EFFECT OF INSULIN ON CARBOHYDRATE METABOLISM OF THE BIVALVE MOLLUSC MERETRIX CASTA (CHEMNITZ)

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INTRODUCTION

It is well known that in the regulation of carbohydrate metabolism in vertebrates, insulin has a very important role. But little is known regarding the control of carbohydrate metabolism in invertebrates. It is likely that there is a hormonal control of carbohydrate metabolism in crustacea. But we know nothing regarding the regulation of carbohydrate metabolism in molluscs. Holtz and von Brand (1940) pointed out that the constancy of the chief blood constituents in molluscs would imply some sort of regulation. Collip (1923) had earlier suggested that "wherever glycogen occurs there would be insulin not far distant". But the experiments of Collip, Kumagai and Shikanami (1929) have not confirmed the hypothesis. Schwarz (1934) injected insulin into the snail but could not observe any drastic change in the blood sugar level. Wolf-Heidegger (1935) found that insulin had no effect on blood sugar level in the snail Helix pomatia. Ville et al. (1950) found that insulin had no effect upon glucose uptake on glycogen synthesis in muscle slices or gills of the clam Mactra, Pecten or Mya or the muscle slices of the snail Buscyon. Krahl (1961) has stated that, in general, insulin has been found to have no effect on the glucose level in the extracellular fluid of invertebrates or upon glucose uptake of isolated tissues from such organisms.

The present author has been investigating the regulation of carbohydrate metabolism in bivalves. The present account records the observations on the effect of injection of insulin on the glucose level in the blood and on the glycogen content of foot and digestive diverticula in the clam Meretrix casta.

MATERIAL AND METHODS

Specimens of the Meretrix casta were collected from the tidal zone of the Vellar estuary and kept alive in laboratory aquaria. The clams are fairly
hardy and thrive well for a few days under laboratory conditions. Specimens of more or less equal weight and size were selected for the experimental studies. An hour prior to insulin injection, the specimens were transferred to thrice-filtered estuarine water and maintained in condition to ensure that feeding did not take place during the experiments and that changes in the glycogen content could only be due to insulin effect.

Insulin [Boots Pure Drug Co. (P) Ltd., India] dissolved in distilled water was used. Further dilutions from the original concentration were obtained with distilled water. Dosages varying from 0.5 to 4.0 units were injected into the animals. After the injection of insulin, the glycogen content of the foot and digestive diverticula were estimated at hourly intervals. Blood from the same specimens was drawn directly from the heart with a fine syringe for estimation of glucose. For control experiments, distilled water, equal to the volume of insulin, is injected into the specimens. Five separate estimations in each experiment, and for each estimation the tissue from five specimens were pooled together.

For the estimation of blood glucose the micro-method of Hagedorn and Jensen (1923) was followed. The blood protein was precipitated out with zinc hydroxide and the filtrate was subsequently heated with potassium ferricyanide. The amount of ferricyanide reduced was determined by addition of iodide solution and titrating the liberated iodine with sodium thiosulphate.

Tissue glycogen.—Colorimetric micro-method of Kemp and Kits (1954) was employed for the estimation of glycogen in the digestive diverticula and foot. The glycogen from the tissues was extracted by heating the tissues with 5 ml. of deproteinising solution (5% TCA with 0.1% of silver sulphate) for fifteen minutes in a boiling water-bath. The tubes were then cooled and deproteinising solution was added to restore the original volume. After centrifugation, 2 ml. of the supernatant was taken, and 6 ml. of concentrated sulphuric acid was added and heated for 6.5 minutes in a boiling water-bath. The glycogen was hydrolysed by concentrated sulphuric acid into glucose, which in turn reacted with the concentrated sulphuric acid to produce a pink colour. The intensity of the colour is proportional to the concentration of the glucose present, and was estimated in Lange’s photoelectric colorimeter, using 530 m filter. Standard reference curves for known quantity of glucose were drawn and the concentration in the unknown sample was read from the curves. The glucose was converted into glycogen using the glycogen constant.
Effect of Insulin on Carbohydrate Metabolism of Bivalve Mollusc

EXPERIMENTAL RESULTS

The results relating to the effects of insulin injections are shown in Tables I to III and Graphs I and II. They have been found to be statistically significant.

A. Effect of Injection of Insulin on the Level of blood Glucose

**TABLE I**

Effect of injection of different units of insulin on blood glucose level

<table>
<thead>
<tr>
<th>Units of insulin injected</th>
<th>Blood glucose levels in (mg./100 ml.)</th>
<th>mg. per cent. of glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1st hour</td>
<td>2nd hour</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>46·8</td>
<td>46·0</td>
</tr>
<tr>
<td>0·5 Unit</td>
<td>19·5</td>
<td>50·5</td>
</tr>
<tr>
<td>1·0 Unit</td>
<td>19·0</td>
<td>42·0</td>
</tr>
<tr>
<td>2·0 Units</td>
<td>18·0</td>
<td>43·0</td>
</tr>
<tr>
<td>3·0 Units</td>
<td>16·0</td>
<td>41·0</td>
</tr>
<tr>
<td>4·0 Units</td>
<td>12·0</td>
<td>18·0</td>
</tr>
</tbody>
</table>

The normal level of blood sugar in *Meretrix casta* shows much variability ranging from 36 mg. to 61 mg. per 100 ml. with a mean of 47·90±2·5262.

The effects of injection of different doses of insulin on blood glucose level are shown in Table I.

When 0·5 unit of insulin was injected, the glucose level drops down after an hour to 19·5 mg. per 100 ml. Subsequently, recovery sets in and the normal level is restored by about three hours after injection.

The injection of 1 unit shows a similar effect to begin with, but after two hours, there is some fluctuation in the glucose level. This probably corresponds to normal variability of blood sugar found in the species.

Injection of two units also has similar effect. Even when higher doses like 3 and 4 units are used, the initial drop in concentration occurs at the end of an hour after injection.
B. Effect of Insulin on the Glycogen Content of Tissues (Digestive Diverticula and Foot)

Normally, the average glycogen content of digestive diverticula is $1.2855 \pm 0.0683$ mg. per 100 mg. tissue. In the foot, the average glycogen content is $0.4362 \pm 0.0215$ mg. per 100 mg. tissue. During the first hour following injection of 0.5 unit of insulin, while the blood glucose level declines the glycogen content in the digestive diverticula and foot begins to increase (Table II). In the former, the glycogen content attains a peak value an hour after the injection. In the foot the increase continues and the peak is reached in the second hour. In the digestive diverticula the subnormal level of glycogen continues, though with some fluctuation. In the foot, however, the peak is followed by a marked decrease to an almost subnormal level, and this is followed by another phase of increase and decrease of glycogen content. These results are shown in Graph I.

The effects of increased dosage of insulin on the glycogen content of digestive diverticula and foot are shown in Table III, Graph II.

It will be observed that in the foot the glycogen content increases and shows alternate decrease and increase, whereas in the digestive diverticula, there is a perceptible decrease.

### Table II

<table>
<thead>
<tr>
<th>Time in hours</th>
<th>Glycogen content in foot mg./100 mg. of tissue</th>
<th>Control mg./100 mg. tissue</th>
<th>Glycogen content in digestive diverticula mg./100 mg. of tissue</th>
<th>Control mg./100 mg. tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>$0.4362 \pm 0.0215$</td>
<td>.</td>
<td>$1.2855 \pm 0.0683$</td>
<td>.</td>
</tr>
<tr>
<td>1</td>
<td>0.5409</td>
<td>0.4836</td>
<td>1.3060</td>
<td>1.2946</td>
</tr>
<tr>
<td>2</td>
<td>0.6713</td>
<td>0.4943</td>
<td>0.9332</td>
<td>1.3015</td>
</tr>
<tr>
<td>3</td>
<td>0.4134</td>
<td>0.5099</td>
<td>1.0178</td>
<td>1.2494</td>
</tr>
<tr>
<td>4</td>
<td>0.5916</td>
<td>0.4714</td>
<td>0.8368</td>
<td>1.2123</td>
</tr>
<tr>
<td>5</td>
<td>0.4942</td>
<td>0.3987</td>
<td>0.8516</td>
<td>1.1974</td>
</tr>
<tr>
<td>6</td>
<td>0.4739</td>
<td>0.4394</td>
<td>0.6501</td>
<td>1.2746</td>
</tr>
</tbody>
</table>
DISCUSSION AND CONCLUSION

The present study does not lend support to Krahl's statement that insulin does not have any effect on glucose level in invertebrates. Injection of insulin does affect the carbohydrate metabolism. The effect on the blood glucose is typical. There is a fall followed by recovery and increase of dosage produces greater fall of glucose level.
But the effects on the glycogen content in digestive diverticula and foot are interesting and need some explanation. During the first hour, following the insulin injection while there is decline in the blood glucose level, there is an increase in the glycogen content in the digestive diverticula and foot. Evidently, there is at this time a greater uptake and storage in these tissues. Subsequently, when the normal level of blood sugar is being restored, the glycogen content in the digestive diverticula shows perceptible decrease, but in the foot, this is followed by alternate decrease and increase.

GRAPH II. Effect of different dosages of insulin on the glycogen content of digestive diverticula and foot, after 2 hours.
**Effect of Insulin on Carbohydrate Metabolism of Bivalve Mollusc**

**TABLE III**

*Effect after 2 hours of injection of different units of insulin on glycogen content in tissues*

<table>
<thead>
<tr>
<th>Units of insulin injected</th>
<th>Glycogen content in mg./100 mg. tissue in foot</th>
<th>Control mg./100 mg. tissue</th>
<th>Glycogen content in mg./100 mg. tissue in digestive diverticula</th>
<th>Control mg./100 mg. tissue</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0.4362±0.0215</td>
<td></td>
<td>1.2855±0.0683</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.8026</td>
<td>0.5316</td>
<td>1.1887</td>
<td>1.2283</td>
</tr>
<tr>
<td>2</td>
<td>0.6625</td>
<td></td>
<td>1.0779</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.8852</td>
<td></td>
<td>1.2120</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.7969</td>
<td></td>
<td>1.0191</td>
<td></td>
</tr>
</tbody>
</table>

This would imply that the digestive diverticula are a rather labile store of glycogen, readily increasing when the blood glucose level falls and *vice versa*. In the foot, there is at first an increase of glycogen when blood glucose level falls; later, the glycogen shows fluctuations with decrease and increase, which seem to indicate that there may be some regulatory processes. Further investigations are in progress. It is, however, certain that insulin has a kind of differential effect on the glycogen content of digestive diverticula and foot.

**SUMMARY**

The effect of injection of insulin into *Meretrix casta* has been investigated. Insulin lowers the blood glucose level significantly after an hour following the injection; recovery sets in later. Increase of dosage of insulin produces increased lowering of blood glucose.

Injection of insulin produces an increase of glycogen content in the digestive diverticula and foot but there is a differential effect of insulin on the glycogen content of digestive diverticula and foot. The former seems to be a labile storehouse of carbohydrates and the latter a more stable store, with probably some mechanism of regulation.

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