Studies on Carbohydrate Metabolism in Lizards
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Carbohydrate Metabolism, Lizards, Saccharoid Fraction

1. Initial glycogen levels in the liver were found to be significantly higher than in the kidneys of the two species studied. The glycogen levels in kidney were however considerably higher than in some mammalian species.

2. Total reducing capacity, true glucose, saccharoid fraction and titratable acidity increase gradually with concomitant decrease in the glycogen levels when liver and kidney homogenates of *Uromastix* and liver homogenate of *Varanus* were incubated for a period of 4 hours.

3. Total reducing capacity, true glucose and the titratable acidity increase gradually, while the glycogen and the saccharoid fraction remain unchanged when the kidney homogenates of *Varanus* are incubated for 4 hours.

4. Total reducing capacity, true glucose and saccharoid fraction in the blood of *Uromastix* are higher than in *Varanus*. The blood glucose levels in both the species are higher than found in the mammals.

5-7 The extent to which any one or more of these pathways is used by any organism depends upon several factors. Changes in the nature of environmental conditions of the organism are associated with innumerable modifications in the pattern of life. Many adaptive changes of structure and behaviour are related to biochemical modifications which have been associated with the environmental changes. Reptiles occupy a pivotal position in the animal kingdom because they serve as a link between the poikilotherms and the homeotherms. Most of the studies that have been conducted on carbohydrate metabolism in reptiles are related to seasonal variations in blood sugar levels. Blood, glycogen content of liver and kidney and time course of changes in the above parameters and titratable acidity of liver and kidney homogenates of two reptilian species.

Materials and Methods

1. Animals: The lizards of local species of *Uromastix hardwickii* and *Varanus monitor* were collected from fields near Karachi. Animals of both sexes were kept in wooden boxes without food for several days before the experiments. The animals were collected during the period May to October which is the active period of these animals.

2. Preparation of tissue homogenates: Livers of three animals were pooled and a 10% homogenate was prepared in 0.02 M Tris-HCl buffer pH 7.36 + 0.1 M NaCl, 0.0039 M KCl and 0.002 M CaCl₂. In the case of *Varanus* kidneys of three animals and in case of *Uromastix* kidneys of six to eight animals were homogenised in the above buffer to give a 5% homogenate.

3. Studies with tissue homogenates: Samples of liver and kidney homogenates were drawn immediately at zero time and analysed for initial glycogen, glucose, total reducing capacity and titratable acidity. The rest of homogenates were incubated at 37 °C in a water bath with frequent shaking. Samples were drawn after 30, 90, 180 and 240 min and analysed for above parameters.

a) Glycogen in the homogenates was precipitated with ethanol and then determined according to the method of FALES.

b) True glucose was determined by NELSON-SOMogyi method.

c) Total reducing capacity of the homogenates at various time intervals was determined by FOLIN-WU method.
Results

The initial glycogen levels in the liver and kidney of *uromastix* and *varanus* are shown in Table I. The initial glycogen levels were found to be 3.96 and 0.33 mg-% in the liver and kidney of *uromastix* respectively, whereas the liver and kidney of *varanus* were found to contain 4.62 and 0.23 mg-% glycogen respectively. Thus the glycogen concentration in the liver of *uromastix* was 10 times that of its kidney while in *varanus* the glycogen content in liver was 20 times more than in kidney.

Table II shows the time course of changes in carbohydrate content and titratable acidity in the liver homogenate of *uromastix*. The total reducing capacity, true glucose, saccharoid fraction and titratable acidity increased gradually whereas glycogen content decreased. The total reducing capacity and true glucose levels increased from 0.38 to 1.23 mg-% and 0.19 to 0.66 mg-% respectively in 4 hours. The saccharoid fraction was increased 2 1/2 times, from 0.2 to 0.57 mg-%. The titratable acidity increased from 13.9 to 17.0 µeq/100 mg tissue. The glycogen levels were however decreased from 1.97 to 0.5 mg-%.

The time course of changes in carbohydrate content and titratable acidity in the kidney homogenate of *uromastix* is shown in Table III. The true glucose and total reducing capacity are more than doubled in 4 hours. The total reducing capacity was increased from 0.39 to 0.82 mg-%, whereas true glucose increased from 0.18 to 0.45 mg-%. The saccharoid fraction was increased from 0.21 to 0.40 mg-%, similarly titratable acidity increased from 12.75 to 14.2 µeq/100 mg tissue. During this period of time the glycogen level fell from 0.17 to 0.06 mg-% hence it was reduced to about 1/4 in 4 hours.

The time course of changes in carbohydrate content and titratable acidity in the liver homogenate of *varanus* is shown in Table IV. The total reducing capacity and true glucose was increased 3 to 4 folds in 4 hours. The total reducing capacity was increased from 0.67 to 1.87 mg-% whereas true glucose level was increased from 0.28 to 1.17 mg-%. The saccharoid fraction and the titratable acidity also increased from 0.18 to 0.45 mg-%. The saccharoid fraction was increased from 0.39 to 0.70 mg-%. The titratable acidity was increased from 12.1 to 16.0 µeq/100 mg tissue, hence an increase of 32% in 4 hours. The glycogen level registered a sharp decline in the first 90 min (from
Table III. Time course of changes in carbohydrate content and titratable acidity in the kidney homogenate of *uromastix*.

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>Carbohydrates mg glucose/100 mg tissue wet weight</th>
<th>Titratable Acidity (µeq of lactate/100 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Reducing Capacity</td>
<td>True Glucose</td>
</tr>
<tr>
<td>0</td>
<td>0.39 ± 0.01 *</td>
<td>0.18 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.55 ± 0.02</td>
<td>0.25 ± 0.01</td>
</tr>
<tr>
<td>90</td>
<td>0.68 ± 0.01</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>180</td>
<td>0.78 ± 0.01</td>
<td>0.39 ± 0.03</td>
</tr>
<tr>
<td>240</td>
<td>0.82 ± 0.01</td>
<td>0.45 ± 0.05</td>
</tr>
</tbody>
</table>

* Mean ± S.E. Each value is a mean of 4 observations.

Table IV. Time course of changes in carbohydrate content and titratable acidity in the liver homogenate of *varanus*.

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>Carbohydrate mg glucose/100 mg tissue wet weight</th>
<th>Titratable Acidity (µeq of lactate/100 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Reducing Capacity</td>
<td>True Glucose</td>
</tr>
<tr>
<td>0</td>
<td>0.67 ± 0.04 *</td>
<td>0.28 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>1.10 ± 0.06</td>
<td>0.58 ± 0.01</td>
</tr>
<tr>
<td>90</td>
<td>1.43 ± 0.07</td>
<td>0.99 ± 0.06</td>
</tr>
<tr>
<td>180</td>
<td>1.79 ± 0.12</td>
<td>1.12 ± 0.10</td>
</tr>
<tr>
<td>240</td>
<td>1.87 ± 0.12</td>
<td>1.17 ± 0.08</td>
</tr>
</tbody>
</table>

* Mean ± S.E. Each value is a mean of 4 observations.

Table V. Time course of changes in carbohydrate content and titratable acidity in the kidney homogenate of *varanus*.

<table>
<thead>
<tr>
<th>Time [min]</th>
<th>Carbohydrate mg/100 mg tissue wet weight</th>
<th>Titratable Acidity (µeq lactate/100 mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total Reducing Capacity</td>
<td>True Glucose</td>
</tr>
<tr>
<td>0</td>
<td>0.43 ± 0.08 *</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>30</td>
<td>0.40 ± 0.03</td>
<td>0.14 ± 0.02</td>
</tr>
<tr>
<td>90</td>
<td>0.52 ± 0.06</td>
<td>0.18 ± 0.03</td>
</tr>
<tr>
<td>240</td>
<td>0.61 ± 0.06</td>
<td>0.25 ± 0.03</td>
</tr>
</tbody>
</table>

* Mean ± S.E. Each value is a mean of 4 observations.

3.40 to 0.84 mg-%) and was then reduced gradually to 0.30 mg-% at the end of 4 hours incubation period. During the total incubation period the glycogen level was reduced to 1/10 the initial levels. The two reptilian species used in the present studies — namely *uromastix* and *varanus* are taxonomically very closely related to each other. Both of them belong to the natural order Lacertilia and have
adapted for the desert life and live deep in burrows in sandy areas. Inspite of this they do have anatomical and physiological differences distinct enough to place them in two different genera.

The glycogen levels in the kidney and liver tissues (Table I) are very much similar in both the species. The livers of *uromastix* and *varanus* were found to contain 3.96 and 4.62 mg glycogen per 100 mg wet wt of tissue. These values are similar to those observed in other animal species. WEBER and CANTERO\textsuperscript{15} reported 4 mg-% glycogen in rat liver while LYON and PORTER\textsuperscript{16} reported 5.07 mg-% in mice liver. In human beings the liver glycogen content is 3.15 mg-%\textsuperscript{17} while in a lizard *Varanus gresesus*, it is 4.4 mg-%\textsuperscript{18}. The glycogen content in kidneys of both the reptiles is considerably less in comparison to that of their livers. This is not surprising because liver plays an important role in accumulating and storing the excess sugar from the blood in the form of tissue glycogen. This reserve carbohydrate is released into the blood circulation when sugar level falls below a critical level in the blood. Such a regulatory mechanism by the liver has been known for a long time.

Although the levels of liver glycogen in various species are about the same, there is a considerable difference in the glycogen levels of the kidneys of different species. KREBS et al.\textsuperscript{18} have reported 0.01 mg-% glycogen in rat kidney but higher glycogen content in the kidneys of reptiles has been reported\textsuperscript{19}. Perhaps the high glycogen content in the kidneys of these reptiles indicates a higher metabolic activity in this tissue as compared to mammals.

During the course of incubation of the liver homogenate of *uromastix* (Table II) over a period of 4 hours there was a gradual drop in the glycogen content while the total reducing capacity, true glucose, saccharoid fraction and titratable acidity increased concomitantly. During the process of glycolysis when glycogen breaks down, pyruvate and lactate will be accumulated, along with an increase in the free glucose and sugar phosphate levels. The total reducing capacity usually includes besides the true glucose a number of other components including sugar phosphates\textsuperscript{20}. The difference between the total reducing capacity and true glucose is referred to as saccharoid fraction. During the breakdown of glycogen the increase in saccharoid fraction indicates the accumulation of sugar phosphates. Since the end product of glycolysis is the pyruvic and/or lactic acid, the increase in titratable acidity therefore is not surprising.

Similar pattern of changes in total reducing capacity, true glucose, saccharoid fraction, titratable acidity and glycogen was observed in the liver homogenate of *varanus* (Table IV).

The time course of changes in carbohydrate content and titratable acidity in the kidney homogenate of *uromastix* (Table III) show that total reducing capacity, true glucose, saccharoid fraction and titratable acidity increased gradually with time whereas glycogen decreased. Time course of changes in total reducing capacity, true glucose, saccharoid fraction, titratable acidity and glycogen in the kidney homogenate of *varanus* is shown in Table V. A gradual increase in total reducing capacity, true glucose and titratable acidity was noted whereas the saccharoid fraction and glycogen content showed no significant change.

Kidney homogenates of *uromastix* and *varanus* showed a different pattern of changes. Increase in total reducing capacity, true glucose, and saccharoid fraction was more in case of *uromastix* than in *varanus* and specifically saccharoid fraction showed no significant change in the latter.

The total reducing capacity, true glucose and the saccharoid fraction were higher in the blood of *uromastix* than in *varanus* (Table VI). These levels are however significantly higher than those found in mammals\textsuperscript{21}. The saccharoid fraction is related to the blood sugar level and varies with total reducing capacity. This is in agreement with the findings of KHAN and RAHMAN\textsuperscript{22} who reported that the change in the value of saccharoid fraction was similar to that of sugar and the behaviour was similar in normal as well as in diabetes.

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The Effect of some Sodium Substitutes on the Receptor Potential of the Crayfish Photoreceptor Cell

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Receptor potential, Na⁺-substitutes, ion dependence, photoreceptor cell, Crustacea

Isolated crayfish retinas were perfused with four solutions in which Li⁺, NH₄⁺, Tris H⁺ and glucose were substituted for the sodium ions in the physiological salt solution.

The changes of the extracellularly recorded receptor potential (ReP) evoked by short or long stimuli were measured. The changes in the shape of ReP by test solutions were different for each Na-substitute.

For lithium ions as a Na-substitute (Tab. I and Fig. 3) the plateau value hₑ was considerably decreased (to ~20%) contrary to the peak-amplitude hₘₐₓ which even slightly increased.

Ammonium ions show quite a different effect than all the other substitutes. The ReP is decreased strongly and irreversibly (Tab. II and Fig. 4). When Tris (hydroxymethyl-ammoniummethane-hydrochloride) is substituted for Na, hₘₐₓ decreased to about 60 per cent and the plateau is even more reduced (to 20 per cent; Tab. III). Only the recovery-value for hₑ (50% smaller) is markedly different contrary to our former experiments where choline was used as Na-substitute ( decrease 20%).

Glucose as a substitute for sodium chloride caused strongly decreased peak-amplitude hₘₐₓ 26% (Tab. IV and Fig. 9). Increased osmotic pressure due to excess glucose causes irreversible damage of the ReP (Tab. V). All the changes except those produced by NH₄⁺ were reasonably reversible. The results can be explained by the following assumptions:

a) the maximum of the ReP is caused mainly by an increase in the permeability of the cell membrane for sodium. Ca⁺- and Mg⁺-ions also contribute to it to a certain degree.
b) The plateau value of the ReP to long light stimuli is determined:
   1. by the sodium concentration gradient,
   2. by active transport processes,
   3. by the Ca²⁺- and Mg²⁺-gradients,
   4. the chloride gradient may perhaps contribute to this value.

It is generally accepted that the receptor potential of the invertebrate retina is caused by ion currents across the visual cell membrane. In this process positive ions permeating from the external medium into the cell play a decisive role.

In earlier publications we have reported ion substitution experiments in the crustacean retina (Stieve¹ ²). When external sodium was replaced by choline, we observed effects which were at least in part a specific result of the substitute ion. The present paper deals with a series of experiments performed with different sodium substitute ions in order to determine more exactly the specific role of sodium in the process leading to the receptor potential.

One should keep in mind that sodium ions play a different role in the visual system of invertebrates as compared to vertebrates; and that they possibly per-