β-GLUCURONIDASE AND ESTERASE ACTIVITIES OF RAT TESTIS AFTER X-RAY TREATMENT

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Summary. The β-glucuronidase and esterase content of rat testes after X-ray treatment has been studied. It was found that together with the tubular damage induced by the radiation there was a definite and progressive increase of β-glucuronidase activity. Variations in the acetic and valeric esterase activities were not clearly defined. As the radiation causes tubular cell destruction while the Leydig interstitial cells remain apparently undamaged, the authors think that the enzymatic variations may be related to a hyperfunctional state of the interstitial cells during the destructive action of the X-rays on the tubular epithelium.

INTRODUCTION

A vitamin E deficient diet, when fed for a prolonged time to young male rats, produces damage of the seminiferous epithelium (Mason, 1925). It has been demonstrated that such a diet also causes an early rise and a subsequent decline of the β-glucuronidase and esterase enzymatic activities of the testes (Arata, Santoro, Severi & Pecora, 1962a). As the Leydig cells do not seem to be affected by the diet, at least from a morphological viewpoint, it is possible that they might be responsible for the variation of these enzymatic activities.

To elucidate the relationships existing among β-glucuronidase, esterase, tubular epithelium damage and interstitial cells, we have investigated the effects of X-ray treatment of the scrotal area of rats on the β-glucuronidase and esterase activities of the testes. The testicular lesions produced by X-rays differ from those due to vitamin E deficiency in that the former involve first the immature, then the mature cells of the seminal line, while the latter involve first the mature and then the immature cells. At a later date, however, both treatments produce an apparently similar picture: the cells of the seminal line have disappeared while the interstitial tissue is well preserved.

MATERIALS AND METHODS

Thirty Long-Evans adult male rats, weighing 230±20 g were used. Ten were kept as a control and killed by decapitation on the 15th day from the beginning of the experiment. Twenty were anaesthetized with an intraperitoneal injection of sodium methyl-thioetil-2'-pentilthiobarbiturate (Diogenal, 20 mg/100 g body weight) and their scrotal area exposed to 600 r of X-rays under the following...
conditions: scrotal area exposed through an opening in a lead plate shielding the rest of the body; 80 kV; 6 mA; the radiation filtered through a 3 mm thick aluminium filter.

The rats thus treated were divided at random into five groups of four animals, each group being killed by decapitation every 10 days, starting 10 days after irradiation.

The testes of the experimental and control animals were removed, weighed and fragments were taken for histological studies and fixed in Bouin’s solution. The seminal vesicles were drained of any fluid, gently blotted and weighed. Duplicate samples of testicular homogenates were used for each β-glucuronidase and esterase determination. β-glucuronidase activity was measured according to Fishman’s micromethod (Fishman, Springer & Brunetti, 1948) using phenolphthalein glucuronate (Light & Co, Colnbrook) as substrate. A unit of enzymatic activity was defined as the amount of enzyme liberating 1 µg of phenolphthalein in 1 hr under the conditions employed.

Esterase activity was measured by the method described by Huggins & Lapides (1947), using p-nitrophenol acetate and p-nitrophenol valerate as substrates, prepared by Spasov’s technique. A unit of enzymatic activity was defined as the amount of enzyme liberating 1 µmole of p-nitrophenol in 20 min under the conditions of the method. The β-glucuronidase and esterase concentrations have been expressed here in terms of units per total wet weight of testis.

The results of the enzymatic assays were submitted to analysis of variance and to Student’s ‘t’ test.

RESULTS

The testicular lesions produced by the X-ray treatment are illustrated in Plate 1 and may be summarized as follows:

Ten days after irradiation the number of spermatogonia is greatly reduced, especially in the tubules close to the testicular surface. The other cells of the seminal line appear to be normal. The lumen of some tubules is filled with desquamated cells and with an amorphous eosinophilic substance. Alterations of the basal membrane or interstitial tissue are not noticeable.

Twenty days after irradiation, very few tubules present a normal appearance; most of them contain only spermatozoa and an amorphous substance of granular and filamentous aspect, the other cells of the seminal line having disappeared. Occasionally Sertoli cells and large macrophages are seen. The interstitial tissue seems undamaged.

Thirty days after irradiation, a decrease in the diameter of the tubules is evident and a few spermatozoa and Sertoli cells are recognizable near the basal membrane. The Leydig cells appear normal and are clearly visible, either because of an increase in their number or because of reduction in the size of the tubules.

Forty days after irradiation, the volume of the testes is greatly reduced and the tubules consist of a thickened basal membrane with few scattered Sertoli cells and spermatogonia, the latter occasionally in mitosis. The Leydig cells appear hyperplastic, while an eosinophilic, amorphous substance is visible in the intertubular spaces.
Fig. 1. Section of testis from rat 10 days after X-ray irradiation (x 75). Note particularly in some tubules the lumen occupied by desquamating cells and in others the absence of spermatogonia.

Fig. 2. Same testis as Fig. 1 (x 360). Lumen of a tubule: spermatogonia, spermatocytes, spermatids and spermatozoa are mixed with amorphous substance.

Fig. 3. Section of testis from rat 40 days after X-ray irradiation (x 75). Note particularly the intertubular cells.

Fig. 4. Same testis as Fig. 3 at higher magnification (x 210).

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Fifty days after irradiation the picture does not differ considerably from the one seen previously, except for the greater frequency of spermatogonia in mitosis. In rare tubules the regenerative process is more evident and it is even possible to observe two superimposed rows of spermatogonia.

The weights of experimental and control rats, the weights of the testes and seminal vesicles and the results of the enzymatic assays expressed in percentage values and per total weight of the testes are presented in Tables 1 and 2.

**Table 1**

**ENZYME ACTIVITY IN RELATION TO TOTAL WEIGHT OF TESTIS**

<table>
<thead>
<tr>
<th>Group†</th>
<th>Days after radiation</th>
<th>Average body wt (g ± s.d.)†</th>
<th>Average wet wt of testes (mg ± s.d.)</th>
<th>Average wet wt of seminal vesicles (mg ± s.d.)</th>
<th>ß-Glucuronidase activity (units/100 total wt of testes ± s.d.)</th>
<th>Acetic esterase activity (units/100 total wt of testes ± s.d.)</th>
<th>Valeric esterase activity (units/total wt of testes ± s.d.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>214 ± 21</td>
<td>2154 ± 279</td>
<td>510 ± 48</td>
<td>631 ± 170*</td>
<td>1225 ± 744*</td>
<td>428 ± 157</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>216 ± 59</td>
<td>1477 ± 292</td>
<td>601 ± 32</td>
<td>1122 ± 787</td>
<td>725 ± 412</td>
<td>248 ± 182</td>
</tr>
<tr>
<td>3</td>
<td>30</td>
<td>272 ± 54</td>
<td>1512 ± 332</td>
<td>580 ± 38</td>
<td>1587 ± 384</td>
<td>504 ± 119</td>
<td>138 ± 63**</td>
</tr>
<tr>
<td>4</td>
<td>40</td>
<td>237 ± 32</td>
<td>889 ± 155</td>
<td>640 ± 40</td>
<td>2239 ± 872**</td>
<td>435 ± 120</td>
<td>281 ± 54</td>
</tr>
<tr>
<td>5</td>
<td>50</td>
<td>251 ± 37</td>
<td>776 ± 100</td>
<td>710 ± 62</td>
<td>2124 ± 689**</td>
<td>727 ± 58</td>
<td>411 ± 44*</td>
</tr>
<tr>
<td>Control</td>
<td>—</td>
<td>209 ± 30</td>
<td>2231 ± 222</td>
<td>606 ± 30</td>
<td>1273 ± 393</td>
<td>386 ± 186</td>
<td>294 ± 82</td>
</tr>
</tbody>
</table>

* P < 0.05;  ** P < 0.01.
† Each group consists of four rats.
‡ Standard deviation was calculated according to the following expression: s.d. = \( \sqrt{\frac{\sum x^2}{n-1}} \) where x = deviation from group mean.

**Table 2**

**ENZYME ACTIVITY BY UNIT WEIGHT OF TESTIS TISSUE**

<table>
<thead>
<tr>
<th>Group*</th>
<th>ß-Glucuronidase activity (units/100 mg fresh tissue ± s.d.)</th>
<th>Esterase activity (units/100 mg fresh tissue ± s.d.)</th>
<th>Acetic</th>
<th>Valeric</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>30·5 ± 10·6</td>
<td>56·3 ± 29·8</td>
<td>22·4 ± 6·8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>78·4 ± 28·4</td>
<td>47·5 ± 22·2</td>
<td>16·2 ± 10·8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>126·0 ± 30·1</td>
<td>40·2 ± 10·0</td>
<td>9·7 ± 6·3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>242·0 ± 87·3</td>
<td>51·7 ± 10·1</td>
<td>32·2 ± 6·6</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>275·0 ± 17·4</td>
<td>94·5 ± 8·7</td>
<td>53·6 ± 7·1</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>47·9 ± 21·0</td>
<td>21·0 ± 10·0</td>
<td>13·0 ± 4·0</td>
<td></td>
</tr>
</tbody>
</table>

* Each group consists of four rats.

**DISCUSSION**

Examination of the results shows that after the X-ray treatment the weight of testis is sharply diminished by almost complete destruction of the seminiferous epithelium (a phenomenon previously observed by others), while the interstitial cells survive and function well, at least as far as can be judged by their histological picture and by the weight of the seminal vesicles during the whole period of the experiment.

The enzymatic content of the testis tissue varies, more especially with respect to ß-glucuronidase activity; the variation is less well defined with respect to the esterase activity.

A*
After an initial decrease, β-glucuronidase activity tends to increase progressively to the end of the experiment, reaching double the normal values. As shown in Tables 1 and 2, this is clearly evident whether the results are expressed in percentage values of the wet weight of testicular tissue or in total wet weight of testis.

Changes in esterase activity are not so clear and significant. Expressed as percentages the results suggest that, after an initial increase and a subsequent decrease (around the 20th to 30th day after radiation), a sharp increase of activity occurs around the end of the experiment.

When the values are expressed in total wet weight of testis, the phenomenon is not at all the same, and one observes only a conspicuous increase of the acetic esterase activity around the 10th and 50th day of experiment, while the valeric esterase activity diminishes noticeably around the 30th day and increases above the normal values around the 50th day.

Keeping in mind the small numbers of animals in the experimental groups, the enzymatic values have been analysed statistically. As mentioned above the analysis of the variance and Student’s ‘t’ test have been performed.

In the analysis of variance of the enzyme activities per total wet weight of testis, the value of $P$ was less than 0·001 in the case of β-glucuronidase activity, and between 0·05 and 0·01 in the case of the acetic and valeric esterase activity.

This first analysis gives us the rough indication that a definite influence of the experimental factors does exist. So, to ascertain in which test group a statistically significant variation of values in respect of the control did exist, we have compared the averages obtained from the data of the control animals and those of each experimental group.

The determination of these parameters has given statistical meaning to the values of β-glucuronidase activity observed on the 10th, 40th and 50th day of the experiment, while the acetic esterase activity gave significant values only on the 10th day, and the valeric esterase activity on the 30th and 50th day after irradiation.

From this statistical analysis it can be assumed that the X-ray treatment caused in the testis tissue of our animals a striking and progressive increase of β-glucuronidase activity beginning from the 30th day of irradiation. Of the acetic esterase activity it is only possible to say that there is an initial rise; there is a significant decrease in valeric esterase activity around the 30th day and a definite increase towards the end of the experiment.

Comparison of these results with those obtained by submitting albino rats to a vitamin E deficient diet for 6 to 7 months shows that the β-glucuronidase activity in the two experimental situations follows the same pattern, so that it seems possible to correlate the isolated lesion of the testicular seminiferous epithelium with the variation in β-glucuronidase activity.

It is not easy to explain the enzymatic variation, since very little is yet known about the biochemical value of these enzymes in the testis.

Concerning the esterases—as we observed in previous investigations on normal rats (Arata, Santoro, Severi & Pecora, 1962b)—those contained in the gonads of irradiated animals acted on the acetate rather than on the valerate $p$-nitrophenol substrate. Verne & Hébert (1952a, b) and Huggins & Moulton (1948)
found that enzymes of the esterase type are contained in the interstitial cells of Leydig, and, as they believe that the enzymatic concentration would directly depend on the grade of the functional maturity of such cells, they maintain that these enzymes in the testis would in a sense express the endocrine activity of the organ. Hence, it would appear that the esterase activity observed in the testis of the irradiated rat could be the indirect expression of a particular behaviour of the Leydig cells during the radiation damage. This is also supported by the histological researches of Verne & Hébert (1952a, b) on artificially cryptorchid rats, showing that in the retained testis, with an isolated destruction of the seminal epithelium, there is a remarkable increase in the esterase activity.

A similar hypothesis to the one just put forward for the esterase activity could be formulated for the testicular enzymatic activity of the β-glucuronidase type, which according to Hayashi, Ogata, Takuro & Kawose (1958) and ourselves (Arata et al., 1962b), seems to be connected with the function of the Leydig cells.

It therefore seems worth while to ascertain further the relationship between these enzyme activities and the seminiferous and interstitial cells of the testis.

REFERENCES


