THE EFFECT OF TESTOSTERONE ON
CELL RENEWAL AND MITOTIC CYCLES IN SEX
ACCESSORY GLANDS OF CASTRATED MICE

By
Pentti Tuohimaa and Mikko Niemi

ABSTRACT

The effect of testosterone propionate on cell renewal rate and mitotic cycle of castrated mice was investigated. 0.25 mg of the hormone was given thrice at 24 h intervals and 50 μc of ³H-thymidine was injected simultaneously with the last hormone injection. After this the mice were killed at 1 to 2 h intervals. Ventral prostates and seminal vesicles were weighed and the thymidine incorporation into their epithelia determined in radioautograms. A piece of proximal small intestine was used as reference tissue.

The labelling index in the castrated control animals was 1.3 for the seminal vesicle; 68 h after testosterone treatment it was 44. The figures for the ventral prostate were 1.9 and 37, and for the small intestine 44 and 57. The mitotic index in the prostate of the experimental animals and that in the intestine of all the animals was 0.5; in the seminal vesicle it was 0.4. The mitotic index in the accessory glands of the control mice was very low. The specific activity of DNA per nucleus was higher in the hormone treated than in the control animals.

The cell cycle (T_C) in the prostate was shortened from about 900 h to 20 h after testosterone. The synthetic phase (T_S) was shortened from 16 to 7 h, presynthetic (T_G1) phase from 900 to 10 h, whereas the postsynthetic phase (T_G2) remained unchanged. The corresponding figures for the seminal vesicle were: T_G2 from 4.5 to 0.5 h, T_S from 12 to 7 h, T_G1 from 900 to 10 h and T_C from 900 to 18 h. The changes in the intestinal epithelium were less marked: T_C from 22 to 16 h, T_S from 8.5 to 8 h, T_G1 from 12 to 6 h, while T_G2 remained unchanged.

This investigation was supported in part by research grant DRG-940 from the Damon Runyon Memorial Foundation.
Oestrogens and testosterone are considered to be mitogenic hormones, because of their general stimulating action on the mitotic activity of the body tissues (Allen 1958). The action of oestrogens has been explored in great detail by Epifanova (1966) who showed a marked shortening after oestrogen treatment of all the phases in the cell cycle of the epithelium in the uterus and cornea. On the other hand, testosterone is well known to stimulate mitotic activity in the male accessory sexual glands (Bullough & van Oordt 1950). The mitosis index increases within 30 h after a single testosterone injection into castrated mice and remains high up to 66 h (Allen 1958). This activation seems to be dose-dependent (Robson et al. 1965). However, in none of the previous communications has the effect of testosterone on the various mitotic phases been described, and thus the work presented in this paper has been considered of interest. As specific target organs for androgenic stimulation, the ventral prostate and the seminal vesicles were investigated, and the epithelial cells of the small intestine served as a non-specific tissue reference.

**MATERIAL AND METHODS**

35 adult (3 months) white mice were castrated. Two weeks after the operation, 18 of them were given 0.25 mg of testosterone propionate (Neo-Hombreol®, Organon) in 0.25 ml of sesame oil subcutaneously three times at 24 hourly intervals. Simultaneously with the last testosterone injection, 50 µc of ³H-methyl thymidine (Radiochemical Centre, Amersham, England, specific activity 5.0 c/mm) was injected. 17 mice served as castrated controls and were injected with sesame oil only; the method of administration in these cases and dose of thymidine given was the same as that of the experimental animals.

The animals were kept in a separate quiet room. Thymidine injection was given at 8 p.m. and the animals were killed 1 to 2 hours later and after that within 24 hours.

The accessory glands were prepared as a single block and the urethra was ligated. In addition, a piece from the proximal part of the small intestine was excised. The tissues were fixed in 4 per cent neutral buffered formaldehyde for 48 hours, after which the ventral prostate and the seminal vesicles were dissected free and weighed. The histology was performed in the usual way. 5 µ paraffin sections were used, and the radioautograms were prepared by dipping them into Ilford G-5 liquid emulsion as described by Joftes & Warren (1955). The sections were dried for 3 h under a cold air fan, and exposed for 10 days. The radioautograms were developed in Kodak's D-19b X-ray developer for 50 sec, washed with 3 per cent acetic acid and fixed in Amfix. The sections were finally stained with haematoxylin and eosin.

The radioautograms were evaluated by counting the number of labelled epithelial cell nuclei per 1000 cells. A cell was considered as labelled when at least 5 silver grains were present over its nucleus. From each animal altogether 30 to 50 mitotic figures were analyzed, except for the accessory glands of the control animals, where only 10 mitoses could be studied. All the cells in either meta-, ana- or telophase were considered as being under mitosis. The duration of the mitotic phases was determined according to the percentage of labelled mitoses; the synthetic period was estimated at the 50 per cent level of labelled mitoses (Rajewsky 1966).
RESULTS

Weight changes. An extensive weight increase in the accessory sexual glands was observed after testosterone treatment (Table 1); the weight of the ventral prostate increased by an average of 53 per cent and that of the seminal vesicles by 90 per cent. The weight increase of the prostate occurred sooner than that of the seminal vesicles, as the weight increment during the last 24 hours of the experiment was not more than 12 per cent. On the other hand, the largest weight increment of the seminal vesicle, was about 140 per cent, during the last 24 hours of the experiment.

Labelling and mitotic indices. The labelling index of the seminal vesicles of the control mice was 1.3 ± 0.3. In the testosterone treated experimental mice it was 44 ± 2.5, 20 hours after the thymidine injection. The corresponding

Table 1.

Weight of the sex accessory glands of the control and testosterone treated mice.

<table>
<thead>
<tr>
<th>Hours after ³H-thymidine labelling</th>
<th>Weight of the ventral prostate (mg)</th>
<th>Weight of the seminal vesicle (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>control</td>
<td>experimental</td>
</tr>
<tr>
<td>1</td>
<td>32.6</td>
<td>63.0</td>
</tr>
<tr>
<td>2</td>
<td>23.2</td>
<td>44.0</td>
</tr>
<tr>
<td>3</td>
<td>44.8</td>
<td>57.0</td>
</tr>
<tr>
<td>4</td>
<td>35.0</td>
<td>53.0</td>
</tr>
<tr>
<td>5</td>
<td>38.6</td>
<td>74.0</td>
</tr>
<tr>
<td>6</td>
<td>36.0</td>
<td>50.0</td>
</tr>
<tr>
<td>7</td>
<td>40.6</td>
<td>64.0</td>
</tr>
<tr>
<td>8</td>
<td>39.4</td>
<td>61.0</td>
</tr>
<tr>
<td>9</td>
<td></td>
<td>54.0</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>63.0</td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>65.0</td>
</tr>
<tr>
<td>12</td>
<td>42.2</td>
<td>43.0</td>
</tr>
<tr>
<td>14</td>
<td>31.0</td>
<td>54.0</td>
</tr>
<tr>
<td>16</td>
<td>33.4</td>
<td>51.0</td>
</tr>
<tr>
<td>18</td>
<td>38.6</td>
<td>60.0</td>
</tr>
<tr>
<td>18</td>
<td>53.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>49.0</td>
<td>52.0</td>
</tr>
<tr>
<td>22</td>
<td>30.0</td>
<td>80.0</td>
</tr>
<tr>
<td>24</td>
<td>32.0</td>
<td>52.0</td>
</tr>
<tr>
<td>26</td>
<td>42.6</td>
<td></td>
</tr>
<tr>
<td>Mean ± standard error of the mean</td>
<td>37.8 ± 1.8</td>
<td>57.8 ± 2.3</td>
</tr>
</tbody>
</table>
figures for the ventral prostate were $1.9 \pm 0.5$ and $37 \pm 3.2$. The labelling index of the small intestine increased from $44 \pm 3.0$ to $57 \pm 2.7$.

The mitotic index of the prostate was about 0.5 in the testosterone treated animals and in the small intestine of all the animals; in the seminal vesicle of the experimental mice it was 0.4. The mitotic indices of the accessory glands in the control mice were very low (from 0.01 to 0.001).

*The cell cycle.* In Fig. 1 the labelling of the mitoses in the seminal vesicles of both the control and the testosterone treated mice is given 26 h after thymidine injection; a similar curve illustrating the prostate can be seen in Fig. 2. A second cell cycle was obtained only in the accessory glands of the experimental animals during the period of investigation. However, in the case of the small intestine, the appearance of the second labelling phase occurred even in the control mice during the time of the experiment (Fig. 3).

In Table 2 the times are given for various phases in the cell cycle as calculated from the curves for the labelling of the mitoses. It must be pointed out, however, that the total time of the cycle ($T_C$) as well as that of the post-mitotic presynthetic phase in the control mice were obtained by using the following equations given for the steady cell populations by *Epifanova* (1966).

![Figure 1](image-url)

*Fig. 1.*

*Figs. 1 to 3:* The figures depict the percentage of labelled mitoses following the injection of tritiated thymidine. Each symbol represents a single animal; 30 to 50 mitoses were scored in each experimental animal and 10 in the control animals.
Prostate

**Fig. 2.**

Small intestine

**Fig. 3.**
### Table 2.
The average time (h) of different phases in the mitotic cycle.

<table>
<thead>
<tr>
<th></th>
<th>( T_{G2} )</th>
<th>( T_S )</th>
<th>( T_{G1} )</th>
<th>( T_C )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Seminal vesicle</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>4.5</td>
<td>12</td>
<td>900$ )</td>
<td>900$ )</td>
</tr>
<tr>
<td>Hormone treated</td>
<td>0.5</td>
<td>7</td>
<td>10</td>
<td>18</td>
</tr>
<tr>
<td><strong>Ventral prostate</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>2.5</td>
<td>16</td>
<td>900$ )</td>
<td>900$ )</td>
</tr>
<tr>
<td>Hormone treated</td>
<td>2.5</td>
<td>7</td>
<td>10</td>
<td>20</td>
</tr>
<tr>
<td><strong>Small intestine</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>1</td>
<td>8.5</td>
<td>12</td>
<td>22</td>
</tr>
<tr>
<td>Hormone treated</td>
<td>1</td>
<td>8</td>
<td>6</td>
<td>16</td>
</tr>
</tbody>
</table>

\( T_{G2} = \) postsynthetic premiotic period  
\( T_S = \) synthetic period  
\( T_{G1} = \) post mitotic presynthetic period  
\( T_C = \) the whole cell cycle  
$ = \) the values are calculated from the equation of the steady state cell population and are rounded off to the nearest hundred.

\[
\frac{n_s}{N} = \frac{T_S}{T_C}
\]

\[
T_{G1} = T_C - (T_{G1} + T_S + T_M)
\]

\[
T_M = \text{mitotic index} \times T_C
\]

where \( n_s \) is the number of labelled cells, \( N \) the total number of cells and \( T_M \) the duration of the mitoses.

*The specific activity.* The activity per nucleus of deoxyribonucleic acid (DNA) was 35 ± 4 grains in the accessory glands of the testosterone injected animals, 5 h after thymidine injection; 17 h later it had decreased by about 15 per cent. The corresponding value for the control animals was about 18 grains and remained unchanged throughout the experiment. On the contrary, the grain density was very high in the epithelial cell nuclei of the intestine 5 h after thymidine injection, making the grain counting impossible, but 17 h later only about 13 grains were found per nucleus.

### DISCUSSION

The results presented clearly demonstrate that testosterone propionate is capable of shortening significantly all the phases in the cell cycle independent of
whether the cell was considered as a specific target for the hormone. The most marked change was observed in the $T_{G1}$ phase, whereas the $T_{G2}$ phase was least altered. Our results thus indicate that the shortening of the cell cycle by testosterone is a summation effect of sortenings in all phases of the cycle. The components responsible for a total shift in $T_C$ has previously been discussed and some investigators, e.g. Defendi & Manson (1963), think that only changes in the $T_{G1}$ phase can affect it, while others (Bresciani 1965) believe, like we do, that all the phases influence $T_C$.

As well as a shortened $T_C$, the proliferative pool of the cell population can, of course, influence the speed of proliferation of a cell population. Rajewsky (1966) believes that a shortening of $T_C$ is more important than either cell destruction or an increased proliferating pool. However, Epifanova (1966) was able to demonstrate that oestrogen caused a significant increase in the proliferating pool of the uterine epithelium of castrated mice whereas that of the cornea remained unchanged. It would also have been interesting to investigate the size of this pool in the male accessory glands, but the very long $T_C$ of the castrated control animals makes it impossible to obtain a suitable figure of reference.

The mammalian seminal vesicle is often considered as a static organ without any significant cell renewal. However, our observations indicate that even in a castrated animal the gland shows some mitotic activity. It seems probable, moreover, that DNA is coupled with the metabolic activity of the gland as already suggested by Ahlström et al. (1944) and later confirmed by Pelc (1958) and Pelc & Gahan (1959). This is illustrated in our experiment by a higher labelling index than would be expected on the basis of DNA duplication only. As a result of this, the estimated duration of cell cycle in control animals is too short. On the other hand, the rate of cell renewal in the experimental animals is uncertain, especially as far as the seminal vesicles are concerned, because the weight increase is almost exponential during the period of observation. Correspondingly, $T_C$ should be calculated by using an equation for the exponentially growing cell population, i.e. $T_C$ should be multiplied by 0.693 (Lennartz & Maurer 1964). However, in our experiments the accessory glands were weighed with their secretions, and both the size of the epithelial cells and the amount of secretion seemed to increase considerably as judged by the histological evaluation. The very rapid weight increase may therefore indicate an increased cell function rather than an exponential cell proliferation.

The mitotic activity is clearly dependent on the time of the day. In a mouse, it is lowest before and after noon, when the animal is asleep. During the night and when asleep the activity is highest. To eliminate the influence of variations in day rhythm, the duration of $S$-period was obtained from animals killed during the night.

The mechanism of the mitotic action of testosterone is still unknown. It
might depend on the known effect of testosterone on the arterial blood supply of the secondary sexual organs (Reynolds et al. 1942). On the other hand, in vitro cultivation of the prostatic gland has shown high mitotic indices even without testosterone (Simnet & Morley 1967). This could be interpreted as showing an absence of a specific prostatic chalone in the culture medium. Testosterone might also be capable of eliminating the effect of chalones in other tissues, as its effect was clearly evident in the present study, even on a non-specific target tissue.

The results show that the maximal rate of proliferation after testosterone in the ventral prostate and the seminal vesicle occur at different times. This is against the concept proposed by Epifanova (1962), where the mitogenic effect of sexual steroids are assumed to be produced by mediators like adrenaline or glucocorticoids.

There is no doubt that the opinion with regard to the mitotic activity of the accessory glands presented in this paper does not describe the condition outside the limits of the period used in the investigation. Indeed, Sheppard et al. (1965) observed that after 7 days of testosterone treatment, the accessory glands of castrated mice hardly incorporated any thymidine at all. This shows the difficulty of interpretation when results produced by different hormone treatment are to be compared. Immediately after the hormone treatment the non-functional mature cells of the target organ became mitotically active progenitor cells, which later moved over to functionally active but mitotically inactive cell types (Bullough 1965). Our present results differ from those obtained by Morehead & Morgan (1967) in castrated rats, but the labelling indices obtained after testosterone treatment are at the same level as those found by Pelc & Gahan (1959) in similar experimental conditions in mice.

An interesting finding was the increase in the specific activity of DNA in the testosterone treated animals. It seems that the effects of testosterone and oestrogens differ in this respect from each other, since the latter produced no increase in the synthesis of DNA (Epifanova 1966).

REFERENCES

Bullough W. S.: Cancer Res. 25 (1965) 1688.

703

Received on October 14th, 1967.