Groups did not differ significantly in the outer diameter of blastocysts and in the diameter of embryonic disc. There was great variability within group and within the same litter in the size of blastocysts and their survival. Implantation and normal gestation in the rabbit could be maintained, after ovariectomy and salpingectomy, by injection of exogenous progesterone.

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Effect of Alloxan on Malic Dehydrogenase Activity of Microdissected Mammalian Pancreatic Islets.* (28746)

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Dunn *et al*(1) first reported that alloxan injection caused a selective necrosis of the pancreatic beta cells, with consequent induction of diabetes. It had been suggested that alloxan directly or indirectly inactivates a critical sulphydryl enzyme(s) within the beta cell(2). More recent studies, however, indicate that alloxan may kill the beta cells by altering the permeability of the cell membrane. Tracer doses of alloxan apparently do not enter the beta cell(3). However, concentrations of alloxan equivalent to the diabetogenic dose, increased the *in vitro* permeability of fish islet tissue to mannitol, a substance which is normally restricted to the extra cellular space(4).

Hitherto, it had not been possible to study directly the changes in the mammalian islet tissue following alloxan administration. The techniques developed by Lowry and coworkers(5) permit microdissecting, weighing and enzymatic analysis of submicrogram quantities of tissue. Using these techniques, we have undertaken a systematic study of the changes in the enzyme activity in the rat islet tissue following administration of diabetogenic agents.

We are reporting here the changes observed in malic deyhdrogenase (MDH) activity of the microdissected islets following alloxan administration.

Materials and methods. The male rats used were litter mates, 2 to 3 months of age, bred in this laboratory. All animals were fasted overnight, and blood samples were taken from the tail vein for glucose determination(6). Experimental animals were given a single intravenous injection of alloxan (40 mg/kg body weight(7)), whereas controls were injected with distilled water. One animal from each litter was always used as a control. The litter mates were decapitated (by guillotine) at various intervals (24, 48, 72 and 168 hours) after alloxan injection. In another series of experiments, litter mates were sacrificed at 12 and 18 hours after alloxan. The pancreas was removed, and quickly frozen in liquid nitrogen (−196°C). As a rule, the blood sugar level at time of sacrifice was over 300 mg/100 ml. The frozen tissue was sectioned at 20 μ in a

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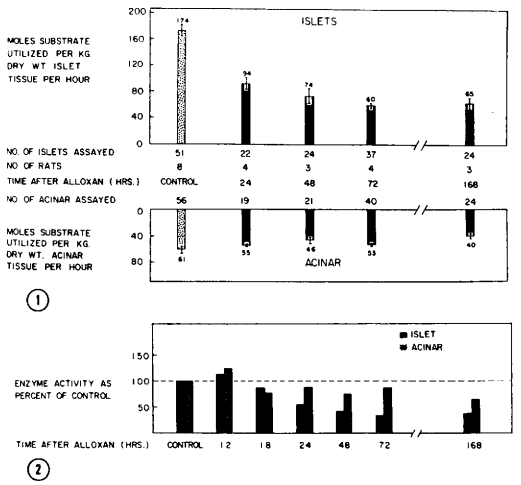

FIG. 1. Effect of alloxan on malic dehydrogenase (MDH) activity of microdissected islet and acinar tissue. Number of top of each bar represents mean values; vertical line indicates standard error of mean. The decrease in malic dehydrogenase activity in the islets after alloxan administration was significant at all periods ($p = <0.001$).

FIG. 2. Relative changes in malic dehydrogenase (MDH) activity of islet and acinar tissue after alloxan injection. Determinations at 0, 24, 48, 72 and 168 hours were carried out in a single experiment using litter mates. Determinations at 12 and 18 hours were carried out in a second series of litter mates and values were compared to the respective controls.

cryostat at –20°C. The sections were placed in aluminum holders, dried under vacuum at –40°C, and the frozen dried sections were stored in glass jars *in vacuo* at –20°C. On the day the islets were to be dissected, the jars containing the sections were brought to room temperature under vacuum before removing the tissue sections from the vacuum chamber. The islets were microdissected free from acinar tissue under a binocular microscope at a magnification of 60 ×. The microdissected tissue was weighed on a quartz fiber balance(8). Weight range of the tissue was 0.01 to 0.1 μg. A mechanical loading device(9) was used to place the tissue in the small tubes used in the subsequent analysis.

The MDH activity of the tissue was determined, with some modifications, by the fluorometric method described by Lowry and co-workers(9,10).

Results and discussion. The malic dehydrogenase activity of the normal rat islets was about 3 times greater than that of the acinar tissue (Fig. 1). Lacy(11) also observed a similar ratio of MDH activity in the "beta" and acinar cells of rabbit pancreas. Our absolute values for MDH activity in the normal rat islet and acinar tissue are slightly lower than the corresponding values in the rabbit as reported by Lacy (loc. cit.) and in rats as reported by Kissane *et al*(12).

The MDH content of the islet was decreased following alloxan administration. Although the enzyme activity was not changed significantly at 12 hours, a slight decrease ($p = <0.05$) was observed between 12 and 18 hours (Fig. 2); whereas at 24 hours the MDH activity had decreased to about 54% of the normal value (from 174 to 94 moles per kg dry weight of tissue per hour, $p = <0.001$). The enzyme activity of the islets declined progressively and at 72 hours after alloxan, it was about 35% of the normal (60 moles per kg dry weight of tissue per hour). In the pancreas of the rats treated with alloxan 7 days previously, very few islets could be identified; enzyme activity in the microdissected islets was about 37% of the normal.

By contrast, the MDH activity of the acinar tissue was not affected at 24 hours after alloxan (90% of normal controls) or at 72 hours (87% of controls, Fig. 1). However, at 7 days when the rats had lost considerable weight (compared with controls), MDH activity of the acinar tissue was decreased to about 66% of that of the normals ($p = <0.01$).

Lazarus and co-workers(13), using a histochemical method, reported that adenosine triphosphatase (ATPase) activity in the beta cell cytoplasm is decreased within 15 minutes after alloxan injection. Since the ATPase is an -SH dependent enzyme, these workers have suggested that the loss of activity could be due to a reduction in the enzyme -SH groups, or alternatively, that alloxan reacts with this enzyme to form a product that ultimately leads to cell destruction. Although MDH is likewise an -SH dependent enzyme (14), our studies indicate that the islet MDH activity did not show any significant change within the first 12 hours after alloxan

administration. Since the half-life of alloxan at 37°C and pH of 7.4 is approximately one minute(15), if the alloxan inactivated MDH by combining with the essential -SH groups, the activity of this enzyme should have decreased in the period immediately after alloxan administration.

In contrast to the changes in MDH activity in the rat islet tissue following alloxan administration, the insulin content was unchanged at 24 hours. By 48 hours, however, the insulin content was less than 5% of the normal values(16). The observed decrease in MDH activity in the islet tissue may be a late manifestation of the death of the beta cell brought about by altered membrane permeability(4).

Our findings that MDH activity in the islet decreases after selective destruction of the beta cells suggested that the concentration of MDH in the beta cells of the islets is greater than that in the alpha cells.

Summary. Malic dehydrogenase activity was determined in the microdissected islets obtained from normal and alloxanized rats. There was no change in the enzyme activity up to 12 hours after alloxan administration. At 24 hours after alloxan, enzyme activity was decreased to about half the value found in the normal; at 72 hours it was decreased to one-third. By contrast, enzyme activity in the acinar tissue was not changed at these time periods, but was significantly decreased at 168 hours after alloxan administration. The malic dehydrogenase activity in the islet is about 3 times greater than that in the acinar pancreas; enzyme activity of the beta cell appears to be considerably greater than that in the alpha cell.

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Search for Virus in Human Malignancies. 3. Sex Segregation and Neoplasia in ICR/Ha Mice.* (28747)

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A previous report from this laboratory(1) described findings in a controlled study to recover viruses in ICR/Ha mice from a variety of human malignant tissues. These studies revealed a high incidence of spontaneous malignancies, especially mammary

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