Toxic effects of DDT, malathion and mercury on the tissue carbohydrate metabolism of *Sarotherodon mossambicus* (Peters)

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**Abstract.** Toxic stress due to DDT, malathion and mercury on the tissue carbohydrate metabolism of *Sarotherodon mossambicus* revealed the following manifest effects: (i) concentration of free sugars in the liver and muscle increased due to mobilisation of it from its bound form, glycogen and (ii) the normal carbohydrate metabolic pathway was altered indicating a switch over to anaerobic state involving the conversion of sugars into more lactate via pyruvate.

**Keywords.** Toxicosis; lactic acidosis; stress syndrome; extracarbohydrate source.

1. Introduction

Studies on blood sugars of fishes exposed to toxicants have demonstrated that tissue carbohydrate sources are mobilised resulting in what is known as hyperglycemia (Holmberg *et al* 1972; Grant and Mehrle 1973; McLeay and Brown 1975; Watson and McKeown 1976). The changes which the free sugars undergo after their mobilisation from bound state remain to be understood. Studies on fishes subjected to physical stress have revealed that lactic acid and pyruvic acid levels are altered following the changes in the tissue carbohydrates (Black 1957; Black and Barrett 1957). The above studies also suggested that lactate and pyruvate concentrations in tissues serve as good indices to assess the stress manifestations. Very little is known about the effects of toxic stress on these intermediary metabolites of carbohydrates. Hence, in the present study, the effects of DDT, malathion and mercury (mercuric chloride) on the blood and tissue carbohydrates and their intermediate metabolic derivatives namely lactic and pyruvic acids were determined.

2. Materials and methods

Specimens of *Sarotherodon mossambicus* were obtained from the fresh water ponds of Tamil Nadu State Fisheries Research Station, Chetpet, Madras. They were maintained in large aquaria (110 × 85 × 75 cm) for a period of two weeks for acclimation as suggested by Chavin and Young (1970). During the period of acclimation, the fishes were fed with dried prawn powder mixed with cooked rice. Water was changed on alternate days. Well water was used for both acclimation. Water temperature was maintained at 30 ± 1°C. As mentioned above, 3 sets, each set comprising as 0-01 ppm DDT, 0-95 ppm malathion and 0-1 ppm mercuric chloride concentrations. The pesticides were the analar grade mercuric chloride.
was used as the source of mercury. The sublethal concentrations were deduced by multiplying an application factor of 0.25 to the LC<sub>50</sub> values of the above compounds.

The fishes used for the above sublethal experiments varied in weight from 15–20 g. Every 24 h, the exposed fishes were transferred into fresh solutions of toxicants. The fishes were fed during the experimental periods. The surviving fishes in each treatment and control were sampled at intervals of 24 h, 7 and 15 days. Before the removal of tissues (liver and muscle) blood was collected from the fishes by tail severance. Potassium oxalate (0.8%) mixed with ammonium oxalate (1.2%) was used as the anticoagulant for rinsing the pipette before withdrawing blood. Blood and tissue lactate were determined by the method of Barker and Summerson (1940). Pyruvic acid was determined following the procedure of Friedemann and Haugen (1943). Tissue glycogen and free sugars were estimated by using the anthrone reagent (Roe 1955; Caroll et al 1956). All determinations were carried out in Bausch and Lomb Spectronic 20 Spectrophotometer. The values obtained for each toxicant at the 3 intervals were compared with the corresponding control values by Student's t test. The significance was attached to differences at 0.05 level.

3. Results

The results on tissue carbohydrate metabolites are given in table 1. In the liver of DDT exposed fishes 66% depletion of the total initial level was noticed after 15 days. In contrast, the muscle glycogen content showed 90% depletion. The free sugars in liver increased after 24 h and 7 days. In muscle it increased after 24 h and 15 days with a decline in between at 7 days. The liver and muscle glycogen in malathion exposed fishes decreased by 65 and 85% respectively at the end of 15 days. The glycogen in the liver of mercury exposed fishes showed 80% decline while its depletion in muscle was significant even after 24 h. The free sugars in the liver and muscle of these two groups also remained at a higher level corresponding to the glycogen decrease. In the muscle of these groups the free sugars increased at the end of 15 days.

In the liver, significantly higher levels of lactic acid persisted at all the 3 intervals in DDT and malathion exposed fishes while in mercury exposed ones its level increased significantly after 24 h and 7 days but became insignificant after 15 days from that of the control. The pattern of changes in blood lactic acid levels in malathion and mercury exposed groups conformed to its corresponding levels in the muscle at all intervals. On the contrary, in DDT exposed fishes, there was no conformity in the blood and muscle lactate levels, except at 24 h. Tissue pyruvic acid increased following the depletion of glycogen.

4. Discussion

In the control group, the lactic acid in blood and muscle showed differences between the 3 intervals viz., 24 h, 7 and 15 days. The above variations could be due to changes in the normal metabolism of the fishes and also to the smaller blood volume by which the exchange of metabolites from and into the tissues is mediated. Previous studies also reported fluctuations and transitory rise and fall of blood sugars and tissue lactate in normal fishes (Black and Barrett 1957; Chavin and Young 1970).
Table 1. Effects of toxicants on metabolites of S. mossaebicus.

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Glycogen</th>
<th>Free sugars</th>
<th>Pyruvic acid</th>
<th>Lactic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Liver</td>
<td>Muscle</td>
<td>Liver</td>
<td>Muscle</td>
</tr>
<tr>
<td></td>
<td>(µg/100 mg wet wt.)</td>
<td>(µg/100 mg wet wt.)</td>
<td>(µg/ml)</td>
<td>(µg/ml)</td>
</tr>
<tr>
<td>Control</td>
<td>24 h</td>
<td>5.83±0.72</td>
<td>0.15±0.02</td>
<td>3.41±0.48</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.82±1.07</td>
<td>0.18±0.03</td>
<td>3.45±0.51</td>
</tr>
<tr>
<td></td>
<td>7 Days</td>
<td>3.27±0.44</td>
<td>0.14±0.03</td>
<td>3.85±0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.78±0.35</td>
<td>0.23±0.02</td>
<td>4.05±0.34</td>
</tr>
<tr>
<td></td>
<td>15 Days</td>
<td>3.79±0.93</td>
<td>0.18±0.02</td>
<td>3.84±0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.81±0.35</td>
<td>0.24±0.01</td>
<td>4.02±0.30</td>
</tr>
<tr>
<td>DDT</td>
<td>24 h</td>
<td>5.74±1.18</td>
<td>0.74±0.05</td>
<td>4.73±1.26</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5.71±1.53</td>
<td>0.79±0.05</td>
<td>4.70±1.28</td>
</tr>
<tr>
<td></td>
<td>7 Days</td>
<td>3.81±0.94</td>
<td>0.23±0.03</td>
<td>4.31±0.34</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.83±0.35</td>
<td>0.24±0.01</td>
<td>4.08±0.31</td>
</tr>
<tr>
<td></td>
<td>15 Days</td>
<td>4.32±1.09</td>
<td>0.29±0.04</td>
<td>4.82±1.18</td>
</tr>
<tr>
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<td></td>
<td>3.43±0.35</td>
<td>0.24±0.01</td>
<td>4.08±0.31</td>
</tr>
<tr>
<td>Melathion</td>
<td>24 h</td>
<td>8.93±0.92</td>
<td>9.88±0.92</td>
<td>11.44±1.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9.74±1.14</td>
<td>9.71±1.14</td>
<td>11.43±1.15</td>
</tr>
<tr>
<td></td>
<td>7 Days</td>
<td>3.77±1.09</td>
<td>9.77±1.09</td>
<td>11.44±1.17</td>
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<td></td>
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<td>3.77±1.09</td>
<td>9.77±1.09</td>
<td>11.44±1.17</td>
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<tr>
<td></td>
<td>15 Days</td>
<td>5.29±1.92</td>
<td>10.77±1.92</td>
<td>13.77±2.92</td>
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<tr>
<td></td>
<td></td>
<td>5.29±1.92</td>
<td>10.77±1.92</td>
<td>13.77±2.92</td>
</tr>
<tr>
<td>Mercury</td>
<td>24 h</td>
<td>2.26±0.93</td>
<td>0.99±0.37</td>
<td>0.20±0.02</td>
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<tr>
<td></td>
<td></td>
<td>2.26±0.93</td>
<td>0.99±0.37</td>
<td>0.20±0.02</td>
</tr>
<tr>
<td></td>
<td>7 Days</td>
<td>1.93±0.99</td>
<td>0.97±0.35</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.93±0.99</td>
<td>0.97±0.35</td>
<td>0.18±0.02</td>
</tr>
<tr>
<td></td>
<td>15 Days</td>
<td>1.94±0.96</td>
<td>0.98±0.35</td>
<td>0.18±0.02</td>
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<tr>
<td></td>
<td></td>
<td>1.94±0.96</td>
<td>0.98±0.35</td>
<td>0.18±0.02</td>
</tr>
</tbody>
</table>

*Significant (P<0.05). Values are given as Mean ± SD.
Hence the levels of metabolites in experimental groups were compared to the corresponding levels in the control fish at the respective time intervals.

Increase of lactic acid in tissues is attributed to the inadequacy of oxygen supply in cells to cope up with complete breakdown of carbohydrates to carbon di-oxide and water (Seskin and Levine 1947). Hence the persistence of tissue lactate in fishes exposed to DDT and malathion in the present study implies hypoxia. Significant decrease in the oxygen consumption of the whole animal and corresponding decrease in the succinic dehydrogenase (SDH) enzyme activity in the liver and muscle of DDT and malathion treated fishes also support the above implication of tissue hypoxia (Ramalingam 1980, 1985).

Moreover the persistence of higher levels of lactic acid in both muscle and liver of DDT group while in the liver of malathion exposed fishes at all time intervals may also be due to impairment in the diffusion of it from the above tissues. Such impairment in the diffusion of tissue lactate into the blood has been reported in stressed fishes. The stasis in the portal vessels has been demonstrated to enhance the above (Heath and Pritchard 1965). The histopathological changes in the liver of DDT and malathion exposed fishes which revealed occlusion of red blood cells in the periportal vessels of liver also indicated heart’s congestion and stasis during toxicity in this species (Ramalingam 1985).

In fishes exposed to mercury, both liver and muscle showed no build up of lactate at the end of 15 days. This indicates a mechanism in Hg-exposed fishes for the elimination of lactic acid. The possibility of lactic acid being excreted in the urine of fishes could be inferred from the studies of Karuppannan and Kutty (1978) who have shown that *Tilapia mossambica* (*Sarotherodon mossambicus*) is capable of excreting as much as 25 μg/kg/h of lactic acid under forced swimming stress. In addition to the excretion of lactic acid, evidences are also available which suggest that it could be eliminated through gills (Bates and Vinsonhaler 1956). Studies on anxiety neurosis stress in human also demonstrated that lactic acid accumulates in the tissues, in to couple with cations such as Ca$^{2+}$, Mg$^{2+}$ and K$^{+}$ (Wallace 1975).

Tars levels in the present study reveal that in addition to glycogen other stores bound to protein or protein itself may also be involved in the increase in fishes under toxic stress. The increase in 15 days in the muscle of DDT and malathion groups hydrate sources contributing to free sugars pool studies (Coleman 1968). Significant qualitative changes in the 5 and 15 days of exposure the sparing
Metabolic effects of toxicants on *S. mossambicus*

Metabolic effects of DDT and Malathion on *S. mossambicus*

TOXICANT

DDT & MALATHION

FISH

PROTEINS (NEO-GLUCOGENESIS)

TISSUE GLUCOGEN LIVER & MUSCLE

BOUND HEXOSAMINES

TISSUE FREE SUGARS

BLOOD SUGAR (HYPERGLYCEMIA)

PYROVIC ACID

REDUCTION (LIDH)

KREBS CYCLE

BLOOD

GILLS

ELIMINATION THROUGH ION EFFLUX

LACTIC ACID

TISSUES

RED OXIDATION

CO₂ + H₂O

KIDNEYS

EXCRETION URINE

Figure 1.

The lethality to fishes by toxicants may be due to the dysfunctioning of 5 different routes R-I, R-II, R-III, R-IV and R-V through which the intermediate metabolites are either used or eliminated in the normal unstressed fishes. The results of the present investigation, revealing a striking similarity to the features of stress syndrome of Selye (1950a, b), could be ascribed to hormonal imbalance.

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