STUDIES ON THE MECHANISM OF ALLOXAN HYPOGLYCEMIA

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It is well known that when alloxan is injected intravenously into rabbits, dogs, and monkeys an initial hyperglycemia is followed by a transient hypoglycemia, leading to death of the animals from hypoglycemic convulsions. If the hypoglycemia is prevented by repeated intravenous injections of glucose, the animals survive and permanent hyperglycemia and diabetes develop.

The cause of the transient hypoglycemia has been differently explained by different workers. While Goldner and Gomori (1), Ridout et al. (2), Kennedy and Lukens (3), and Banerjee (4) consider that the alloxan hypoglycemia is pancreatic in origin owing to the release of preformed insulin from the necrosed islets, Houssay et al. (5), Wrenshall (6), and Carrasco-Formiguera (7) are of opinion that the cause of this hypoglycemia is extrapancreatic. In experiments with dogs Houssay et al. (5) observed hypoglycemia when alloxan was injected half an hour after the animals were pancreatectomized. Wrenshall (6) removed the pancreas of dogs 9 hours after the animals had received injections of a diabetogenic dose of alloxan and determined its insulin content. No significant difference in the insulin content as compared to normal controls was observed, which indicated that no measurable quantity of insulin was released into the circulation. Wrenshall further observed that when dogs were made resistant to insulin by injections of anterior pituitary extract a diabetogenic dose of alloxan significantly lowered the blood sugar level to that of normal controls. Carrasco-Formiguera (7) clamped the pancreaticoduodenal blood vessels in three dogs before the diabetogenic dose of alloxan was injected. Although all of the animals failed to develop diabetes, all showed pronounced hypoglycemia. In experiments with rabbits Carrasco-Formiguera observed that when rabbits received injections of epinephrine 1 hour prior to the injection of a diabetogenic dose of alloxan most of the animals failed to develop diabetes, but all of them had pronounced hypoglycemia. Goldner and Gomori (8) could not confirm the claims of Houssay and others (5–7). They observed no hypoglycemia after injection of diabetogenic doses of alloxan in dogs in which the pancreas was removed 30 minutes or several days or weeks prior to the injection. When the blood
vessels supplying the pancreas were clamped prior to the injection of alloxan, the dogs which failed to develop diabetes did not show any hypoglycemia. In their experiments with rabbits Goldner and Gomori, however, confirmed the observations of Banerjee (4) who showed that partially

pancreatectomized rabbits failed to develop severe hypoglycemia, did not have hypoglycemic convulsions, and all of them survived without injection of glucose and developed diabetes. These findings indicated that the hypoglycemia depended upon the amount of insulin available in the pancreas.

**Fig. 1.** Blood sugar curves of rabbits after injection of alloxan (200 mg. per kilo). Prior to the injection of alloxan the rabbits were fasted for 7 days and each received by injection a daily dose of 100 mg. of phlorhizin for 7 days.
The present communication is intended to throw further light on the mechanism of alloxan hypoglycemia. Several rabbits were fasted for a week and injected with phlorhizin in order to lower the insulin secretion of the pancreas; the effects of the injection of diabetogenic doses of alloxan on the blood sugar level in those animals were studied.

![Graph](http://www.jbc.org/)

**Fig. 2.** Glucose tolerance curves of a rabbit. The dash curve represents the test made before the animal was fasted and phlorhizinized; the solid curve indicates the test made after the rabbit was fasted and phlorhizinized for 7 days.

**EXPERIMENTAL**

Four healthy female Himalayan rabbits, varying in weight between 1280 and 1530 gm., were housed in separate metabolism cages. The animals were fasted for a period of 7 days but were allowed to drink water during this period. Each animal received a daily intramuscular injection of 100 mg. of phlorhizin suspended in olive oil for these 7 days. All of the animals excreted sugar in the urine by the 2nd day of the experiment. On the 8th day alloxan in a dose of 200 mg. per kilo of body weight was injected into the marginal ear vein of all the rabbits and the blood sugar was estimated according to the method of Hagedorn and Jensen (9) in samples of blood.
taken both before and at varying intervals up to 24 hours after the injection of alloxan. The blood sugar curves of the animals are shown in Fig. 1.

In order to study the utilization of glucose by a phlorhizinized rabbit fasted for 7 days, a glucose tolerance test was performed in a rabbit fasted overnight. The rabbit was fasted and then given a daily injection of 100 mg. of phlorhizin for a period of 7 days as described before. On the 8th day the glucose tolerance test was performed as follows: The rabbit was fed a 50 per cent solution of glucose in a dose of 1 gm. per kilo of body weight. Samples of blood were taken both before and at intervals of half an hour up to 2\(\frac{1}{2}\) hours after the glucose feeding. Blood sugar was determined as before. The results are shown in Fig. 2.

Two rabbits were made diabetic by intravenous injection of alloxan. After 7 days a diabetic type of glucose tolerance curve was obtained.

### Table I

**Blood Sugar Values of Alloxan-Diabetic Rabbits before and after Intravenous Injection of Alloxan**

<table>
<thead>
<tr>
<th>Weight of rabbit</th>
<th>Fasting blood sugar</th>
<th>Blood sugar after injection of alloxan</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(\frac{1}{2}) hr.</td>
</tr>
<tr>
<td>gm.</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1490</td>
<td>233</td>
<td>512</td>
</tr>
<tr>
<td>1500</td>
<td>118</td>
<td>204</td>
</tr>
</tbody>
</table>

Alloxan (200 mg. per kilo) was then injected in both of these rabbits and samples of blood were drawn at varying intervals up to 6 hours after the injection of alloxan. The blood sugar values are given in Table I.

### Results

Phlorhizinized rabbits fasted for 7 days showed a diabetic type of glucose tolerance curve. Injection of alloxan into such rabbits produced an initial hyperglycemia, but no hypoglycemia was observed in any one of them. 24 hours after the injection of alloxan all of the rabbits excreted sugar in the urine and the blood sugar level was high, indicating that all of the animals developed diabetes.

Injection of alloxan into rabbits made diabetic by a previous injection of alloxan caused a further rise in the blood sugar level, and even 6 hours after the injection of alloxan the blood sugar level was much above the fasting blood sugar value.
DISCUSSION

Normal rabbits develop hypoglycemic convulsions within 2 to 4 hours after the intravenous injection of a diabetogenic dose of alloxan (200 mg. per kilo) (4). All of the four rabbits which were phlorhizinized and fasted for a period of 7 days showed initial hyperglycemia, contrary to the findings of Goldner and Gomori (10), but failed to develop hypoglycemia even 5 hours after the injection of alloxan. All of the animals survived the next day without injection of glucose and showed marked hyperglycemia and glycosuria and developed diabetes. A glucose tolerance test in one such rabbit gave a diabetic type of curve, indicating that a fasted phlorhizinized rabbit has possibly less insulin for the utilization of glucose. Du Vigneaud and Karr (11) observed that rabbits fasted for 7 days or longer did not utilize glucose as normal animals do. The injection of phlorhizin to the fasted rabbit led to the excretion of glucose in the urine, which further enhanced the carbohydrate starvation, leading possibly to further diminution in the insulin secretion of the pancreas. Injection of alloxan in two alloxan-diabetic rabbits failed to lower the blood sugar level from the initial fasting value. The absence of hypoglycemia after the injection of a diabetogenic dose of alloxan in fasted and phlorhizinized rabbits and in rabbits made diabetic by previous injection of alloxan, conditions in which there is less insulin in the pancreas, seems to indicate that the cause of alloxan hypoglycemia is pancreatic in origin and not extrapancreatic as claimed by Houssay and others (5-7).

SUMMARY

1. Alloxan (200 mg. per kilo) was injected intravenously into four rabbits which were fasted and then received a daily injection of 100 mg. of phlorhizin for a period of 7 days. No hypoglycemia was observed in any of the rabbits.

2. Glucose tolerance tests were performed in a rabbit, both before and after the animal was phlorhizinized and fasted for 7 days. A diabetic type of glucose tolerance curve was observed when the animal was phlorhizinized and fasted.

3. Alloxan was injected into two rabbits made diabetic by a previous injection of alloxan. Alloxan did not lower the blood sugar level from its initial fasting value.

4. The alloxan hypoglycemia as observed in rabbits seems to be pancreatic in origin.

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