PHYSIOLOGICAL EFFECTS OF THE PINEAL GLAND IN ANDROGEN-STERILIZED FEMALE RATS

By

Russel J. Reiter

ABSTRACT

The influence of early androgen treatment, light deprivation (by blinding), pinealectomy and superior cervical ganglionectomy on the reproductive system of female rats was tested. Early postnatal treatment of rats with testosterone propionate caused adult rats to exhibit the characteristic signs of androgen sterilization; these included polyfollicular ovaries, normal-sized uteri and persistent vaginal cornification. If early androgen-treated rats also were blinded the ovaries were smaller in size and contained fewer follicles, the uteri were greatly reduced in size and the incidence of vaginal oestrus was decreased by approximately 50%. If in addition to blinding, androgen-sterilized animals were subjected to either removal of the pineal gland or superior cervical ganglia, the reproductive organs and the vaginal smears were indistinguishable from those of testosterone-treated rats with eyes. These data indicate that the inhibitory influence of blinding on the pituitary-ovarian axis was mediated through the sympathetic nervous system and the pineal gland. The restraining influence of light deprivation on the growth of the reproductive organs was not permanent as illustrated by the fact that if these animals were kept to 120 days of age the ovaries and uteri grew to the same level as those of pinealectomized control rats.

The cyclic changes characteristic of reproductive activity of adult female rats can be interrupted by the injection of a variety of androgens or oestrogens into such animals early in life (Barraclough 1961). Apparently, these steroids, when administered to new born rats, permanently alter neural centers regulating gonadotrophin secretion and result in an anovulatory syndrome which can be characterized by polyfollicular ovaries which lack corpora lutea and a vaginal mucose which is permanently cornified.
The overt signs of androgen sterilization are suppressed by depriving animals of light either by blinding or by exposing them to total darkness (Hoffmann et al. 1968). The mechanisms whereby the absence of light restricts the manifestation of these signs remain unclear but preliminary studies indicate that the pineal gland may be involved (Reiter et al. 1968a). The pineal gland, which is at least partially controlled by photic stimuli (Reiter & Hester 1966; Reiter et al. 1968b; Wurtman 1967) is most active during darkness and presumably secretes a gonad-inhibiting substance which impedes gonadotrophin synthesis or release from the pituitary gland or its action at the level of the gonads (Reiter & Fraschini 1969) and in this manner prevents the occurrence of some of the reproductive changes normally associated with the androgen-sterilized rat. The following report describes a series of experiments which were designed to further test the relationships of blinding and androgen sterilization and attempt to define, more precisely, the role of the pineal gland in the response.

**METHODS**

A total of 392 female Sprague-Dawley rats were used in the investigations; after weaning they were housed 2 or 3 per cage in windowless rooms under controlled lighting (14 h of light per day) and temperature (22 ± 1°C) conditions. Artificial illuminations was provided by 40 W «cool white» fluorescent bulbs yielding an intensity of 40 to 100 footcandles at the level of the animals. All animals were fed with standard laboratory chow.

In the initial experiment 81 animals were used. Of these, 48 randomly selected females from 11 litters were given a single injection of 1.0 mg of testosterone propionate (TP) in 0.1 ml of sesame oil at 5 days of age. Following its administration, collodion was placed on the site of injection to prevent escape of the injected material from the wound. The young were returned to their mothers after reduction of litter size to 8–10 animals. At the time of weaning (21–23 days of age), all TP-treated rats were either blinded, pinealectomized or sham pinealectomized, the operations being done either alone or in combination. Three groups of non-androgen treated animals were also utilized. The resulting groups are shown in Table 1. In this and the subsequent two experiments, all non-pinealectomized animals were sham operated. However, since this procedure had no apparent effect on the results it is not listed in the tables nor will it be discussed. The techniques for pinealectomy (Hoffman & Reiter 1965) and blinding (Reiter & Hester 1966) have been described elsewhere. All operations were performed with animals under sodium pentobarbital anaesthesia. Beginning at 59 days of age and continuing until the time of necropsy, daily vaginal smears were taken by lavage. These were allowed to air dry and were stained with toluidine blue. All animals were killed at 73 days of age by exsanguination under ether anaesthesia. The ovaries, uterus and pituitary glands were weighed and preserved for histological study by fixation in Bouin's fluid. Numerical data were tested for significance using the Student's t test.

Since the innervation of the pineal gland in the rat is derived from the superior cervical ganglia (Kappers 1965) and because the sympathetic fibers to the pineal seem to be of paramount importance in regulating pineal activity (Axelrod & Wurtman...
1968; Reiter 1967; Wurtman 1967; Wurtman et al. 1964), the second experiment was
devised to study the effect of bilateral superior cervical ganglionectiony (SCG) on the
reproductive response of TP-treated blinded rats. The procedures were similar to those
of the previous study. TP was injected into 5-day-old females and the operations,
i.e., sham or real pinealectomy, SCG or blinding, were done on 22–23 day old rats.
The various treatment resulted in 8 groups of rats (Table 2). The technique for re-
moval of the superior cervical ganglia was similar to that used in an early study
(Reiter 1967). Daily vaginal smears were taken for 2 weeks before necropsy, the
animals being killed at 75 days of age. Tissues and data were treated as in exper-
iment 1.

To test whether the inhibitory influence of the pineal gland on the hypothalamo-
hypophysis-gonadal axis of the TP-treated eyeless rat represented a permanent sup-
pression of gonadotrophins, a third experiment was conducted utilizing 234 animals.
The animals were divided into three groups: untreated; TP-treated and blinded; TP-
treated, blinded and pinealectomized. The non-pinealectomized animals were sham
operated. The course of treatment was similar to that employed in the two earlier
studies. Beginning at 45 days of age and continuing at 15 days intervals thereafter,
animals (9–17 per group) from each of the categories were killed. Organs were weighed
and retained for histological study.

RESULTS

1. The effects of blinding, pinealectomy and early TP-treatment on body
weight increase and on the pituitary-gonadal axis in female rats (Table 1)

Removal of the eyes (Group 2) was followed by a significant depression in
body weight increase unless the animals were also pinealectomized (Group 3).
The restraining influence of blinding on the growth of the rats was apparent
also in the TP-treated rats; this was likewise reversed by pineal ablation.
Barraclough (1961) reported that early androgen treatment causes the rats to
grow faster than normal. This tendency was apparent in the present experi-
ment, i.e., TP-injected animals with eyes (Groups 4, 5, and 7) were invariably
heavier than rats not receiving the steroid although the increase was never of
sufficient magnitude to be statistically significant.

Although both the absolute and relative organ weights are listed in Table 1
the following discussion will include remarks made without reference directly
to absolute or relative weights. This is done since, with few exceptions, if the
absolute weight of an organ was significantly different from that of the con-
trol value the relative organ weight of the experimental animal also differed
significantly from the relative organ weight of the control group.

Characteristically, unless animals were pinealectomized (Group 3) blinding
delayed the maturation of the ovaries (Group 2). Even though the ovaries of
blinded rats were somewhat smaller, they were microscopically similar and
could not be routinely distinguished from those of untreated controls (Fig. 1).
Table 1.
Influence of blinding, early androgen treatment and pinealectomy on body weights, absolute and relative (in parentheses) organ weights and vaginal smears of rats.

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>N</th>
<th>Body weight (g)</th>
<th>Organ weights</th>
<th>C. I. 1)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ovaries</td>
<td>Uterus</td>
</tr>
<tr>
<td>1. None</td>
<td>15</td>
<td>205 ± 5</td>
<td>58.8 ± 1.8</td>
<td>327 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(28.7 ± 0.9)</td>
<td>(160 ± 12)</td>
</tr>
<tr>
<td>2. Blinded</td>
<td>9</td>
<td>183 ± 4†</td>
<td>45.7 ± 3.1†</td>
<td>231 ± 21†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(25.0 ± 2.0)</td>
<td>(126 ± 12)*</td>
</tr>
<tr>
<td>3. Blinded; pinealectomized</td>
<td>9</td>
<td>203 ± 5</td>
<td>65.1 ± 2.2</td>
<td>345 ± 14</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(32.1 ± 1.5)</td>
<td>(170 ± 10)</td>
</tr>
<tr>
<td>4. TP-treated</td>
<td>8</td>
<td>210 ± 9</td>
<td>31.0 ± 1.7†</td>
<td>319 ± 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(14.8 ± 0.9)*</td>
<td>(152 ± 9)</td>
</tr>
<tr>
<td>5. TP-treated; pinealectomized</td>
<td>10</td>
<td>219 ± 7</td>
<td>36.7 ± 2.3†</td>
<td>321 ± 9</td>
</tr>
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<td></td>
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<td></td>
<td>(16.6 ± 1.2)*</td>
<td>(147 ± 6)</td>
</tr>
<tr>
<td>6. TP-treated blinded</td>
<td>16</td>
<td>179 ± 6†</td>
<td>17.3 ± 1.7†</td>
<td>132 ± 19†</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(9.7 ± 0.9)*</td>
<td>(74 ± 11)*</td>
</tr>
<tr>
<td>7. TP-treated; blinded; pinealectomized</td>
<td>14</td>
<td>211 ± 9</td>
<td>33.7 ± 1.9†</td>
<td>297 ± 13</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(16.0 ± 1.0)*</td>
<td>(140 ± 8)</td>
</tr>
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</table>

1) Cornification index – per cent of vaginal smears containing cornified cells; range in parentheses.
† Significantly different from absolute mean weight of untreated controls (group 1).
* Significantly different from relative mean weight of untreated controls (group 1).
Early androgen treatment (Group 4), however, has marked gross and microscopic effects on ovarian morphology (Barraclough & Gorski 1961; Flerkó et al. 1967; Harris 1964; Jacobson 1964). These changes are obvious in Fig. 2 which shows an ovary of a rat treated neonatally with TP. The absence of corpora lutea, which accounts for the small size, and the plethora of vesicular follicles were characteristic; removal of the pineal gland (Group 5) did not interfere with reproductive influences of early androgen treatment.

Growth of the ovaries in TP-treated rats that were also blinded was severely retarded; in the present experiment the mean ovarian weight was 17.3 mg at 73 days of age (Group 6), about one-third the control value. In addition to being grossly smaller, ovaries from these rats contained fewer than the normal number of large antral follicles usually seen in ovaries of androgen-sterilized rats and moderate amounts of interstitial tissue (Fig. 3). Pinealectomized rats treated in this manner (Group 7) had ovaries (Fig. 4) that were as large and microscopically indistinguishable from those of TP-treated rats with eyes (Groups 4 and 5).

As with the ovaries, the mean uterine weight of eyeless rats (Group 2) was significantly depressed; this effect was reversed by pinealectomy (Group 3). Since the polyfollicular ovaries of androgen-sterilized rats secrete sufficient amounts of oestrogen, uterine weights are maintained at about the control level regardless of whether the animals are (Group 5) or are not (Group 4) pinealectomized. The smallest uteri were found in those animals that were eyeless and had received TP at 5 days of age (Group 6); uteri from these animals also were microscopically infantile in appearance. Pineal ablation in blinded TP-treated rats (Group 7) resulted in uteri that were similar to those treated with only TP.

Pituitary growth followed a pattern similar to that of the uteri. Removal of the eyes alone (Group 2) or in combination with androgen treatment (Group 6) curtailed pituitary maturation. In both cases (Groups 3 and 7, respectively) absence of the pineal allowed normal pituitary growth. Vaginal smear information further supports the conclusion that blinding caused a suppression of the pituitary-ovarian axis unless the rats were pinealectomized. The cornification index, which is the per cent of vaginal smears that contained cornified cells, is listed in Table 1. Forty-five per cent of the smears from normal rats contained sloughed cornified epithelial cells. All of these occurred during the prooestrus and oestrous phases of the oestrus cycle. Blinding alone or with pinealectomy did not appreciably alter the vaginal smears. TP-treatment, of course, induce persistent vaginal cornification with 93% of the smears showing cornified cells (Group 4); this was not influenced by pineal removal. If, however, androgen-treated rats were blinded, the incidence of oestrus smears containing cornified cells was reduced to 52% (Group 6). The inhibitory effect of blinding on the cornification index of TP-treated rats were negated by
pinealectomy, with 91% of the smears from animals of this group containing cornified cells (Group 7).

2. The effects of blinding, bilateral superior cervical ganglionectomy and early TP-treatment on body weight increase and on the pituitary-gonadal axis in female rats (Table 2)

The results from this experiment were similar to those of the initial study. Hence, the data are discussed in a more cursory manner. Although the mean body weight of the androgen-treated animals (Group 6) was less than that of any other group it was not significantly different from that of controls.

Ovarian weight was not influenced by superior cervical ganglionectomy (SCG) alone (Group 2) or when combined with pineal removal (Group 3) and, similarly, neither of these treatments (Groups 5 and 6) reversed the inhibitory effect of the TP-treatment on ovarian growth. As in the first study, the growth of the gonads was markedly retarded in TP-treated, light-deprived rats (Group 7). Interestingly, SCG was as effective as pinealectomy in countering this regressive response. Microscopically, the ovaries of androgen-treated, eyeless rats were similar to those seen in experiment one; they possessed fewer than the normal number of vesicular follicles and moderate amounts of interstitial tissue. After SCG (Group 8) the ovaries were characteristically polyfollicular and were devoid of corpora lutea.

Gross uterine weights were not influenced by the various combinations of treatments with the exception of animals that were androgen-sterilized and blinded (Group 6). The importance of the sympathetic innervation to pineal function is further illustrated by the fact that sympathetic denervation of the pineal rendered it incapable of causing ovarian inhibition (Group 8).

As with the uteri, only in the early androgen-injected blinded rat did the

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**Fig. 1.** An ovary of an untreated rat killed at 73 days of age. Corpora lutea, vesicular follicles and small amounts of interstitial tissue are obvious. × 15.

**Fig. 2.** Ovarian structure of a 73-day-old rat that had been treated neonatally with testosterone. Absence of corpora lutea and the large number of vesicular follicles are characteristic. × 15.

**Fig. 3.** Ovary of a TP-treated, blinded rat killed at 73 days of age. Corpora lutea are still absent but there are many fewer antral follicles than in ovaries of TP-treated rats with eyes (cf. Fig. 2). Interstitial tissue is relatively abundant. × 15.

**Fig. 4.** A polyfollicular ovary taken from an androgen-sterilized rat that was both blinded and pinealectomized. It is obvious that the ovarian effects of blinding have been counteracted by pineal removal. Ovaries from these rats were similar to those of TP-treated, non-blinded rats. × 15.
Table 2.
Influence of blinding, early androgen treatment, superior cervical ganglionectomy and pinealectomy on body weights, absolute and relative (in parentheses) organ weights and vaginal smears of rats.

<table>
<thead>
<tr>
<th>Group and Treatment</th>
<th>N</th>
<th>Body weight (g)</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ovaries</td>
<td>Uterus</td>
</tr>
<tr>
<td>1. None</td>
<td>9</td>
<td>201 ± 8</td>
<td>66.9 ± 3.5</td>
<td>336 ± 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(33.3 ± 2.1)</td>
<td>(167 ± 9)</td>
</tr>
<tr>
<td>2. Ganglionectomized</td>
<td>8</td>
<td>213 ± 8</td>
<td>65.3 ± 3.6</td>
<td>320 ± 16</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(30.7 ± 1.7)</td>
<td>(150 ± 10)</td>
</tr>
<tr>
<td>3. Ganglionectomized; pinealectomized</td>
<td>8</td>
<td>217 ± 9</td>
<td>61.8 ± 2.2</td>
<td>340 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(28.5 ± 1.4)</td>
<td>(157 ± 8)</td>
</tr>
<tr>
<td>4. TP-treated</td>
<td>10</td>
<td>206 ± 5</td>
<td>31.8 ± 2.0†</td>
<td>309 ± 18</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(15.4 ± 1.1)</td>
<td>(150 ± 10)</td>
</tr>
<tr>
<td>5. TP-treated; ganglionectomized</td>
<td>12</td>
<td>219 ± 10</td>
<td>34.7 ± 1.8†</td>
<td>322 ± 15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(15.8 ± 1.1)</td>
<td>(147 ± 6)</td>
</tr>
<tr>
<td>6. TP-treated; ganglionectomized; pinealectomized</td>
<td>11</td>
<td>216 ± 5</td>
<td>31.0 ± 1.9†</td>
<td>298 ± 26</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(14.4 ± 0.9)</td>
<td>(138 ± 0.9)</td>
</tr>
<tr>
<td>7. TP-treated; blinded</td>
<td>9</td>
<td>191 ± 6</td>
<td>21.9 ± 1.4†</td>
<td>154 ± 15†</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>(11.5 ± 0.7)</td>
<td>(80 ± 8)</td>
</tr>
<tr>
<td>8. TP-treated; blinded; ganglionectomized</td>
<td>10</td>
<td>217 ± 8</td>
<td>31.8 ± 1.7†</td>
<td>303 ± 15</td>
</tr>
<tr>
<td></td>
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<td>(14.7 ± 0.8)</td>
<td>(140 ± 9)</td>
</tr>
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1) Cornification index – per cent of vaginal smears containing cornified cells; range in parentheses.
† Significantly different from absolute mean weight of untreated controls (group 1).
* Significantly different from relative mean weight of untreated controls (group 1).
pituitary glands fail to grow normally, this also being negated by denervation of the pineal gland (Group 8).

The vaginal smear data were similar to those of experiment one. The cornification indices were similar in untreated (Group 1), ganglionectomized (Group 2) and ganglionectomized pinealectomized (Group 3) rats. All non-blinded animals treated with TP (Groups 4, 5 and 6) exhibited persistent vaginal cornification. Blinding curtailed markedly the frequency of cornified cells in vaginal smears to TP-treated rats (Group 7) but this was counteracted by pineal denervation (Group 8).

3. The long term effects of blinding, pinealectomy and early TP-treatment on body weight increase and the growth of the ovaries, uteri and pituitary glands (Fig. 5)

In this study 3 groups of rats were utilized: untreated; TP-treated and blinded; TP-treated blinded and pinealectomized. At 45 days of age the mean body weights of the 3 groups of animals (based on Student’s t test) did not differ significantly (Fig. 5, A). Due to a lag in growth, however, at 60 and 75 days of age mean body weights of androgen-treated eyeless rats were less than those of the other 2 groups. At 90 days of age and thereafter until the termination of the experiment, body weights were similar for all groups.

Fig. 5.

Body (A), ovarian (B), uterine (C) and pituitary (D) weights at various ages in three groups of rats: untreated control rats (●); TP-treated, blinded rats (□); TP-treated, blinded pinealectomized rats (○). Each point represents 9–17 animals. Mean weights that differ significantly ($P < 0.001$) from those of untreated control rats are marked with an asterisk.
As seen in the first two studies, ovarian weight after TP-treatment was altered appreciably even in rats with eyes. If rats were additionally blinded, the ovaries grew even more slowly so that they were significantly smaller \((P < 0.05)\) than those of TP-treated, blinded pinealectomized rats (Fig. 5, B). This difference persisted until 90 days of age at which time the ovaries of TP-treated, blinded and TP-treated, blinded, pinealectomized rats were equivalent in size; ovaries from these groups never approached the size of those in untreated rats.

As indicated by uterine weights (Fig. 5, C), oestrogen secretion was severely reduced in androgen-sterilized rats after removal of the eyes. Uteri in these rats remained smaller than those of untreated and pinealectomized controls until 120 days of age.

The pituitary glands were the only organs studied that did not recover from the pineal inhibitory influence by 120 days after birth (Fig. 5, D). Even at this age pituitary glands of light-deprived, androgen-treated rats were one-half the size of those of the two control groups; mean hypophyseal weights in the two control groups never differed significantly.

**DISCUSSION**

The restraining influence of blinding on body weight increase is well documented and is known to be prevented by pineal ablation \((Reiter et al. 1968b)\). However, whether or not substances produced by the pineal of eyeless animals have a specific effect on growth retardation is a sequel of the suppression of other endocrine organs, *e.g.*, the thyroid gland, requires consideration.

The gonadotrophin deficit produced by treating female rats shortly after birth with testosterone has been extensively studied \((Barracough 1967; Harris 1964; Jacobson 1964)\). The best evidence available indicates that androgen, given to rats during the first week of life, causes irreversible damage to the neural centres which, after adulthood, are responsible for the cyclic release of luteinizing hormone (LH). This alteration in hypothalamic development accounts for the syndrome associated with androgen sterilization \((Barracough & Gorski 1961)\). The response does not involve the pineal since removal of this organ is without effect on the reproductive physiology of androgen-treated rats. On the other hand, early TP-treatment apparently makes the hypothalamus, and/or other portions of the brain, highly sensitive to the pineal substance in blinded female rats. Whereas light deprivation in non-androgen treated rats had only moderate effects on reproduction, when blinding was combined with early TP-treatment, reproductive changes were generally pronounced. Throughout the three experiments, the ovaries and uteri of eyeless, TP-treated rats were grossly smaller and microscopically altered when com-
pared with those of pinealectomized control rats. The changes that occurred in these animals cannot be explained solely on the basis of an additive effect of the two treatments since TP-injection alone had no influence on uterine weight. On the other hand, light deprivation in such animals severely depressed uterine growth when compared with the effect of blinding in non-androgen treated rats.

Sympathetic neural regulation of endocrine organs is generally exerted by an effect on the vasculature of the organ. The minimal rate of blood flow through the pineal gland of the rat is greater than that of all other endocrine organs (Goldman & Wurtman 1964) and yet it is apparently only slightly altered after sympathetic denervation (Goldman 1967). In at least several mammals, including the rat, many of the postganglionic fibers originating within the superior cervical ganglia reportedly terminate directly on pineal parenchymal cells rather than in the perivascular space (Bostelmann 1968; Kappers 1965; Wartenburg & Gusek 1965). The sympathetic fibers participate in the regulation of the metabolic activity of the pineal as evidenced by the fact that in bilaterally superior cervical ganglionectomized rats light, perceived by the eyes, is no longer capable of inhibiting melatonin synthesis within the pineal gland (Axelrod & Wurtman 1968; Wurtman et al. 1964). Similarly, the pineal of the blinded golden hamster loses its gonad-inhibiting influence after sympathetic denervation (Reiter 1967; Reiter & Hester 1966). The data reported here are consistent with the findings in the hamster and support the conclusion that the neural fibers, derived from the sympathetic ganglia within the neck, have an influence on the synthesis or the secretion of the gonad-inhibiting principle from the rat pineal. In fact, in the present study SCG was equally as effective as removal of the pineal gland itself in preventing the inhibitory effect of light deprivation on the reproductive organs of androgen-sterilized rats.

At least two possible explanations are available for the eventual growth (by about 120 days) of the reproductive organs of blinded androgen-treated rats to the level of those in TP-treated, non-blinded or TP-treated, blinded, pinealectomized rats (Fig. 5, B and C). After long term stimulation the pineal gland either discontinues secretion of the active principle or the inhibited site overcomes the suppressive influence of the pineal, i. e., it becomes less sensitive to the pineal principle. A similar phenomenon occurs in hamsters where blinding alone is followed by approximately a 20 week period of total gonadal involution with an eventual return of the gonads to the adult condition (Reiter 1969). What the metabolic status of the pineal gland is during gonadal growth in either of these species is unknown.

The site at which the pineal substance acts has not been identified although several areas, namely the midbrain and the median eminence, have been implicated (Anton-Tay et al. 1968; Fraschini et al. 1968). It is generally conceded that the administration of TP to neonatal rats alters the development
of the preoptic-anterior hypothalamus and possibly, when administered in high doses such as used in these studies, the ventromedial-arcuate nuclear complex as well (Barraclough 1967). Since this treatment «sensitizes» the hypophyseo-gonadal axis to the pineal substance one is tempted to conclude that the gonad-inhibiting principle also may act on these altered sites. This suggestion requires considerably more evidence, however, before it can be made with any degree of certainty.

ACKNOWLEDGMENT

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