Zinc Metabolism and Dibutyl Phthalate-Induced Testicular Atrophy in the Rat

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It is an established fact that zinc is essential for the maintenance of normal testicular function (Gunn & Gould, 1970). Further, the protective role that zinc plays on testicular atrophy caused by cadmium (Parizek, 1957) prompted us to look at the possible role of zinc metabolism on the action of another causative agent of atrophy, namely dibutyl phthalate. The method adopted was to measure the excretion of $^{65}$Zn in the urine and its tissue distribution and half-life in the testes, liver and kidneys.

Fig. 1. $^{65}$Zn excretion in dibutyl phthalate-treated rats

The results are expressed as a percentage of the control (corn oil)-rat excretion value. Day 0 corresponds to the comparison of excretion values before the commencement of treatment. Results for days 1–9 represent the amounts of $^{65}$Zn excreted on daily treatment with 2 g of dibutyl phthalate/kg (as described in the text) compared with the equivalent daily excretion in the corn-oil-treated controls.
Table 1. Effect of dibutyl phthalate administration on the half-life of total $^{65}$Zn content of liver, kidney and testes

Rats were injected with $^{65}$Zn and treated with dibutyl phthalate as described in the text. A zero-day killing was carried out and the time-period required to clear half of that tissue content determined by killings between 1 and 14 days.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver</td>
<td>4.1 (3.1–4.9)</td>
<td>5.3 (3.7–7.1)</td>
</tr>
<tr>
<td>Kidney</td>
<td>2.8 (2.5–3.1)</td>
<td>3.9 (3.1–4.3)</td>
</tr>
<tr>
<td>Testes</td>
<td>&gt;14</td>
<td>5.5 (5.2–6.1)</td>
</tr>
</tbody>
</table>

Charles River Sprague–Dawley rats, 3–4 weeks old (body wt. 60–90 g), were allowed food and water ad libitum in all experiments. $^{65}$Zn was injected subcutaneously in the intrascapular region of the rat at a dosage of 25 $\mu$Ci/kg. The dibutyl phthalate was dissolved in corn oil and administered daily for the desired period of treatment by oral intubation at a dosage of 2 g/kg. Controls received an equivalent volume of corn oil.

Groups of rats were placed in Metabowls and 24 h urine specimens were collected from control and treated animals over a period of 4–9 days. Over the 9-day period it was found in the treated animals that there was an increased urinary excretion of $^{65}$Zn centred over the first 4 days, which then tapered off until no apparent difference between the two groups was evident (Fig. 1). Accordingly, in subsequent studies a 4-day period of treatment was adopted. At the end of this period of treatment, considerable testicular atrophy was evident (approx. 30% loss in weight of testes). It was apparent in all studies that the maximum excretion of $^{65}$Zn occurred on the second day of treatment (156–166% of the control value) and that overall there was a 38–45% increase in $^{65}$Zn excretion over the 4-day period.

It was then decided to see whether this increased zinc excretion was reflected in the $^{65}$Zn concentrations in the testes, liver and kidney. The rats were treated with dibutyl phthalate and $^{65}$Zn as described previously and then killed by cervical dislocation after 2 and 4 days of treatment. The respective tissues were then excised and prepared for radioactivity counting. It was found that in the testes even after 2 days of treatment there was a significant ($P<0.05$) decrease in the concentration of zinc in the testes. At this stage atrophy was minimal, with a decrease in testes weight of approx. 10%. After 4 days the decrease in the $^{65}$Zn concentration in the testes was approx. 23% of the control value ($P<0.001$). In all these studies there was no decrease in the $^{65}$Zn concentration in the kidney or liver; if anything, there was a slight increase.

Dibutyl phthalate treatment also markedly altered the half-life of $^{65}$Zn in the testes. The results (Table 1) showed that the half-life of $^{65}$Zn in the testes of control animals exceeded 14 days and that dibutyl phthalate administration decreased by over 50% the half-life of $^{65}$Zn in the testes. This effect was not observed in the liver or kidney.

These findings indicate that zinc metabolism of the testes is markedly affected by the administration of dibutyl phthalate. Preliminary results suggest that a considerable degree of protection is afforded by the co-administration of zinc with dibutyl phthalate.
