PROLIFERATIVE EFFECT OF TESTOSTERONE PROPIONATE ON HUMAN VAGINAL EPITHELIUM

BY

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with a statistical appendix by

A. A. Weber\textsuperscript{2}) and R. Borth.

Most of the contradictory effects of testosterone on the female genital organs are due to the fact that this hormone affects the intact and the castrated female in a different manner. It is not within the scope of this paper to review the extensive experimental studies in the intact animal, nor the clinical results in menstruating women, since no satisfactory conclusions about the actions of testosterone can be drawn in the presence of the ovaries which also produce effects on the uterus and vagina.

The purpose of the present investigation was to study the effect of the male hormone on the vagina of castrated and menopausal women, and to give a reliable method for assessing the trophic changes in the epithelium.

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Information provided by experimental work indicates that testosterone produces a direct proliferative effect on the vagina of spayed animals (Clarke & Selye, 1942, Deanesly & Parkes, 1936, Korenchevsky, Dennison & Hall, 1937, Lucchetti, 1939, Nathanson, Franseen & Sweeney, 1938, Nelson & Merkel, 1937, Noble, 1939, Salmon, 1938). In the human subject the results appear contradictory; several investigators have observed a proliferative effect following small and/or large doses of testosterone in menopausal and castrated women (Boger, 1946, Greenblatt, 1943, Nathanson & Towne, 1939, Pundel, Rakoff, 1943, Rakoff, Feo & Goldstein, 1944, Salmon, 1937, Salmon, 1941, Sannicandro, 1946). Other workers did not obtain such changes on atrophic epithelium (Abel, 1945, Berlind, 1941, Mocquot & Moricard, 1936, Moricard & Saulnier, 1939, Rothermich, 1939, Shorr, Papanicolaou & Stimmel, 1938). A detailed investigation seemed therefore necessary.

MATERIAL AND METHODS

Our initial studies were performed on a group of twelve patients, 54—85 years of age, in hospital for treatment of atrophic vaginitis, cystitis, fibroma, ovarian tumour or cancer of the breast. Five women were castrated; the duration of the menopause was more than ten years in six patients, and four years in one patient.

Testosterone propionate3) (TP) was administered in doses of 25 mg. i. m. every other day to ten women, and every day to two patients — up to a total of 125 mg. Vaginal biopsies and, in some instances, endometrial biopsies were performed before and after treatment, and repeated about one week after the last injection of TP (Fixation: Bouin, Stain: Hematoxylin-Eosin and Best’s Carmine). Vaginal pH, vaginal flora, and basal temperature were determined throughout the period of observation. Vaginal smears from the posterior fornix were

3) Perandren (Ciba), generously supplied by Dr. K. Miescher and Prof. R. Meier, Ciba Aktiengesellschaft, Basel, Switzerland.
taken before treatment, before each injection, and repeated 2 days and 6—11 days after the administration of 125 mg. TP. In each instance, 1 or 2 smears were fixed and stained as described by Papanicolaou (1942), and one slide was stained with the iodine vapor technique of Mack et al. (1942).

Preliminary data are given for three patients with recurrent mammary cancer, treated for a period of 1—2 months with a total of 1250 mg. TP. Vaginal smears were taken twice weekly.

The following histological criteria have been used to assess trophic changes: the height of the vaginal epithelium, differentiation in three layers, activity of the basalis, abundance in glycogen deposition, and density and vascularisation of the subjacent connective tissue.

The cytological changes were evaluated by the cellular index, by the nuclear, acidophilic and glycogen indices, and by the general appearance of the smear.

To determine the cellular index, the proportion of superficial to intermediate and basal cells was obtained by counting one hundred cells in at least two different areas. In difficult smears, showing crowding or irregular grouping, the counting was done on 3, 4 or even 5 groups of one hundred cells. The results were compared in order to test the reliability of the method. The average values of two counts chosen at random\(^4\) are given as final results. They show the following approximate standard deviations\(^5\):

\[
\begin{array}{cccccc}
\text{Average values in per cent} & 0 & 5 & 10 & 20 & 30 & 40 \\
\hline
\text{Standard deviations in per cent} & \pm & 0.5 & 3 & 4 & 5.5 & 6 & 6.5 & 7
\end{array}
\]

In this report only the percentages of superficial cells are given. The differentiation of large basal and intermediate cells might be a question of personal interpretation.

\(^4\) Using the random numbers in Fisher & Yates, 1948.

\(^5\) See statistical appendix.
The nuclear index was obtained by counting pyknotic and vesicular nuclei in one hundred superficial cells in at least two different areas. The results, in percentage of pyknotic nuclei, were found to have the following approximate standard deviations:

<table>
<thead>
<tr>
<th>Average values in per cent</th>
<th>0</th>
<th>5</th>
<th>10</th>
<th>20</th>
<th>30</th>
<th>40</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>100</td>
<td>95</td>
<td>90</td>
<td>80</td>
<td>70</td>
<td>60</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Standard deviations in per cent</th>
<th>± 0.5</th>
<th>3</th>
<th>4.5</th>
<th>6</th>
<th>7</th>
<th>7.5</th>
</tr>
</thead>
</table>

The acidophilic index gives the proportion of acidophilic to basophilic superficial cells in per cent (average values).

The glycogen index, as suggested by Mack et al. (1942) is expressed in grades from I to IV.

Stress was laid on vaginal smear studies which, in addition to biopsies, are particularly suitable for following the trophic changes in the epithelium. Repeated controls reveal the slightest variation in the cytological pattern, and the different states of proliferation can be observed more distinctly and compared more easily than in biopsies. Accurate cellular and nuclear counts were thought to be necessary to establish an objective basis for comparison and to exclude errors dependent on subjective determinations. We tried, though not always successfully, to avoid incidental factors which might modify the epithelial response.

**CASE REPORTS AND RESULTS**

To allow of the evaluation of the hormonal effect, all cases are reported in detail (see Table 1). Genital status, duration of menopause, clinical diagnosis and treatments given at the same time as testosterone, are mentioned before the histological and cytological results, which are given for the following periods of treatment:
Period I = before treatment
Period II = 2—3 days after 50 mg. TP
Period III = 2—3 days after 125 mg. TP
Period IV = 6—11 days after 125 mg. TP

In nearly all the cases the epithelial changes resulting from testosterone administration were striking. The biopsies showed an increased height of the vaginal epithelium up to about twenty layers, a differentiation in three layers with the development of a previously absent intermediate layer, and an enlargement of the cells. In some instances mitoses were observed in the basal layer. The subjacent connective tissue showed a decreased density and wider vascular lumina. One castrate with simultaneous X-ray treatment showed only a slight thickening of the already moderately developed epithelium. In all eight women with atrophic smears before treatment, a statistically significant increase in the number of superficial cells was apparent when only 50 mg. of testosterone had been given. Values of 100 per cent superficial cells were reached or approached in five cases after the administration of 125 mg. TP (see Figs. 1 a, 2 a, 3 a, 4 a). The two cases treated at five-day intervals showed a similar striking increase, though they did not reach the 100 per cent level, the dosage/time factor probably being not sufficient for a full physiological response. In one castrate the smear contained 60 per cent superficial cells after 125 mg; treatment was not continued. — The regressive changes one week after cessation of treatment were moderate. As expected, no change occurred in the cellular index in three women with superficial smears before treatment. In one case with persistent leucorrhoea the smears gave no information, and the effect of treatment was determined from biopsies (see Figs. 5 and 6).

The nuclear changes may be summarized as follows: three smears with many pyknotic nuclei showed a transitional, statistically significant decrease in their number and an increase in vesicular nuclei after 50 mg. of testosterone. With the subsequent differentiation of a newly built superficial layer
Figs. 1—4.
Case 647/48, Mrs. D., age 70 years. Smears in figs. a are stained as described by Papanicolaou, 1942; smears in figs. b are stained as described by Mack et al., 1942.

Vaginal smears before TP treatment. (a) Showing numerous leucocytes and predominance of basal cells. (b) Glycogen index I—II.

Vaginal smears two days after 50 mg TP. (a) Appearance of large superficial cells with vesicular nuclei. (b) Increased number of large glycophilic cells. Glycogen index II—III.
Vaginal smears two days after 125 mg TP. (a) The atrophic type as shown in fig. 1 is converted to a superficial smear type. (b) Deeply stained cells rich in glycogen. Glycogen index IV.

Vaginal smears nine days after 125 mg TP. (a) Maintenance of superficial type showing few leucocytes and large basophilic cells. (b) Smear taken following biopsy. Slight regression in glycogen content, glycogen index III—IV.
Figs. 5 & 6.  
Case 128/49, Mrs. M., age 85 years. Biopsies stained with Best's carmine.

Fig. 5.  
Vaginal biopsy before TP treatment showing atrophic epithelium with scanty glycogen.

Fig. 6.  
Vaginal biopsy three days after 125 mg TP, showing well developed epithelium with abundant glycogen deposition.
the pyknotic nuclei increased and, in some cases, their number exceeded the initial values. In four smears with a low percentage of pyknotic nuclei, a steady increase in their number to about 60 per cent was noticed during treatment. Two cases with moderately developed epithelium showed no significant nuclear changes and the results in two cases were discarded because of simultaneous X-ray treatment.

Abundant glycogen deposition in all three layers of the vaginal epithelium is the most striking effect of testosterone administration. In biopsies it is most spectacular in the well-developed intermediate layer. In vaginal smears it is easily demonstrated in the deeply stained superficial cells (see Figs. 1 b, 2 b, 3 b, 4 b).

The acidophilic index showed no significant changes during or after treatment.

In seven cases the smears became clean and dry with the progressive reduction in leucocytes. In four cases no decrease in the number of leucocytes was observed, although a rise in glycogen content occurred simultaneously in three of them.

Variations in vaginal acidity, vaginal flora and basal temperature, as noted in the clinical observations, could not be correlated with the testosterone treatment. The indicator-paper-method used for vaginal pH measurements was not satisfactory.

Endometrial biopsies showed no changes in the atrophic histological pattern.

The results of biopsies and vaginal smears showed a more or less marked proliferation of the vaginal epithelium in all patients. In some women this was not so marked as after treatment with oestrogen. An increase in the glycogen deposition occurred in all cases. Testosterone, however, did not have a fully »oestromimetic« effect. The pyknotic nuclei never became as prominent or as small as after treatment with oestrogens. Moreover a significant increase in acidophilic cells was never observed. As this might have been due to the small doses used, we examined the smear changes in three more pa-


In these patients no significant rise in acidophilic or nuclear indices occurred during TP administration.

**DISCUSSION**

Greenblatt (1943) reports an oestrogenic effect of testosterone in 2 menopausal patients with atrophic smears before pellet implantation (400 mg.). The average absorption from a 100 mg. pellet is 0.3 mg. per day. In menopausal or castrated patients with moderately mature smears no effect was observed with testosterone. Nathanson & Towne (1939) observed smear-changes in 2 radiological and 2 surgical castrates, 20—41 years old. The smears before treatment were of the menopausal type with slight cornification in 3 patients. In 2 cases there was complete cornification after 115 mg. TP given over 2½ weeks and 125 mg. TP given over 2 weeks, and the two other cases showed marked changes from the pre-treatment appearance after 207.5 mg. TP given over 4 weeks and 112.5
mg. TP given over 2½ weeks. Boger (1946) administered 1230 mg. methyl testosterone (MT) (corresponding to 410 mg. TP) to a 27 years old patient within 80 days after a bilateral ovariectomy. Thereafter, the smear showed a full oestrogenic effect. It is possible that methyl testosterone prevented the regressive changes in the epithelium, although, without treatment, atrophy might not have occurred within 80 days after castration. Salmon (1941) reports smear changes from the typical negative to the characteristic oestrogen type in 8 out of 43 post-menopausal and castrate patients, but states that only high doses of the order of 50—100 mg. TP per day for at least 3 weeks were effective, except in one case reported in 1937 (465 mg. TP per 27 days, Salmon, 1937). 10—25 mg., 2 or 3 times weekly or daily, in several cases for 4—9 months, did not produce oestromimetic effects. No more detailed dosage/time values are given for these 35 patients. Moreover there is no indication as to whether these smears were of the superficial menopausal type and only failed to show cornification. Smears were stained with aqueous fuchsin (Salmon, 1936). Pundel (in the press) obtained proliferation and glycogen deposition in 4 castrated women treated with doses of 600—6000 mg. TP for 6 weeks to 10 months. The smears consisted of large basal and intermediate cells, and biopsies showed hypertrophy of the basal and intermediate layers, with reduction or complete absence of the superficial layer. In our series, after 125 mg. TP we never found that the superficial layer was absent in a well developed epithelium, nor a failure of differentiation into basophilic superficial cells in the smears. Sannicandro (1946) demonstrated proliferation and glycogen deposition in vaginal biopsies of 4 castrates, treated with 100—120 mg. TP for 10 days. Smears were not taken. Zambelli (1939) reports healing of cervical erosions following the local application of testosterone (in a glycerine-gelatine or a vaseline-lanolin vehicle). Rakoff (1943) and Rakoff et al. (1944) found that submucosal injection of 25 mg. TP in the vagina of a castrate resulted in some local hyperplasia. When given parenterally in doses up to 300 mg. over a period of 4—6
weeks similar results were not obtained. Abel (1945) treated 4 radiological castrates and 1 menopausal woman with $3 \times 50$ mg. TP weekly or $2 \times 10$ mg. MT daily. After 4—6 weeks of treatment the smear was still moderately oestrogenic in 1 patient. Four women, not controlled before treatment, showed atrophic smears with a tendency to mucification in 3 of the patients. The vulvo-vaginal tissue remained succulent, and not atrophic as might have been expected after X-ray treatment and radium. We wonder if patients who have received concentrated doses of X-rays and radium are suitable for studying the changes in the vaginal epithelium. Rothermich (1939) studied the effect of TP on the vaginal epithelium in 11 patients selected from an involutional melancholia group. Among 5 patients, menopausal for a few months, vaginal bleeding occurred during treatment in 3; they were apparently not in a well-established menopause and consequently regressive changes in the vaginal epithelium occurred. 5 patients, aged 46 to 55 years, had been in the menopause for 15—48 months, and 1 woman was castrated. These patients, 5 of whom had atrophic smears, received 600 mg. TP over a period of 6 weeks; the doses were then adjusted according to the clinical condition. At least one week after receiving a total of 890—1870 mg. TP, the vaginal smears indicated atrophic changes. The question arises: do patients with psychological disturbances react to endocrine therapy in the same way as normal patients? All the patients mentioned were younger and had been in the menopause for a shorter period than those in our own series. Berlind (1941) administered 30—50 mg. MT daily for 1—2 months to 37—48 years old climacteric women, who responded with regressive vaginal changes. Mocquot & Moricard (1936), as well as Moricard & Saulnier (1939) did not observe vaginal proliferation in castrates after the administration of 30 mg. testosterone acetate per month; these doses were probably inadequate. Shorr, Papanicolaou & Stimmel (1938) administered 25 mg. TP per day to 3 menopausal and 2 castrated women for an unknown period of time. Smears remained menopausal throughout the treatment, though the go-
nadotrophin excretion decreased. Carter, Cohen & Shorr (1947) mention smear changes in menopausal women observed by Herrmann, Adair & Woodward. We did not find any details in this report.

Some of the papers mentioned above give only scanty information as observations on vaginal changes are added merely as marginal notes to extensive clinical studies. Others summarize findings from a non-homogeneous material. Control smears were not always taken before, and smears were not repeated several times during and after treatment. Smears may have been contaminated with basal cells from cervical erosions. No difference is made between climacteric women and those in a well-established menopause. Only a few authors give definite indications as to the type of smear found. Some of the oestrogen deficient and menopausal smears mentioned, may have been of the superficial type, as is found in a high percentage of menopausal smears taken from women between 40 and 60 years old. We have also found that testosterone does not stimulate the production of cornified smears in these cases, and the conclusion that proliferation has not been produced can only be made from biopsy material. We do not want to lay particular emphasis on this absence of cornification, as we may not have continued treatment for a sufficiently long period. But our experiments certainly indicate that there is a qualitative difference between androgenic and oestrogenic effects. Acidophilic cells, present in small numbers, showed a tendency toward regression, except in one case receiving vaginal douches; in the three cases with prolonged treatment not the slightest change in the basophilic picture was observed. Although both hormones had proliferative properties, proliferative is not a synonym for oestromimetic. The finding of cornified cells might have been due to the erroneous interpretation of smears, improperly stained (no differential stains) or dried before fixation. Irritating external factors, e.g. douches and suppositories, may also induce the presence of a high percentage of acidophilic cells, thus complicating the evaluation of vaginal smears.
In addition to these questions which arise from the difficulty of reconstructing conditions at the time of observation, there are still other problems which await clarification. Do surgical castrates react to androgens differently from women castrated with X-rays who still possess an intact interstitial ovarian tissue? In our series there was no difference in the response to TP between menopausal and recently castrated women. In one patient, however, castrated 12 years previously, we observed a slower and incomplete response to TP. Further studies will show if this lies within the range of physiological variation, or if women castrated for several years show a significant difference, as compared with young or menopausal patients who have been oophorectomized shortly before TP treatment.

As to the mode of action of TP, various possibilities have been suggested. Numerous clinical observations indicate that there is an inhibitory effect of androgens on pituitary gonadotrophic function. The reduction of FSH excretion in castrates and menopausal women (Laroche, Simmonet & Bompard, 1938, Nathanson & Towne, 1939, Rothermich & Foltz, 1940, Salmon, 1937, Shorr, Papanicolaou & Stimmel, 1938) and in menstruating women (Loeser, 1940), as well as a negative AZ-reaction in 2 pregnant women (Adair, 1947) were observed during androgen therapy. Simultaneous administration of gonadotrophins and testosterone might show that the ovaries are not directly inhibited. Some authors, on the basis of animal experiments suggest that there is a direct antagonism between androgens and oestrogens (Courrier, 1943, Markee, 1940, Robson, 1936, Steinkamm & Meckies, 1939, Wolfe & Hamilton, 1937). In ovariecetomized and menopausal women they obtained inhibition of oestrogenic effects by the simultaneous administration of testosterone (Ferin, 1946, Rothermich, 1939, Rothermich & Foltz, 1940, Shorr, Papanicolaou & Stimmel, 1938). The same »neutralisation« may be expected with normally circulating oestrogens of ovarian or other origin, and appears to be at least partly responsible for the repressive vaginal changes which follow TP administration in cyclic
and climacteric women, particularly in those with signs of hyperoestrogenism. But in menopausal women, in whom a crowded superficial smear type is considered to be an index of persistent oestrogenic activity, testosterone does not bring about atrophy. This superficial smear type therefore appears to be due not to an oestrogenic effect, but to the action of other substances, possibly androgens. The suggestion that a direct transformation of androgens into oestrogens occurs is not supported by our own observations, as we did not find that TP produced any oestromimetic effect on the vaginal epithelium.

In conclusion it can be said that the androgens produce different effects in menopausal women and in women with some ovarian oestrogenic activity. In the latter they induce regressive vaginal changes in doses of 300—500 mg. per month. The observations of other workers suggest that the doses used are of secondary importance in menopausal women. Our own experience with medium doses is restricted to three cases. From our results with small doses of TP it is apparent that 125 mg. TP induce a rapid proliferation of the vaginal epithelium of castrated and menopausal women.

STATISTICAL APPENDIX

For the statistical analysis, the cellular indices of nine cases (No. 271/48, 647/48, 878/48, 15/49, 766/48, 59/49, 452/48, 703/48 and 356/48) and the nuclear indices of seven cases (the same, but excluding No. 59/49 and 356/48) are used, after transforming the original percentage data \( p \) into degrees \( \Phi \) by means of the relationship \( \Phi = \sin \sqrt{p} \) (Fisher & Yates, 1948; Kendall, 1946).

The analysis of variance of the differences between duplicate counts of the same smear reveals no significant effect of either periods (see p. 269) or patients. The exceptional cases 437/49 and 439/49, who had received daily injections, are not included in the following analysis as their duplicate differences are not consistent with those of the other cases; the data show a significant effect of the dosage/time factor.
Cellular Index.

The two missing values of the cases 59/49 and 356/48 (period II) are calculated in such a way that the interaction periods \( \times \) patients becomes a minimum (Kendall, 1946), giving \( p_{II} \) = 65 per cent and 100 per cent, respectively. Then the usual analysis of variance can be performed.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares (( \Phi ))</th>
<th>Mean Square (( \Phi ))</th>
<th>F</th>
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<tr>
<td>Periods</td>
<td>3</td>
<td>3113.964</td>
<td>1037.988</td>
<td>8.08 **</td>
</tr>
<tr>
<td>Patients</td>
<td>8</td>
<td>12737.675</td>
<td>1592.209</td>
<td>12.406 **</td>
</tr>
<tr>
<td>Interaction Periods ( \times ) patients</td>
<td>22</td>
<td>2823.491</td>
<td>128.340</td>
<td>8.761 **</td>
</tr>
<tr>
<td>Duplicates</td>
<td>34</td>
<td>498.075</td>
<td>14.649</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>67</td>
<td>19173.205</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

** \( P < 0.01 \).

1. As the interaction is significantly greater than the variance due to duplicates, it follows that different patients respond differently from one period to another.

2. Both the effect of the periods (treatment) and the influence of the patients are significantly greater than the interaction.

3. The average values of \( \Phi \) for the four periods are:
   \( \Phi_i = 62.03 \), \( \Phi_{II} = 70.77 \), \( \Phi_{III} = 77.61 \) and \( \Phi_{IV} = 78.39 \); and the corresponding levels of significance for the difference between two periods are: 7.83 at \( P = 0.05 \), and 10.64 at \( P = 0.01 \). It follows that the treatment causes a significant increase of the cellular index, \( \Phi_i \) being significantly smaller than \( \Phi_{II} \) (\( P < 0.05 \)), \( \Phi_{III} \) (\( P < 0.01 \)) and \( \Phi_{IV} \) (\( P < 0.01 \)). The increases from period II to period III or IV are too small to be significant.

4. Considering the individual instead of the average differences (\( \Phi_{III} - \Phi_i \)), the levels of significance are 7.77 at \( P = 0.05 \), and 10.45 at \( P = 0.01 \), and we have:
<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Duration of menopause</th>
<th>Genital condition</th>
<th>Clinical diagnosis</th>
<th>TP treatment</th>
<th>Concomitant treatment</th>
<th>Period</th>
<th>Cell index</th>
<th>Nucl. index</th>
<th>Acido-philic index</th>
<th>Glycogen index</th>
<th>Leucocytes</th>
<th>Histology of vaginal biopsies</th>
</tr>
</thead>
<tbody>
<tr>
<td>271/48 M.A.</td>
<td>61a</td>
<td>13a</td>
<td>intact genital organs</td>
<td>breast ca post op.</td>
<td>125 mg in 10 days</td>
<td>antistine</td>
<td>I</td>
<td>67</td>
<td>67</td>
<td>4</td>
<td>II-III (+)</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>647/48 D.</td>
<td>70a</td>
<td>26a</td>
<td>intact genital organs</td>
<td>hydrenephrosis chronic cystitis</td>
<td>125 mg in 11 days</td>
<td>pyridacil urotropin</td>
<td>I</td>
<td>62</td>
<td>53</td>
<td>9</td>
<td>I-II ++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>878/48 K.</td>
<td>57a</td>
<td>4a</td>
<td>intact genital organs</td>
<td>fibroma, cardiac insufficiency</td>
<td>125 mg in 10 days</td>
<td>coramin digitalin euphyllic, benerva</td>
<td>I</td>
<td>72</td>
<td>25</td>
<td>0</td>
<td>II-III ++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>15/49 Sch.</td>
<td>65a</td>
<td>15a</td>
<td>bilateral ovariect &amp; subtotal hysterectomy 1949</td>
<td>bilat. ovari cysts post op.</td>
<td>125 mg in 9 days</td>
<td>bellerpal vaginal douches, vag. suppositories</td>
<td>I</td>
<td>73</td>
<td>74</td>
<td>11</td>
<td>II-III ++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>766/48 W.</td>
<td>75a</td>
<td>23a</td>
<td>bilat. ovariectomy 1936</td>
<td>vaginitis luetic aortitis Wa +</td>
<td>125 mg in 10 days</td>
<td>digilanid</td>
<td>I</td>
<td>36</td>
<td>78</td>
<td>20</td>
<td>I-II ++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>59/49 G.</td>
<td>71a</td>
<td>29a</td>
<td>panhysterectomy 1949</td>
<td>bilat. ovari ca post op. 1949</td>
<td>125 mg in 10 days</td>
<td>coramin, X-rays</td>
<td>I</td>
<td>42</td>
<td>0</td>
<td>0</td>
<td>I-II ++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Case</td>
<td>Age</td>
<td>Stage</td>
<td>Type of Tumor</td>
<td>Treatment</td>
<td>Follow-up</td>
<td>Comments</td>
<td></td>
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<tr>
<td>437/49 P</td>
<td>64</td>
<td>I</td>
<td>Ovarian cyst post op. 1949</td>
<td>X-rays</td>
<td>IV</td>
<td>++</td>
<td>1 day after 75 mg. no biopsies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>439/49 Ch</td>
<td>75</td>
<td>I</td>
<td>Bilateral ovarian cancer, ascites, vaginitis</td>
<td>Spasmocoalgin</td>
<td>III-IV</td>
<td>++</td>
<td>1 day after 50 mg. no biopsies</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>452/48 M</td>
<td>58</td>
<td>I</td>
<td>Breast cancer post op.</td>
<td>Euphyllin, cedilanid</td>
<td>II-III</td>
<td>++</td>
<td>Moderate development with abundant glycogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>703/48 Y</td>
<td>61</td>
<td>I</td>
<td>Prostate cancer, ovarian tumor</td>
<td>Dicodid, allonal, penicillin</td>
<td>II-III</td>
<td>+++</td>
<td>Moderate development with abundant glycogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>356/48 B</td>
<td>54</td>
<td>I</td>
<td>Bilateral ovarian cancer &amp; uterine metastases post op.</td>
<td>Benerva, becozym X-rays, diathermia vaginal douches</td>
<td>III-IV</td>
<td>+++</td>
<td>Thicker epithelium, well-developed superficial layer, glycogen scanty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>128/49 M</td>
<td>85</td>
<td>II</td>
<td>Urethral polyp, vaginitis</td>
<td>Coramin, bladder washings</td>
<td>II</td>
<td>+++</td>
<td>Atrophic epithelium, glycogen scanty</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>III</td>
<td></td>
<td>Leucocytes</td>
<td>III</td>
<td>+++</td>
<td>Regenerated epithelium, abundant glycogen</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>IV</td>
<td></td>
<td></td>
<td>IV</td>
<td>+++</td>
<td>No regression</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The increase of the cellular index from period I to period III is highly significant in six cases. *The three cases with an initial cellular index \( (p_1) \) near 100 per cent do not show any significant changes, neither increase nor decrease.*

5. *The accuracy of duplicates* is measured by the corresponding mean square, and hence the standard deviation of \( \Phi \), \( \sqrt{14.649} = \pm 3.83 \). Returning to \( p \), *the standard deviations for different values of the cellular index* are obtained, as given approximately in the table on page 267.

**Nuclear Index.**

Just as for the cellular index, the usual analysis of variance can be performed.

<table>
<thead>
<tr>
<th>Source of Variation</th>
<th>Degrees of Freedom</th>
<th>Sum of Squares ( \Phi )</th>
<th>Mean Square ( \Phi )</th>
<th>( F )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Periods</td>
<td>3</td>
<td>1941.503</td>
<td>647.834</td>
<td>5.082*</td>
</tr>
<tr>
<td>Patients</td>
<td>6</td>
<td>2824.128</td>
<td>470.688</td>
<td>3.692*</td>
</tr>
<tr>
<td>Interaction Period ( \times ) patients</td>
<td>18</td>
<td>2294.755</td>
<td>127.486</td>
<td>7.290**</td>
</tr>
<tr>
<td>Duplicates</td>
<td>28</td>
<td>489.655</td>
<td>17.488</td>
<td>—</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>7550.041</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

* \( P < 0.05 \).
** \( P < 0.01 \).

1. *Different patients respond differently from one period to another.*

2. *Both the effect of periods (treatment) and the effect of patients are significantly greater than the interaction.*
3. The average values of $\varphi$ for the four periods are: 
$\varphi_1 = 46.42$, $\varphi_2 = 38.39$, $\varphi_3 = 53.64$, and $\varphi_4 = 51.60$; and the corresponding levels of significance for the difference between two periods are 8.97 at $P = 0.05$, and 12.28 at $P = 0.01$. It follows that the treatment causes a significant increase in the nuclear index from period II onwards; the initial decrease from period I to period II is significant only at $P \sim 0.07$.

4. Considering the individual differences for each patient, we have the following significant effects:

<table>
<thead>
<tr>
<th>Effect</th>
<th>Case No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease from period I to</td>
<td>271/48 647/48 878/48 15/49 766/48 452/48 703/48</td>
</tr>
<tr>
<td>period II</td>
<td></td>
</tr>
<tr>
<td>Increase from period I to</td>
<td>*       *       *</td>
</tr>
<tr>
<td>period III and/or IV</td>
<td></td>
</tr>
</tbody>
</table>

* Significant either at $P = 0.05$ or $P = 0.01$.

5. The standard deviations of duplicates, as derived from the standard deviation of $\varphi$, $\sqrt{17.488} = \pm 4.18$ are given approximately in the table on page 268.

**SUMMARY**

125 mg. testosterone propionate produced vaginal proliferation and glycogen deposition in 12 menopausal and castrated women.

The trophic changes were evaluated by means of vaginal biopsies and smears.
A cellular index, and nuclear, acidophilic and glycogen indices were established as an objective basis for the comparison of smears. The value of, and the significance of the TP effect on cellular and nuclear indices were demonstrated by statistical analysis.

The mode of action of androgens in women is discussed.

ACKNOWLEDGEMENTS

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