ON THE MECHANISM OF ALLOXAN HYPOGLYCEMIA

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Intravenous injection of alloxan into rabbits, rats, dogs, monkeys, etc., produces a brief hyperglycemia, then a transient hypoglycemia, which in the case of rabbits is severe enough to cause convulsions and death, unless counteracted by repeated injections of glucose, and finally a permanent diabetic hyperglycemia.

The cause of the transient hypoglycemia has had various explanations. Goldner and Gomori (1, 2), Kennedy and Lukens (3), and Banerjee (4) consider that the secondary hypoglycemia is pancreatic in origin, due to the liberation of insulin by the β cells of the islets undergoing destruction. Houssay and his associates (5), however, attribute the hypoglycemia to an extrapancreatic effect, viz., lack of glucose production by the liver.

Recently, Banerjee and Bhattacharya (6) observed no hypoglycemic convulsions in rabbits fasted and phlorhizinized for a period of 7 days and then injected with diabetogenic doses of alloxan. The present paper is an extension of this study of the mechanism of alloxan hypoglycemia. The effect of the injection of diabetogenic doses of alloxan on blood sugar levels has been investigated in rabbits with yellow phosphorus liver damage, with persistent phlorhizin glycosuria, and after fasting. A comparison has also been made of the glycogen content of livers of rabbits after periods of fasting and during convulsive seizures due to injections of diabetogenic doses of alloxan or due to excessive doses of insulin.

EXPERIMENTAL

Five healthy Himalayan rabbits, each approximately 2 kilos in body weight, were kept in separate metabolism cages. Liver damage was produced in all the animals by the oral administration of two to six doses of yellow phosphorus (7), each dose being 3 mg. per kilo. The phosphorus was given twice or three times per week as a 0.25 per cent solution in olive oil. Liver damage was indicated by a diabetic type of glucose tolerance curve (8). Glucose tolerance curves of three of the animals both before and after the administration of phosphorus are compared in Fig. 1. After liver damage had been established in the animals, alloxan in a dose of 200 mg. per kilo was injected into the marginal ear veins of all the animals and the blood sugar values were determined at varying intervals of time up to

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Blood sugar was determined according to the method of Hagedorn and Jensen (9).

Another set of six rabbits, also kept in separate metabolism cages, was given seven daily injections of 100 mg. of phlorhizin suspended in olive oil. All the animals excreted sugar in the urine by the 2nd day of the experi-
ment. After seven doses of phlorhizin, glucose tolerance was determined in three of the animals (Fig. 2). Alloxan in diabetogenic doses was then injected intravenously into all the animals. Blood sugar was determined in samples taken both before and at varying intervals up to 24 hours after the injection of alloxan (Table II).

**Table I**

*Effect of Injection of Diabetogenic Doses of Alloxan on Blood Sugar Levels in Rabbits with Livers Damaged with Yellow Phosphorus*

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Blood sugar after injection of alloxan, mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr.</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>105</td>
</tr>
<tr>
<td>3</td>
<td>69</td>
</tr>
<tr>
<td>4</td>
<td>95</td>
</tr>
<tr>
<td>5</td>
<td>92</td>
</tr>
</tbody>
</table>

**Fig. 2.** Glucose tolerance curves of three rabbits phlorhizinized for 7 days. Glucose tolerance was determined by the oral administration of 2 gm. of glucose per kilo of body weight.

Three more rabbits were fasted for 7 days but were allowed to drink water during this period. Glucose tolerance was determined in these fasted animals, as well as the effect of the injection of diabetogenic doses of alloxan.

Nine rabbits were fasted overnight. On the following day alloxan in
diabetogenic doses (200 mg. per kilo) was injected into a group of three, so that the animals were in convulsions approximately 24 hours after food had been withdrawn. Insulin (10 units) was administered to a second group of three, so that these rabbits were also in convulsions approximately 24 hours after food had been withdrawn. The remaining three animals were simply fasted for the 24 hour period. All the animals were sacrificed by a sudden blow on the head. The throat was cut open, the liver rapidly removed, and a weighed portion was put into boiling 60 per cent potas-

**Table II**

*Effect of Injection of Diabetogenic Doses of Alloxan on Blood Sugar Levels in Phlorhizinized Rabbits*

<table>
<thead>
<tr>
<th>Rabbit No.</th>
<th>Blood sugar after injection of alloxan, mg. per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 hr.</td>
</tr>
<tr>
<td>1</td>
<td>87</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>95</td>
</tr>
<tr>
<td>4</td>
<td>105</td>
</tr>
<tr>
<td>5</td>
<td>92</td>
</tr>
<tr>
<td>6</td>
<td>98</td>
</tr>
</tbody>
</table>

**Table III**

*Liver Glycogen Levels in Rabbits after 24 Hour Fasting Period, with and without Convulsions Induced by Alloxan and by Insulin*

Three rabbits in each group.

<table>
<thead>
<tr>
<th>Normal</th>
<th>During convulsions due to injection of</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm. per cent</td>
<td>Diabetogenic doses of alloxan</td>
</tr>
<tr>
<td>2.5</td>
<td>4.03</td>
</tr>
<tr>
<td>2.2</td>
<td>4.30</td>
</tr>
<tr>
<td>2.8</td>
<td>4.98</td>
</tr>
</tbody>
</table>

sium hydroxide. The glycogen was precipitated, hydrolyzed, and estimated according to the method of Evans, Tsai, and Young (10). The results are given in Table III.

**Results**

Rabbits with livers damaged with phosphorus showed a diabetic type of glucose tolerance curve with fasting blood sugar values almost normal (Fig. 1). Intravenous injection of diabetogenic doses of alloxan into four such rabbits produced an initial hyperglycemia but no hypoglycemia
Rabbit 3 showed practically no fluctuation of blood sugar up to the 9th hour after the injection of alloxan. All of the rabbits showed high blood sugar and excreted sugar in the urine after 24 hours.

Rabbits phlorhizinized for 7 days gave glucose tolerance curves corresponding to those in normal animals (Fig. 2). Intravenous injection of diabetogenic doses of alloxan into such rabbits produced an initial hyperglycemia and a mild hypoglycemia 6 or 7 hours after the injection but never produced hypoglycemic convulsions (Table II). All of the animals survived the next day without injection of glucose and developed diabetes.

Rabbits fasted for 7 days gave the diabetic type of glucose tolerance curve, due possibly to a reduced insulin content of the pancreas (11). All of the fasted rabbits developed hypoglycemic convulsions within 4 to 6 hours after the intravenous injection of diabetogenic doses of alloxan.

The glycogen content of the livers of rabbits in convulsions due to the injection of diabetogenic doses of alloxan was found to be several times greater than that of livers of rabbits in convulsions due to the injection of insulin. The glycogen content of livers of normal rabbits fasted for 24 hours was lower than that of rabbits in convulsions due to the injection of alloxan but greater than that of rabbits in convulsions due to the injection of insulin (Table III).

**DISCUSSION**

Normal rabbits develop hypoglycemic convulsions within 4 to 6 hours after the intravenous injection of diabetogenic doses (200 mg. per kilo) of alloxan. None of the rabbits with livers damaged with yellow phosphorus developed hypoglycemia, even 9 hours after the injection of alloxan. All of the animals survived without injections of glucose and developed diabetes. Phosphorus damages liver by destroying parenchymatous tissues and hampers the process of glycogenesis (8). The process of glycogenolysis in the liver is consequently rendered more or less invalid, for in the absence of glycogenesis there is no scope for glycogenolysis. Animals with severe liver damage consequently exhibit a diabetic type of glucose tolerance, as was confirmed in our experiments.

Houssay et al. (5) are of the opinion that the secondary alloxan hypoglycemia is extrapancreatic in origin and that it is probably due to a temporary blockage of glycogenolysis in the liver. Others believe that the hypoglycemia is pancreatic in origin and that it results from the release of preformed insulin in the necrosed islets of the pancreas. In rabbits with livers damaged with phosphorus the process of hepatic glycogenolysis, as explained above, is rendered more or less invalid, but the pancreas is left intact, as evidenced by the approximately normal values for fasting blood sugar. Injection of alloxan into such rabbits should have produced hypo-
glycemia if its origin were pancreatic, for the β cells were destroyed as usual, as was demonstrated by the onset of diabetes after 24 hours. On the other hand, there should have been no hypoglycemia if it were hepatic in origin, due to a temporary blockage of glycogenolysis in the liver. The results obtained show that the cause of the secondary hypoglycemia is extrapancreatic and probably hepatic in origin.

The results in Table III show that insulin hypoglycemia and convulsions are associated with a decreased glycogen content of the liver, whereas alloxan hypoglycemia and convulsions are associated with an increased level of hepatic glycogen. This tends to show that the hypoglycemia and convulsions due to injections of insulin and alloxan, respectively, are probably of different origin. Insulin hypoglycemia is due to excessive utilization of glucose in the tissues leading to depletion of the glycogen content of the liver, whereas alloxan hypoglycemia is probably due to a blockage of glycogenolysis in the liver leading to the accumulation of extra glycogen.

Phlorhizinized animals have no dearth of insulin and the islets are not damaged in any way, as shown by the fact that the glucose tolerance curves are almost normal and by the fact that the omission of phlorhizin rapidly restores the animals to the normal state. The effect of phlorhizin is only to lower the renal threshold with resulting excretion of sugar in the urine. Loss of sugar in the urine, however, has the same effect on the liver as diabetes produced by pancreatectomy or administration of alloxan. Liver glycogenolysis compensates in part for the sugar lost and this leads to depletion of liver glycogen. Injection of alloxan in phlorhizinized rabbits destroyed the β cells as usual, but there was only limited hypoglycemia, if any. These results also agree with the hypothesis that the hypoglycemia is not pancreatic in origin, that the phenomenon is somehow associated with the process of glycogenolysis in the normal liver, and that it is probably due to a blockage of glycogenolysis. Alloxan cannot possibly check the process of glycogenolysis in livers in which the process has already been carried to an abnormal extreme, as in phlorhizinized animals.

Inhibition of hepatic glycogenolysis may explain the results obtained by Houssay et al. (5) who reported hypoglycemia by the injection of alloxan into dogs pancreatectomized half an hour earlier; that is, in dogs in which the livers were not fully diabetic. The same authors, however, could not produce hypoglycemia in dogs 24 hours after pancreatectomy, by which time the livers certainly were diabetic. This may also explain the results of Banerjee (4) and Goldner and Gomori (2) who observed that half pancreatectomized rabbits failed to develop hypoglycemic convulsions (although they developed moderate hypoglycemia) after the injection of alloxan. The results obtained by Banerjee and Bhattacharya (6) were possibly due not to the combined effect of fasting and of phlorhizin in re-
ducing the insulin content of the pancreas but to the effect of phlorhizin alone in rendering the liver more or less diabetic, as has been established in the present paper. Fasting alone led to impaired carbohydrate utilization, due possibly to the reduced insulin content of the pancreas. Fasting could not, however, lessen the severity of alloxan hypoglycemia.

SUMMARY

1. Intravenous injection of diabetogenic doses (200 mg. per kilo) of alloxan into rabbits, in which the livers were damaged by the oral administration of yellow phosphorus, did not produce hypoglycemia.

2. Intravenous injection of diabetogenic doses of alloxan into rabbits with persistent glycosuria due to daily administration of phlorhizin for 7 days also failed to produce any marked hypoglycemia.

3. Rabbits fasted for a period of 7 days developed hypoglycemia convulsions within 4 to 6 hours after the intravenous injection of diabetogenic doses of alloxan.

4. The glycogen content of the livers of rabbits is increased in alloxan hypoglycemia, whereas it is decreased in insulin hypoglycemia.

5. Alloxan hypoglycemia, as observed in rabbits, appears to be extrapancreatic and is probably due to a temporary blockage of liver glycogenolysis.

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