EFFECT OF TESTOSTERONEPHENYLPROPIONATE AND
19-NORTESTOSTERONEPHENYLPROPIONATE ON THE SEMINAL
VESICLES, THE LEVATOR ANI MUSCLE AND
THE MAMMARY GLANDS OF
CASTRATED MALE RATS*

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ABSTRACT

The response of the seminal vesicles, the levator ani muscle and the mammary glands to testosteronephenylpropionate (TPP) and 19-nortestosteronephenylpropionate (19-norTPP) was studied in castrated male rats. The development of these structures was compared with that found in male rats with intact testes. The main results were as follows:

1) Daily injections of 0.01 mg of TPP produced slight weight increase in the seminal vesicles and levator ani muscle and stimulated a slight but obvious lobule-alveolar development in the mammary glands. The same dose of 19-norTPP produced only a minimal weight increase in the seminal vesicles but produced an obvious development of the levator ani muscle and the mammary glands.

2) Daily injections of 0.05 mg of TPP caused a marked weight increase in the seminal vesicles and levator ani muscle and produced a marked lobule-alveolar development in the mammary glands. The same dose of 19-norTPP produced only a slight weight increase in the seminal vesicles but brought about a marked development of the levator ani muscle and the mammary glands.

3) Daily injections of 0.5 mg of 19-norTPP caused a marked development of the seminal vesicles comparable to that found in rats with intact testes. The levator ani muscle and the mammary glands after this treat-

* In this paper testosteronephenylpropionate and 19-nortestosteronephenylpropionate will be abbreviated to TPP and 19-norTPP, respectively.
ment were, however, much more stimulated than in rats with intact testes. These results indicate 1) that the ratio between the effects of these compounds on the seminal vesicles and on the levator ani muscle depends on the dose-level and 2) that the development of the mammary glands is correlated more to the growth of the levator ani muscle than to the development of the seminal vesicles.

It is well known that androgens, besides having an action on sex specific tissues and other glands of internal secretion, also affect general body processes such as growth and the metabolism of protein. This effect on growth and protein metabolism is usually referred to as »the anabolic action of androgens«. The first demonstration of such an anabolic action of androgens was reported by Kochakian & Murlin (1935) and Kochakian (1937). They showed that urinary extracts containing androgenic material as well as pure androgenic compounds (e.g. testosterone) caused nitrogen retention in castrated dogs. The same effect has also been demonstrated in other animals and in man (for lit. see Albright 1942-1943; Kochakian 1946, 1950; Dorfman & Shipley 1956). The quantity of nitrogen retained is too great to be explained as an action only on accessory sex organs. The effect appears rather to be a more general one, and it is usually assumed that the nitrogen retention is largely due to a protein anabolic action in the skeletal muscles. This view was primarily based on clinical observations (e.g. Kenyon et al. 1944). Experimental studies on male animals have confirmed that there is an atrophy of skeletal muscles after castration in a variety of animals, but these studies have also revealed that only certain groups of muscles show such an atrophy (e.g. Papanicolaou & Falk 1938; Wainman & Shipounoff 1941; Kochakian et al. 1956).

In the male rat a marked atrophy of the levator ani muscle is found after castration and this atrophy can be prevented by the administration of androgens (Eisenberg et al. 1949). Proceeding from the above-mentioned assumption that the anabolic activity of the androgens is largely due to a myotrophic effect, Eisenberg & Gordan (1950) started to use the growth-promoting action of different androgens on the levator ani muscle as a method for determining their anabolic activity. This test-method has since been widely used, as it has been of great clinical interest to prepare androgenic hormones with high anabolic and low androgenic action.

One effect of androgens which is much less studied than those on the male reproductive sex accessories and protein metabolism is their effect on the structures of the mammary gland. These structures belong to the female reproductive system, and in many species the mammary glands of the male consist only of a very restricted duct system (for lit. see Folley 1952). In some species (e.g. the monkey, the guinea-pig and the rat) the male mammary
glands, however, show a certain degree of development at or after puberty, indicating a stimulating influence from the testes. In the rat the development of the male mammary gland is remarkable. Adult male rats with intact testes show an extensive lobule-alveolar development in the mammary glands (Turner & Schultze 1931; Astwood et al. 1937; Ahrén & Etienne 1957). In addition it has been shown that injections of testosterone and other androgens stimulate lobule-alveolar development in the mammary glands of castrated male and female rats (Selye et al. 1936; Astwood et al. 1937; Ahrén & Etienne 1959).

During the last ten years many experimental and clinical studies have been performed new steroid compounds which show a high anabolic activity (mostly recorded as the effect on the levator ani muscle) and a low androgenic action (mostly recorded as the effect on the prostate or the seminal vesicles). The question of whether these new compounds can stimulate the development of the mammary glands has not been studied. One of these compounds, which is widely used in clinical work, is the phenylpropionate of 19-nortestosterone (19-norTPP). In the present study the effects of different doses of this compound on the seminal vesicles, the levator ani muscle and the mammary glands were studied and compared with the effects of different doses of the phenylpropionate of testosterone (TPP). In addition, the development produced in the seminal vesicles, the levator ani muscle and the mammary glands by these compounds was compared with that found in the same organs of untreated rats with intact testes. Our main interest in these experiments was to investigate 1) whether the effect of 19-norTPP on the mammary glands was similar to that of TPP, and 2) whether the response of the mammary glands to these two compounds was correlated with the response of the seminal vesicles (the androgenic action) or with the response of the levator ani muscle (the anabolic action).

M E T H O D S

Male albino rats from a closed colony were used. This colony is kept at the Leo Pharmaceutical Products Trading Ltd., Denmark, and the animals arrived in our Department at the age of 3–4 weeks. From that time they were given a semi-synthetic diet consisting of 22% casein, 63% wheat starch, 10% arachis oil, 4% salt mixture and supplementary vitamins. This diet has been used by Gustafsson (1959) in studies on germ-free rats, and the composition of the diet is given in detail in a previous paper (Ahrén 1959). The diet was given ad libitum, and the rats were always allowed free access to water.

The seminal vesicles, the levator ani muscle and the mammary glands were studied in the following groups of animals:

Group I: Castrated rats injected with arachis oil, as a control group (10 rats).
Group II: Normal untreated rats with intact testes (12 rats).
Group III: Castrated rats injected with TPP in a daily dose of A) 0.01 mg (8 rats) and B) 0.05 mg (8 rats).

Group IV: Castrated rats injected with 19-norTPP in a daily dose of A) 0.01 mg (8 rats), B) 0.05 mg (8 rats) and C) 0.5 mg (4 rats).

The animals were castrated at the age of 3-4 weeks. After another period of about 3 weeks (the rats then weighed 172.6 ± 2.3 g) the injections were started. The TPP and 19-norTPP were given in arachis oil (T. P. P., Organon, 50 mg/ml and Durabol, Pharmacia, 10 mg/ml, respectively). The commercial preparations were diluted with arachis oil so that the volume injected daily into each rat was 0.05 ml. In Group I (the control group) the rats were injected with 0.05 ml of arachis oil daily. All injections were given intramuscularly with a tuberculin syringe for a period of 28 days.

At the end of the injections the rats were killed by bleeding while under ether anaesthesia, and the mammary glands, the seminal vesicles and the levator ani muscle were dissected out and studied as described below.

The mammary glands. — The male rat has, like the female, six pairs of mammary glands. The glands of the male rat have, however, no nipples and the main duct ends blindly in the dermis at the point where the nipple would normally be found in the female. In the present experiments the third thoracic and the abdominal glands were studied, since these glands are easily identified and dissected out. The glands were studied both as whole preparations and as paraffin sections. For the whole mount preparations the subcutaneous tissue containing the mammary gland was dissected out, stretched on tracing paper, fixed in Carnoy’s solution and stained with galloycyanin chromalum as described by Jacobsohn (1948). The gland was then prepared free from the surrounding tissues under the microscope, cleared in xylol and mounted in toto. As discussed in more detail by Ahrén (1959), this method allows the study of the type and degree of growth and development in the whole gland and not only in a part of it (see Figs. 2-7). These whole mount preparations cannot, however, give any information about the cytological architecture of the glands. To obtain such information, paraffin sections (5-7 µ thick) stained with haematoxylin and eosin were also studied.

Before the start of injections, the third right thoracic mammary gland was extirpated for whole mount preparation. This gland will be referred to as «the control gland». At the end of the experiment, the third left thoracic gland and the left abdominal gland were extirpated for whole mount preparations and the right abdominal gland was extirpated for paraffin section. These glands will be referred to as «the experimental glands».

The seminal vesicles. — These glands were dissected out, emptied of any secretion, blotted and weighed on an analytical balance.

The levator ani muscle. — This muscle was dissected out according to the method described by Eisenberg & Gordan (1950), blotted and weighed on an analytical balance. The muscle was weighed immediately after dissection and also after drying in an oven for 24 hours at 80° C. As the wet/dry ratio was the same in all the rats studied, only the weights found immediately after dissection («the wet weight») will be given in this paper.

In previous papers concerning the effect of various androgenic substances on the seminal vesicles and the levator ani muscle, some authors (e.g. Eisenberg & Gordan 1950; Hershberger et al. 1959) have given and discussed only the absolute weights of these organs. Other authors (e.g. Barnes et al. 1954; Ercoli et al. 1960) have re-
duced the weights of both these organs to mg per 100 g body weight (= relative weights), while Overbeck & de Visser (1957) have given the absolute weights of the seminal vesicles and the relative weights of the levator ani muscle. It seems to us to be more correct to give and discuss the relative weight of an organ only if this organ in the control animals grows in proportion to the increase in body weight during the experimental period. It was therefore necessary for us to investigate whether the seminal vesicles and/or the levator ani muscle increased in weight in the control rats (Group I) during the experimental period (= the period of injections). Ten castrated rats were therefore killed and dissected about 3 weeks after castration (the animals then weighed 172.0 ± 5.1 g). The weights of the seminal vesicles and the levator ani muscle of these 10 rats did not, however, differ significantly from those found at autopsy in the control rats (Group I). Thus, in the control rats of the present experiments the weights of the seminal vesicles and the levator ani muscle did not increase during the experimental period, and only the absolute weights of these organs will therefore be given and discussed in this paper.

RESULTS AND COMMENTS

The data are summarized in Table 1 and Fig. 1. The observations made on the seminal vesicles, the levator ani muscle and the mammary glands will be described in more detail and discussed below. Typical findings in the mammary glands are illustrated by the figures on Plates I–II.

Group I: Castrated rats injected with arachis oil (10 rats)

As can be seen from Table 1 and Fig. 1, the seminal vesicles and the levator ani muscle were very small in these rats.

The control mammary glands showed a moderately extended duct system with an intermediate degree of arborescence (Fig. 2). The ducts were thin and atrophic. Most of the glands did not present any alveoli, but some of them showed a few small groups of alveolar structures. The experimental mammary glands from 8 of these 10 rats were atrophic as the control glands (Fig. 3). This result is in agreement with previous observations (e.g. McEuen et al. 1936; Astwood et al. 1937; Ahrén & Etienne 1957) showing that castrated male rats do not develop, or develop only very few, alveoli in the mammary glands.

In the remaining 2 rats of this group the experimental mammary glands had an exceptional appearance, with moderate to extensive development of alveoli. In large series of castrated male rats, studied during the last 2 years in this laboratory, we have never seen such an extensive alveolar development in the mammary glands after treatment with arachis oil. We can offer no explanation for the mammary gland development in these 2 cases (the seminal vesicles and the levator ani muscle were not stimulated). Unfortunately the adrenal glands were not examined in these rats.
Table 1.
Effects of TPP and 19-norTPP on body weight, seminal vesicles and levator ani muscle of castrated male rats.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mg per day</th>
<th>No. of rats</th>
<th>Body weight at:</th>
<th>Seminal vesicles mg*</th>
<th>Levator ani muscle mg*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Start of inj. g*</td>
<td>autopsy g*</td>
<td></td>
</tr>
<tr>
<td>Normal untreated</td>
<td>–</td>
<td>12</td>
<td>179.2 ± 4.4</td>
<td>271.3 ± 9.5</td>
<td>371.3 ± 29.0</td>
</tr>
<tr>
<td>Arachis oil</td>
<td>–</td>
<td>10</td>
<td>175.5 ± 4.7</td>
<td>254.5 ± 7.1</td>
<td>13.7 ± 0.7</td>
</tr>
<tr>
<td>TPP</td>
<td>0.01</td>
<td>8</td>
<td>163.1 ± 3.3</td>
<td>240.0 ± 4.9</td>
<td>50.7 ± 4.0</td>
</tr>
<tr>
<td>TPP</td>
<td>0.05</td>
<td>8</td>
<td>175.0 ± 6.1</td>
<td>275.6 ± 9.2</td>
<td>51.0 ± 3.8</td>
</tr>
<tr>
<td>19-norTPP</td>
<td>0.01</td>
<td>8</td>
<td>158.1 ± 7.5</td>
<td>249.4 ± 11.2</td>
<td>17.6 ± 1.4</td>
</tr>
<tr>
<td>19-norTPP</td>
<td>0.05</td>
<td>8</td>
<td>169.4 ± 4.3</td>
<td>278.8 ± 6.3</td>
<td>89.2 ± 4.6</td>
</tr>
<tr>
<td>19-norTPP</td>
<td>0.5</td>
<td>4</td>
<td>195.0 ± 5.4</td>
<td>301.3 ± 7.2</td>
<td>383.7 ± 38.2</td>
</tr>
</tbody>
</table>

* Mean ± standard error of the mean.

Group II: Normal untreated rats with intact testes (12 rats)
The seminal vesicles were very large (Table 1 and Fig. 1) and distended with thick secretion. In this connection we thought it of interest to see whether the degree of development of the seminal vesicles in these rats represents the maximal development of this organ. Three normal rats were therefore injected with very high doses of androgens (10 mg testosterone propionate for 21 days). The seminal vesicles of these 3 rats were 2–3 times larger than those found in the 12 untreated rats of the present group. This observation shows that administration of high doses of androgens can produce greater development of the seminal vesicles than those found in untreated adult rats with intact testes.

As can be seen from Table 1 and Fig. 1, the levator ani muscle was large in the 12 rats of the present group.

The control mammary glands were as atrophic as those of the above-mentioned group. The experimental mammary glands showed about the same area as the experimental glands of the control group (Group I), but in contrast to the findings in the above-mentioned group the ducts of the present glands were covered with dense clusters of alveoli (Fig. 4). This results is in agreement with previous observations (e.g. Astwood et al. 1937; Ahrén & Etienne 1957) showing that adult male rats with intact testes have an extensive lobule-alveolar development in the mammary glands.

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Group III: Castrated rats injected with TPP

A) 0.01 mg daily (8 rats). – As can be seen from Table 1 and Fig. 1, the seminal vesicles and the levator ani muscle were only slightly stimulated after this treatment and both these organs were much smaller than in the normal rats with intact testes (Group II).

The control mammary glands of these 8 rats were as atrophic as the control glands of the above-mentioned groups. The experimental glands showed slightly thickened ducts and slight but obvious lobule-alveolar development.

Thus, after treatment with this dose of TPP there was a slight but obvious stimulation of the seminal vesicles, the levator ani muscle and the mammary glands.

B) 0.05 mg daily (8 rats). – The seminal vesicles and the levator ani muscle were markedly stimulated in these rats (Table 1 and Fig. 1). These organs were, however, not as large as in the untreated rats with intact testes (Group II). The weights of both the seminal vesicles and the levator ani muscle in the present 8 rats were about 75% of the weights of the same organs in the rats with intact testes.

The control mammary glands were as atrophic as in the above-mentioned groups. The experimental mammary glands showed extensive lobule-alveolar development (Fig. 5), which in type and degree was very similar to that found in the experimental mammary glands of the untreated rats with intact testes.

These observations show that this dose of TPP markedly stimulated the seminal vesicles, the levator ani muscle and the mammary glands.

Group IV: Castrated rats injected with 19-norTPP

A) 0.01 mg daily (8 rats). – As can be seen from Table 1 and Fig. 1, the seminal vesicles of these rats were only slightly larger than those of the control rats. It is also quite clear that the effect on the seminal vesicles was less marked after this dose of 19-norTPP than after the same dose of TPP (Group III A). At this dose-level 19-norTPP seems therefore to be less androgenic than TPP.

In contrast to the seminal vesicles, the levator ani muscle was found to be clearly stimulated after this treatment (Table 1 and Fig. 1). In all the rats of the present group the levator ani muscle was significantly larger than after treatment with the same dose of TPP.

The control mammary glands were atrophic. The experimental mammary glands were very similar to those found after treatment with the same dose of TPP. In 7 of the 8 rats the mammary glands showed thickened ducts and slight but obvious lobule-alveolar development. In only one rat were there no alveoli in the experimental glands.

Thus, this dose of 19-norTPP had only a minimal effect on the seminal
Fig. 1.

The effect of TPP and 19-norTPP on the development of the seminal vesicles, the levator ani muscle and the mammary glands of castrated male rats. The bars indicate the difference in absolute weights between the experimental groups (= rats injected with TPP and 19-norTPP and normal untreated rats) and the control group (= rats injected with arachis oil). For comparison, the weights of the seminal vesicles and the levator ani muscle of the control group are shown in negative bars to the left in the figure.

The development of the mammary glands in the different groups is illustrated by the following symbols:

stands for the development of the duct system.

symbolizes the degree of development of non-dilated alveoli.

stands as a symbol for dilated alveoli.

vesicles, a moderate but very clear effect on the levator ani muscle and a slight but obvious effect on the mammary glands.
Plate 1.

Fig. 2.

Fig. 3.

Fig. 4.

Fig. 5.

5mm

(WHOLE MOUNTS)

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Comments on Plates I–II.

Figs. 2–7 show whole mount preparations of mammary glands stained with gallocyanin chromalum and photographed at the same magnification (see Plate I).

Plate I.

*Fig. 2:* Castrated male rat. Third right thoracic gland extirpated 3 weeks after castration and before the start of injections. – Atrophic gland. – This gland may be taken as »control mammary gland« to all groups.

*Fig. 3:* (Group I). Same rat as in Fig. 2. Third left thoracic gland extirpated after 28 days of treatment with 0.05 ml arachis oil daily. – Atrophic gland.

*Fig. 4:* (Group II). Untreated male rat with intact testes. Third left thoracic gland extirpated at autopsy. – Marked lobule-alveolar development.

*Fig. 5:* (Group III B). Castrated male rat. Third left thoracic gland extirpated after 28 days of treatment with 0.05 mg TPP daily. – Marked lobule-alveolar development.

Plate II.

*Fig. 6:* (Group IV B). Castrated male rat. Third left thoracic gland extirpated after 28 days of treatment with 0.05 mg 19-norTPP daily. – Marked lobule-alveolar development. Slight dilatation of ducts and alveoli.

*Fig. 7:* (Group IV C). Castrated male rat. Third left thoracic gland extirpated after 28 days of treatment with 0.5 mg 19-norTPP daily. – Extensive lobule-alveolar development. Marked dilatation of ducts and alveoli.
B) 0.05 mg daily (8 rats). – As can be seen from Table 1 and Fig. 1, the seminal vesicles were slightly stimulated after this treatment. The weight of this organ was, however, only about 20% of that found after treatment with the same dose of TPP (Group III B), and about the same as that after treatment with 0.01 mg TPP (Group III A). Thus, at this dose-level 19-norTPP seems also to be definitely less androgenic than TPP.

The levator ani muscle was markedly stimulated after this treatment (Table 1 and Fig. 1). In most of the 8 rats the levator ani muscle was even larger than in the rats with intact testes (Group II), and in all of them this muscle was definitely larger than in the rats treated with the same dose of TPP (Group III B).

The control mammary glands were atrophic. All the experimental mammary glands showed a marked lobule-alveolar development (Fig. 6) comparable to that found in rats with intact testes (Group II) and in castrated rats injected with 0.05 mg of TPP (Group III B). The only difference was that the glands of the present group showed slightly more secretion.

These observations show that this dose of 19-norTPP produced a marked development of the levator ani muscle and of the mammary glands, while the seminal vesicles were only slightly stimulated.

C) 0.5 mg daily (4 rats). – The seminal vesicles of these rats were very large (Table 1 and Fig. 1) and distended by thick secretion. In fact, they were very similar to the seminal vesicles of rats with intact testes (Group II). It is therefore quite clear that 19-norTPP in this dose has a high androgenic activity. From the experiments mentioned above (under Group II) it is, however, obvious that the degree of development of the seminal vesicles found in the present rats does not represent a maximal development of this organ.

The levator ani muscles in these rats were also very large. As can be seen from Table 1 and Fig. 1, they were larger than those of any other group of the present work.

The control mammary glands were atrophic. The experimental mammary glands showed an extensive lobule-alveolar development and marked secretion (Fig. 7). The paraffin sections showed that all mammary gland structures were markedly distended by secretion. The degree of both lobule-alveolar development and secretion was more extensive in these glands than in the mammary glands of rats with intact testes (Group II). A similar effect on the mammary glands of castrated rats has, however, been observed after treatment for long periods with high doses of testosterone propionate (see Ahrén & Etienne 1959).

The results of the present group show that this dose of 19-norTPP markedly stimulated the seminal vesicles, the levator ani muscle and the mammary glands. The stimulating effect of this dose of 19-norTPP seems, however, to be more extensive on the levator ani muscle and the mammary glands than on the seminal vesicles.
It has been reported by Hershberger et al. (1953) and Barnes et al. (1954) that injections of 19-nortestosterone in castrated male rats stimulated a definite growth of the levator ani muscle in doses which were only weakly androgenic as tested by the effect on the seminal vesicles. In 1956 Saunders & Drill observed that treatment of castrated male rats with 0.2–3.5 mg of 17-ethyl-19-nortestosterone for a seven-day period induced a marked growth of the levator ani muscle while the seminal vesicles were only slightly stimulated. From this observation Saunders (1957) concluded that 17-ethyl-19-nortestosterone has »a relatively high anabolic potency and only a weak androgenic action«.

The above-mentioned observations stimulated Overbeek & de Visser (1957) to prepare the phenylpropionate of 19-nortestosterone (19-norTPP) and investigate the effects of this compound on the levator ani muscle and the seminal vesicles of castrated male rats. They found that daily injections of this compound in doses of 12.5–200 µg for 7–14 days stimulated marked growth of the levator ani muscle but only slight development of the seminal vesicles. From this observation they concluded that 19-norTPP is a »very favourable anabolic substance«.

In the present experiments three different dose levels of 19-norTPP (0.01, 0.05 and 0.5 mg daily) were injected into castrated male rats for 28 days. As can be seen from Fig. 1, injections of the two smaller doses produced only very slight development of the seminal vesicles. These observations therefore confirm those of Overbeek & de Visser (1957). Injections of the higher dose of 19-norTPP (Group IV C), however, stimulated an extensive growth of the levator ani muscle as well as of the seminal vesicles. Thus, injected in this dosage, 19-norTPP showed not only high anabolic potency (as determined from the effect on the levator ani muscle), but also high androgenic activity (as determined from the effect on the seminal vesicles). The above-mentioned conclusions of Saunders (1957) and Overbeek & de Visser (1957) concerning high anabolic potency and weak androgenic activity for different 19-nortestosterone derivates seem therefore to be correct only for certain dose levels.

The use of the levator ani muscle test as an index of anabolic activity of androgens has been criticized. As pointed out in the introduction, only some of the skeletal muscles show atrophy after castration. The striated muscles of the perineal complex, including the bulbocavernosus, ischiocavernosus and levator ani muscles, show a remarkable atrophy after castration, and this atrophy can be prevented by the administration of androgens. Owing to this fact, several authors (e.g. Scow 1952; Scow & Hagan 1957; Nimni & Geiger 1957) consider the levator ani muscle to be, in this aspect, not representative of the skeletal muscles of the body but representative of the sex accessories.
of the male. From these and other critical objections, discussed in more detail by Gordon (1957), Russel & Wilhelmi (1958) and Eisenberg (1961), it is quite clear that the levator ani muscle test for anabolic activity can be used only as a qualitative screening test and not – as has widely been the case – as a quantitative one.

In agreement with many previous experimental and clinical reports (e.g. Rubinstein & Solomon 1941; Kenyon et al. 1944; Kochakian 1950; Korner & Young 1955) it was also observed in the present study that androgens in certain doses can stimulate an increase in body weight. Compared with the body weights of the castrated rats injected with arachis oil, a significant increase in body weight was found in the castrated rats injected with 0.05 and 0.5 mg of 19-norTPP or with 0.05 mg of TPP (see Table 1).

The response of the mammary gland structures to 19-norTPP was found to be very similar to that found after administration of TPP. Daily injections for 28 days of 0.01 mg of either TPP or 19-norTPP (Groups III A and IV A) stimulated development of a few small groups of alveoli in the mammary glands. Administration of 0.05 mg of TPP (Group III B) produced a more marked lobule-alveolar development which, quantitatively as well as qualitatively, was similar to that found in untreated rats with intact testes (Group II). Administration of 0.05 mg of 19-norTPP stimulated the same degree of lobule-alveolar development as did the same dose of TPP but, after treatment with 19-norTPP, the ducts and alveoli were more distended by secretion. Daily injections of 0.5 mg of 19-norTPP (Group IV C) produced an even more extensive lobule-alveolar development in the mammary glands, but then the ducts and alveoli were widely distended by secretion. It has been shown in previous experiments that administration of high doses of testosterone propionate can also stimulate marked secretion in the mammary glands of castrated male rats (see Ahrén & Etienne 1959). In the doses used, neither 19-norTPP nor TPP stimulated any growth of the mammary duct system.

When comparing the reaction of the mammary gland to the 2 compounds used with that of the levator ani muscle and the seminal vesicles, respectively, it is obvious that, quantitatively, the mammary gland development followed that of the levator ani muscle more than that of the seminal vesicles (see Fig. 1). Thus, injections of 0.01 mg of 19-norTPP produced only a minimal weight increase of the seminal vesicles, while this treatment stimulated definite growth of the levator ani muscle and produced a slight but obvious development of alveoli in the mammary glands (Group IV A). Furthermore, administration of 0.05 mg of 19-norTPP produced only slight weight increase in the seminal vesicles, while this treatment stimulated marked growth of the levator ani muscle and produced an extensive lobule-alveolar development in the mammary glands. In addition, in the castrated rats treated with 0.5 mg of 19-norTPP the seminal vesicles were of about the same size as in the un-
treated rats with intact testes, while the levator ani muscle and the mammary glands were, after this treatment, much more stimulated than in the untreated rats with intact testes. These observations indicate that the lobule-alveolar development, produced in the rat mammary gland by androgens, follows the anabolic more than the androgenic activity of these compounds.

The fact that the development of the rat mammary gland after administration of androgens seems to be correlated more to the growth of the levator ani muscle than to the development of the seminal vesicles may also be of interest from another aspect. It is well known that androgens can also stimulate considerable development of the seminal vesicles in hypophysectomized animals. It is true that treatment of hypophysectomized rats with testosterone together with anterior pituitary factors (growth hormone and prolactin) can stimulate slightly better growth of the seminal vesicles than treatment with testosterone alone (e.g. Huggins et al. 1955; Chase et al. 1957). But since testosterone alone can stimulate marked development of the seminal vesicles in hypophysectomized rats, this synergistic action of anterior pituitary hormones is, for the growth processes in the seminal vesicles, only slight. For the growth processes in the mammary gland, the synergistic action of anterior pituitary hormones is of quite another significance. Thus, testosterone does not stimulate any lobule-alveolar development in the mammary glands of hypophysectomized rats, as does this compound in rats with intact pituitary gland (e.g. Ahrén & Etienne 1959). In addition, injections of testosterone together with growth hormone in hypophysectomized rats produce an extensive lobule-alveolar development (Reece & Leonard 1942; Ahrén 1959). The presence of growth hormone seems therefore to be necessary for the stimulating action of androgens on the rat mammary gland. The response of the levator ani muscle to androgens in the absence of anterior pituitary hormones has not been studied in detail. From the tables in a paper by Scow (1952) some observations indicate, however, that as for the mammary glands, a synergistic action between androgens and anterior pituitary hormones is of great importance for the growth processes in the levator ani muscle. Further studies of these relationships are in progress in this laboratory.

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