A STUDY OF ALLOXAN-INDUCED "DIABETES" IN THE ESTUARINE CLAM, MERETRIX CASTA (CHEMNITZ)

BY S. KASINATHAN

(Marine Biological Station, Porto Novo, S. India)

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INTRODUCTION

In a previous paper, the author (1963) described that injection of insulin into the clam, Meretrix casta (Chemnitz) produces effects similar to what happens in vertebrates and contrary to the view generally held in regard to the effects of insulin in molluscs. As a continuation of this study in a programme bearing on the regulation of carbohydrate metabolism in clams, the author undertook a study of the effect of alloxan injection in these animals.

In investigations relating to diabetes and regulation of carbohydrate metabolism in vertebrates, the alloxan technique has made a significant contribution. Alloxan, which is structurally related to pyrimidine and is the ureide of mesoxalic acid, was first shown in 1937 to produce hyperglycaemia and death in rabbits. It was later shown that alloxan has a specific destructive effect on beta cells of the islet tissue of pancreas and thereby produces experimental diabetes. This is in contrast to the effect of the diabetogenic hormone from the pituitary or partial pancreatectomy which is said to result in hyperactivity of the beta cells. All these studies have been entirely limited to vertebrates. It was thought, therefore, that alloxan experiments on an invertebrate like the clam, Meretrix casta, might be interesting. Alloxan-diabetes in relation to liver glycogen has been studied in albino rats and the effects of glucose and insulin on the diabetogenic action of alloxan on dogs have been studied by Arteta et al. (1954).

The present account describes the effect of alloxan injection in Meretrix casta.

MATERIAL AND METHODS

Specimens of Meretrix casta were collected from the Vellar estuary. The weight of the specimens used in these experiments varied from 3 gm.

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Crystalline alloxan of analytical purity (kindly donated by Dr. A. E. Mirsky of Rockefeller) was used to produce experimental diabetes. The specimens were starved for 24 hours prior to experimental treatment as starvation is known to confer on animals greater susceptibility to alloxan effect.

In the initial experiments, the alloxan dose injected into the specimens was 1 mg./5 gm. body weight. In subsequent sets of experiments, higher doses varying from 2 mg./5 gm. weight, 3 mg./5 gm. weight and 4 mg./5 gm. weight were used. Alloxan was injected into the foot as quickly as possible. The time required for alloxan effect was determined by trial and by estimating the blood glucose. The normal level of blood glucose in *Meretrix casta* is 47.9 ± 2.52 mg./100 ml. of blood, and when the glucose content rose to 70 mg./100 ml. of blood following the injection of alloxan, it was assumed that the diabetogenic effect had resulted.

The glucose content of the blood and the glycogen content of the foot and digestive diverticula were estimated at intervals. Following an injection of alloxan 1 mg./5 gm. weight, the glucose and glycogen were estimated at four successive hourly intervals. With higher doses, the estimations were done after 2 hours.

**METHODS**

Glycogen was estimated adopting the procedure described by Kemp and Kits (1954) and as described in a previous paper by the present author (1963).

Controls were maintained in all these experiments. In these distilled water was injected, equal in volume to that of alloxan injected.

Besides the experiments with alloxan injection mentioned above, the effect of a toxin, T.A.B. vaccine (*Salmonella typhi*) and also the effect of low temperature like 4° C. on the glycogen content of alloxan diabetic animals was determined.

Experiments were also conducted to determine the minimum time required for the inhibition of diabetes when alloxan injection was preceded by glucose injection into the animal.

**RESULTS AND OBSERVATIONS**

The normal glycogen content of the digestive diverticula and foot is 1.2855 ± 0.068, 0.4362 ± 0.022 respectively. When 1 mg./5 gm. wt, of
TABLE I

*Effect of injection of 1 mg./5 gm. wt. of alloxan on tissue glycogen and blood glucose of Meretrix casta after 24 hours of Starvation*

<table>
<thead>
<tr>
<th>State of the animal</th>
<th>Glycogen in mg./100 mg in Foot</th>
<th>Glycogen in mg./100 mg. in digestive diverticula</th>
<th>Blood glucose mg./100 ml.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st hr.</td>
<td>2nd hr.</td>
<td>3rd hr.</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.436±</td>
<td>0.436±</td>
<td>0.436±</td>
</tr>
<tr>
<td>Alloxan diabetic</td>
<td>0.810±</td>
<td>0.883±</td>
<td>0.551±</td>
</tr>
<tr>
<td>Control</td>
<td>0.494±</td>
<td>0.494±</td>
<td>0.510±</td>
</tr>
</tbody>
</table>

![Graph](image)

Fig. 1. Effect of injection of 1 mg./5 gm. wt. alloxan, on glycogen content in tissues,
Alloxan-Induced "Diabetes" in the Estuarine Clam, Meretrix casta 321

Alloxan is injected, the overall effect is an increase of glycogen both in the foot and digestive diverticula (Table I, Fig. 1). However, there are certain striking differences between what happens in the foot and digestive diverticula. During the first hour, the foot shows a more pronounced increase of glycogen content than the digestive diverticula in which the rise is only slight. In the following hour a pronounced increase is seen in the digestive diverticula, whereas in the foot it is slight. In the third hour, there is a fall of glycogen content in both the tissues but is greater in the foot. In the fourth hour there is a steep rise of glycogen in digestive diverticula but less in the foot. Now the increase in glycogen in both foot and digestive diverticula is higher than in the previous hours.

The injection of increased doses of alloxan also shows some interesting results (Table II, Fig. 2). When 2 mg./5 gm. wt. are injected, the increase

Fig. 2. Effect after 2 hours injection of different doses of alloxan on glycogen content tissues.
### Table II

Effect after 2 hours of injection of different doses of alloxan on glycogen content in tissues of 24 hours starved Meretrix casta (Chemnitz)

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Alloxan diabetic</th>
<th>Control</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 mg.</td>
<td>3 mg.</td>
<td>4 mg.</td>
</tr>
<tr>
<td>Foot (Glycogen in mg./100 mg. wt.)</td>
<td>1.153±</td>
<td>1.202±</td>
<td>1.393±</td>
</tr>
<tr>
<td></td>
<td>0.052</td>
<td>0.082</td>
<td>0.104</td>
</tr>
<tr>
<td>Digestive diverticula (Glycogen in mg./100 mg. wt.)</td>
<td>2.688±</td>
<td>2.723±</td>
<td>2.778±</td>
</tr>
<tr>
<td></td>
<td>0.128</td>
<td>0.171</td>
<td>0.123</td>
</tr>
</tbody>
</table>
in the glycogen after two hours is strikingly greater than the action on 1 mg./5 gm. wt. of alloxan in one hour. It is also observed that the increase is greater in the digestive diverticula than in the foot. When 3 mg./5 gm. wt. is injected, there is only slight increase over what is seen when 2 mg./5 gm. wt. is injected. But the larger doses result in slightly greater increase of glycogen in foot than in digestive diverticula.

**Effect of toxin, T.A.B. vaccine** (Salmonella typhi).—This toxin was injected in two doses of 0.1 ml. each at intervals of 15 minutes into clams, which were starved and treated with alloxan to produce experimental diabetes. The injection of toxin resulted in glycogen deficiency. There was not only no increase of glycogen, but a definite decrease of glycogen below a normal level was observed.

Cold treatment of alloxan diabetic clams, i.e., of subjecting them to 4°C for four hours, also produces a diminution of glycogen content below the normal level. The results are shown in Table III. The changes produced in the foot are slight as compared to those in the digestive diverticula. These experiments indicate that the alloxan effect differs from toxic and cold effects.

### Table III

**Effect of toxin and cold treatment on blood glucose and glycogen content of alloxan-diabetic Meretrix casta (Chemnitz)**

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Alloxan diabetic</th>
<th>Control</th>
<th>Normal</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Toxin Exposure to cold for 4 hours Recovery</td>
<td>Toxin Exposure to cold for 4 hours Recovery</td>
<td>Toxin Exposure to cold for 4 hours Recovery</td>
</tr>
<tr>
<td>Foot (Glycogen in mg./100 mg. wt.)</td>
<td>1.201 ± 0.269 1.123 ± 0.042 1.174 ± 0.074</td>
<td>0.990 ± 0.010 1.211 ± 0.029 1.112 ± 0.019</td>
<td>1.271 ± 0.063 1.152 ± 0.043 1.152 ± 0.061</td>
</tr>
<tr>
<td>Digestive diverticula (Glycogen in mg./100 mg. wt.)</td>
<td>2.093 ± 0.620 2.102 ± 0.087 2.169 ± 0.070</td>
<td>1.833 ± 0.305 2.113 ± 0.069 2.199 ± 0.277</td>
<td>2.846 ± 0.056 2.115 ± 0.034 2.115 ± 0.034</td>
</tr>
<tr>
<td>Blood (Glucose mg./100 ml.)</td>
<td>32 ± 8.4 185 ± 1.5</td>
<td>47 ± 14 68 ± 8.4</td>
<td>48 ± 2.5 48 ± 2.5</td>
</tr>
</tbody>
</table>

**Effect of injection of insulin into the diabetic clam.**—When insulin is injected in alloxan-induced diabetic clam, the glycogen content in foot and digestive diverticula shows a greater increase than in only alloxan treated animals. There is also a concomittant decrease in blood glucose level in these animals as is shown in Table IV.
### Table IV

*Effect of injection of insulin in alloxan diabetic Meretrix casta (Chemnitz)*

<table>
<thead>
<tr>
<th>Tissue</th>
<th>24 hours fast without insulin</th>
<th>24 hours fast with insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Alloxan diabetic</td>
<td>Control</td>
</tr>
<tr>
<td>Foot (Glycogen in mg./100 mg. wt.)</td>
<td>0.883±0.030</td>
<td>0.796±0.025</td>
</tr>
<tr>
<td>Digestive diverticula (Glycogen in mg./100 mg. wt.)</td>
<td>1.836±0.172</td>
<td>1.798±0.126</td>
</tr>
<tr>
<td>Blood (Glucose mg./100 ml).</td>
<td>100±23</td>
<td>46.8±14</td>
</tr>
</tbody>
</table>

### Table V

*The action of glucose upon the effects of diabetogenic doses of alloxan in Meretrix casta*

<table>
<thead>
<tr>
<th>Experimental group</th>
<th>Interval between glucose and alloxan injection</th>
<th>Alloxan dose</th>
<th>No. and sex of treated clams</th>
<th>No. and sex of clams developing diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>1. Previous injection of 5 mg./5 gm. wt.</td>
<td>5 Minutes</td>
<td>1 mg./5 gm. wt.</td>
<td>5</td>
<td>5</td>
</tr>
<tr>
<td>2. Previous injection of:</td>
<td></td>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>(a) 5 mg./5 gm. wt.</td>
<td>15 Seconds</td>
<td>1 mg./5 gm. wt.</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>(b) 5 mg./5 gm. wt.</td>
<td>30 Seconds</td>
<td>Do.</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>3. Injection of alloxan dissolved in 10 ml. of 10% glucose</td>
<td>Do.</td>
<td></td>
<td>5</td>
<td>5</td>
</tr>
</tbody>
</table>
The action of glucose upon the effects of diabetogenic doses of alloxan.— The injection of 5 mg./5 gm. wt. of glucose 5 minutes before alloxan injection inhibited the production of diabetes. A number of experiments were carried out in order to determine the minimum time required for this glucose inhibition of alloxan effect (Table V). The results showed that clams which received a total of 5–10 mg. of glucose 15–30 seconds before the injection of 1 mg./5 gm. wt. of alloxan were completely protected against the diabetogenic action of the latter.

Injection of alloxan in glucose medium into *Meretrix* does not produce diabetes. Arteta *et al.* (1954) have reported that such injections into dogs produce diabetes in males only but not in females.

**DISCUSSION**

The observations recorded above bring out some interesting features of alloxan effect on *Meretrix*.

1. Alloxan injection increases the level of blood sugar. In other words it produces diabetes as it does in vertebrates.

2. As a result of alloxan injection the glycogen content increases in the tissues. A similar effect has been observed by investigators on vertebrates.

3. Alloxan has a differential effect on the glycogen content of foot and digestive diverticula. The response of the foot and digestive diverticula to the alloxan injection is not in the same degree.

4. It would appear as though the two tissues had differential limits to glycogen potential. Glycogen reaches a higher level in the digestive diverticula than in the foot as a result of alloxan injection. The overall range of fluctuation of glycogen content in the digestive diverticula is greater than in the foot.

5. Alloxan effect is not due to a toxicity.

6. The puzzling feature is that while blood glucose is decreased in insulin injection, but increased in alloxan injection, the glycogen content of the tissues is increased both in insulin injection as well as in alloxan injection.

We do not have as yet a satisfactory explanation for the increased glycogen synthesis in insulin injections. Krahl (1961) proposed that insulin acts to bring about alteration of the fine structure of responsive cells, resulting in a decompartment which favours glycogen synthesis, fat synthesis and protein synthesis. As he himself remarked this speculation can neither be
proved nor disproved. "Methods with the potential for localization of individual cellular and hormone molecules will ultimately be required. The mechanism of hormone action has thus become a problem in solid state-physics" (Krahl, 1961).

Weber (1946), Tuerkischcher and Wertheimer (1947) and others, who investigated the alloxan effect in vertebrates, have remarked that the increase in glycogen in alloxan diabetic animal is due to utilisation of precursors for additional synthesis of glycogen. What these precursors precisely are has not been determined. Apart from this, there is an interesting aspect which has to be investigated in Meretrix. Alloxan destroys beta cells in vertebrate pancreas. But on what does it act in Meretrix? This is under investigation.

**Summary**

1. Effects of different concentrations of alloxan on the blood glucose and tissue glycogen levels of the estuarine clam, *Meretrix casta* (Chemnitz), have been investigated.

2. In alloxan-diabetic specimens there is an increase in the glycogen content in the foot and digestive diverticula. The overall increase and the range of fluctuations was greater in the digestive diverticula.

3. Alloxan shows a differential effect on the quantity of glycogen in the tissues.

4. Alloxan effect on *Meretrix casta* is different from toxic and cold effects on the glycogen content.

**Acknowledgement**

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