fuchsin[7].

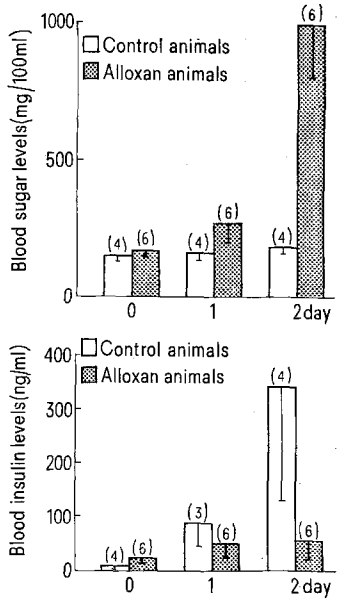

Fig. 1. Levels of glucose and immunoreactive insulin (means ± standard error of the means) in blood from controls and alloxan injected animals. Blood samples on day 0 were taken before the injections. The number of animals in each group is given in parentheses.

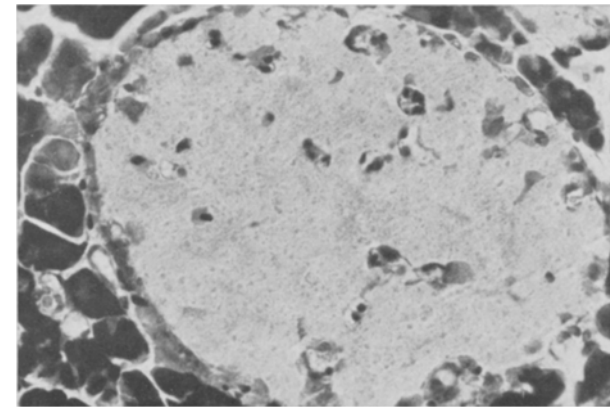

Fig. 2. Chrome-hematoxylin ponceau fuchsin stained section from an alloxan-treated animal. There is a total necrosis of the B-cells in the islet. × 500.

Results. Out of 12 alloxanized animals, 6 died within 48 h after commencement of the experiment. Glucosuria was present within 24 h in all the treated animals and ketonuria occurred in about half of them. As can be seen in Figure 1, the blood sugar level was strongly elevated on the second day after treatment, whereas the insulin level was lower than in the control animals. In the latter animals the blood insulin level rapidly increased when they were allowed free access to food (WESTMAN[8]). Examination of the pancreatic histology showed a severe injury to the islets of Langerhans in the alloxan treated animals. There was an almost total necrosis of the cells in many islets with a thin rim of apparently surviving cells at the periphery (Figure 2). In some of the islets only focal areas were necrotic; the remaining cells having a normal appearance.

Discussion. The present data show conclusively that obese-hyperglycemic mice are not resistant to the diabetogenic action of alloxan and that the mild, non-ketotic diabetes associated with this syndrome[9] can be changed by alloxan treatment into a severe diabetic state. The fulminant disease occurred despite the presence of a considerable circulating insulin level, which further demonstrates the marked insulin resistance of these animals. However, as compared to the controls, the mean insulin level of the alloxan diabetic mice was low at the time of death, indicating that the latter animals had a diminished insulinogenic reserve. This conformed with the finding of degenerative and necrotic islets in the pancreas corresponding to the classical signs of alloxan action previously described in both normal mice and a

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number of other species (FRERICHS and CREUTZFELDT[10]). Amelioration of the diabetic state or of the insulin resistance, like that reported by SOLOMON and MAYER[11] and MAHLER and SZABO[12], was not observed.

The obvious discrepances between our results and those of SOLOMON and MAYER[11] may best be explained by the differences between the two investigations with regard to the route of drug administration and the alloxan doses employed. The former authors gave alloxan intraperitoneally as compared to the intravenous injections given in the present study. Also, the doses used by SOLOMON and MAYER[11] were based on lean body mass, which means that the amount of alloxan given in their study was about half that presently used. Against this background it is concluded that alloxan is diabetogenic in obese-hyperglycemic mice and that it acts by a mechanism similar to that in other species. Since B-cells constitute more than 90% of the islets of obese-hyperglycemic mice, isolated intact islets of such animals should be an excellent preparation for further studies of the mechanism for the selective destruction of B-cells by alloxan (HELLERSTRÖM and GUNNARSSON[13]).

Zusammenfassung. Nachweis, dass fettsüchtige, hyperglykämische Mäuse nach Alloxan-Injektion einen erhöhten Blutzuckerspiegel und herabgesetzte Insulinkonzentrationen im Vergleich mit den unbehandelten Kontrolltieren zeigen. B-Zellen der Langerhansschen Inseln zeigen nach Alloxan nekrotische Veränderungen.

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Chromosomal Accumulation of ^{3}H-Estradiol in Dividing Ovarial Granulosa Cells and Ovarial Squash Preparations

The biological mechanism of action of many steroid hormones has been attributed to an interaction with genetic information in target cell nuclei[1–4]. Steroid hormones can, via such a mechanism, exert an important influence on the transport from the nucleus and accumulation in the cytoplasm of RNA and ribonucleoprotein particles[5].

In target cells most steroids are combined with a specific protein receptor in the cytoplasm and transported into the cell nuclei[6,7]. In the nuclei such steroids can be found associated with chromatin[8,9]. A binding to non-chromosomal proteins may, however, also exist[10].

The present investigation has been performed to see if it is possible by autoradiographical methods to demonstrate an accumulation of a steroid in the chromosomes of a dividing cell of a target tissue. Estradiol was chosen as the steroid and the granulosa cell layer of the ovary was chosen as the target tissue. Estradiol has been shown to be strongly accumulated in the nuclei of the granulosa cell layer of the follicle, which supports the idea that it is a target for estradiol[11,12]. This tissue was chosen because the number of mitoses is easily stimulated and because the cells are large and therefore suitable to study under the microscope.

In 2 female albino NMRI-mice, weighing 20 g each, the follicle growth was stimulated by a s.c. injection of 50 IU pregnant mare's serum (PMS, obtained from AB Ferring, Sweden); 24 h later this treatment was repeated. The mice were at this time also injected s.c. with 1 mCi ^{3}H-estradiol (2,4,6,7-^{3}H-estradiol-17β, spec. act. 110 Ci/m*M*, New England Nuclear; dose per animal: 2,5 μg) dissolved in 0.1 ml dimethyl sulfoxide (DMSO). 1 h later, the mice were injected i.v. with 2.5 mg/kg colchicine, (Sigma Chemical Co., USA), which arrests the mitotic division in metaphase. The mice were killed 3 h after this injection. One ovary in each mouse was taken and cut into 5 pieces and fixed in Levans fixative (60 ml glacial acetic acid, 10 ml 1 *N* hydrochloric acid and 30 ml distilled water) for 20 min. The pieces were then stained with a few drops of orcein (2 g orcein, 60 ml glacial acetic acid and 40 ml distilled water). Thereafter each piece was placed between 2 slides and squashed. The squash-preparations were then subjected to conventional stripping film autoradiography[13] by which stripping film (Kodak AR 10) is stretched on water and floated on to the preparations. Pieces of ovaries from control mice, not injected with ^{3}H-estradiol, were also taken and treated as described above. The other 2 ovaries from the ^{3}H-estradiol-injected mice were cut into small pieces and put in a cold isotonic solution of 1% osmiumtetroxide buffered to pH 7.4 and fixed for 2 h. After dehydration in ethanol the tissue pieces were embedded in Epon. Sections, 1 μm thick, were cut on an LKB Ultrotome and put on glass-slides. Autoradiography was then performed as described for the squash-preparations. The autoradiograms were exposed at −15 °C, the exposure-time being 3–6 months. Since steroid hormones are known to be diffusible during the various steps of the histologic and autoradiographic processing[14], control experiments were undertaken to estimate the loss of radioactivity in the

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