NOTE

Studies on Protein and Nucleic Acid Contents of Toad Testes Following Administration of Sodium Malonate

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Synopsis

An increase in protein and both RNA and DNA contents of toad testes was observed following malonate administration. This rise in testicular protein and nucleoprotein contents after malonate treatment was possibly the result or cause of stimulation of both spermatogenic and steroidogenic activities in toad testes.

The importance of pentose phosphate pathway in gonadal steroidogenesis has been reported by several workers (Deane et al., 1962; McKerns, 1965b; Nielson and Warren, 1965; Savard et al., 1963). The stimulation of pentose phosphate pathway and subsequent increase in nucleoprotein synthesis has been shown by McKerns (1965a) to stimulate the enzyme systems involved in steroidogenesis in the adrenal cortex of rats. The relative stimulation of pentose phosphate pathway over the tricarboxylic acid cycle (TCA cycle) by malonate, has been observed to cause increased steroidogenesis in toad testes (Dey et al., 1971) and in the adrenal cortex (Deb et al., 1971). Steroidogenesis is also found to be increased, following malonate treatment in the ovaries of both mature (Dey and Deb, 1972) and immature (Dey et al., 1972) rats. NADPH generated from the accelerated pentose phosphate pathway after malonate treatment has been considered to be the cause of stimulated steroidogenesis in the above mentioned glands.

But relatively little is known about the protein and nucleoprotein metabolism in the testes after suppression of TCA cycle and simultaneous stimulation of the pentose phosphate pathway in the lower vertebrates. In the present investigation, therefore, experiments have been performed on the effects of suppression of succinic dehydrogenase (SDH) in the TCA cycle by the administration of sodium malonate and the subsequent alterations in the testicular protein, ribonucleic acid (RNA) and deoxy-ribonucleic acid (DNA) contents in the toads (Bufo melanostictus).

Materials and Methods

20 male toads (Bufo melanostictus) weighing between 45–55 g. were collected from their natural habitat during the breeding season. They were divided into two groups, containing equal number of animals in each and were kept in cages, wet with running tap water, for a few days to be acclimatized with the laboratory environment. The experimental group of animals were given sodium malonate injection in the dorsal lymph sac, at a dose level of 80 mg/100 g. body wt. in 0.6% NaCl for 7 consecutive days. The control group of animals were simultaneously injected with the same amount of the solvent only. The animals of both the groups were sacrificed 24 hr after the last injection and the testes were dissected out immediately. Some portion of the testes from each animal was fixed in Carnoy's fluid for histological studies, following the common laboratory procedures. The remaining

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portions of the testes were immediately frozen at \(-10^\circ C\) and later used for biochemical estimation of the protein content (Gornall et al., 1949), and the RNA and DNA content (Schneider, 1946; Ceriotti, 1955; Croft and Lubran, 1965).

**Results**

Treatment with sodium malonate caused increased spermatogenic activity in the tubules and heightened activity of the intertubular cells as evidenced from the histological studies of the toad testes (Figs. 1 & 2). The same treatment also produced an increase in protein and both RNA and DNA contents of the toad testes in comparison to those in control animals (Table 1).

![Fig. 1](image1.png)

**Fig. 1.** The section of a toad testis from the normal group showing all stages of spermatogenesis. Many of the spermatozoa are attached near the basement membrane. \(\times\) 192.

![Fig. 2](image2.png)

**Fig. 2.** The section of a testis from malonate-treated toad showing increased number of spermatogenic cells. Most of the sperms are released into the lumen. Proliferation of Leydig-cells are also evident. Compare with Figure 1. \(\times\) 192.

<table>
<thead>
<tr>
<th>Group</th>
<th>No. of animals</th>
<th>Testes</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>RNA mg/mg wet wt. tissue</td>
</tr>
<tr>
<td>Control</td>
<td>6</td>
<td>17.9 (\pm) 1.19</td>
</tr>
<tr>
<td>Treated</td>
<td>6</td>
<td>25 (\pm) 1.4</td>
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<tr>
<td></td>
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<td>(p &lt; 0.005^*)</td>
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* Statistical analysis by Student's t-test.
The values indicate Mean \(\pm\) S.E.M.
Discussion

The role of pentose sugars generated from the pentose phosphate pathway in the de novo synthesis of nucleoproteins is a well-established fact. Increase in RNA and DNA contents in toad testes after suppression of SDH activity in TCA cycle by malonate, found in the present investigation, may possibly be due to the acceleration of the pentose phosphate pathway in that condition. Similar acceleration of pentose phosphate and subsequent increase in steroidogenesis in the toad testes (Dey et al., 1971) and in the adrenal cortex (Deb et al., 1971) and ovaries of the mature and immature rats (Dey and Deb, 1972; Dey et al., 1972) after malonate (80 mg/100 g. body wt.) treatment has already been reported. Stimulation of ovarian pentose phosphate pathway and steroidogenesis following in vitro addition of sodium malonate in the incubation medium has also been observed (Deb and Dey, 1972). Increased gamatogenic activity as evidenced from the higher concentrations of DNA and RNA as well as cytology of the testes following malonate administration may possibly be due to the increased testicular androgen formation in this condition. Role of androgens in spermatogenesis is well known (Clarmont and Harvey, 1967). The rise in protein content in the toad testes after malonate treatment in the present communication probably suggests, increased synthesis of enzyme proteins, involved in steroidogenesis and the rise in testicular protein content, was probably, the result of increased testicular nucleoprotein synthesis in that condition.

Glucose-6-phosphate dehydrogenase, a key enzyme in the pentose phosphate pathway and Δ⁵-3β-hydroxysteroid dehydrogenase (Δ⁵-3β-OHD) involved in steroidogenesis have been shown to occur both in Leydig cells and spermatogenic cells of toad testes which showed increased activities following acceleration of pentose phosphate pathway after suppression of TCA cycle by sodium malonate (Dey et al., 1971).

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References