The ovary and sexual maturation of the brain

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Pfeiffer (1935, 1936) reported the induction of constant oestrus in female rats following the transplantation of testes from litter-mate males just after birth and noted that the ovaries of these animals did not contain corpora lutea. These changes remained after removal of the testis transplants. The same effects were obtained by Bradbury (1941) following the administration of multiple doses of testosterone propionate. Barraclough & Leathem (1954) found that a single injection of 1 mg of testosterone propionate at 5 days of age led to permanent sterility in female mice, with no corpus luteum formation in their ovaries. Similar results were obtained in rats by Barraclough (1961) with the administration of a single injection of 1·25 mg of testosterone propionate. This permanent change in ovarian function does not appear to be a direct effect upon the ovary (Bradbury, 1941). The masculinizing influence of testosterone propionate appears to be exerted upon the brain, with only an indirect effect upon the anterior pituitary gland by way of the hypothalamus (Adams Smith & Peng, 1966). The influence of the ovary in sexual differentiation of the central nervous system is not clear. This study was undertaken in an endeavour to determine the pattern of sexual maturation of the brain of the female rat ovariectomized at birth and treated with testosterone propionate at different times during the first three post-natal weeks. Different doses of testosterone were administered to see whether dosage influenced the receptive period of the animal’s brain. The receptive period found in ovariectomized animals would allow a comparison with results obtained by workers who had treated normal females with testosterone, so that an assessment could be made as to whether the presence of the ovary influenced the length of this receptive period. Control procedures were planned to allow an observation of the sexual maturation of the brain of ovariectomized females not subjected to androgen treatment.

MATERIALS AND METHODS

Female rats of an inbred Lister strain were used and were fed on a stock diet. The animals were housed in a room in which the temperature was maintained between 21 and 23° C, with 13 h of light and 11 h of darkness in every 24 h.

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Ovariectomy. Newborn litters of rats were sexed and the females were subjected to hypothermia and ovariectomized within 16 h of birth (usually between 2 and 6 h from birth) using the method described by Pfeiffer (1936). The litter size was reduced to eight or nine young which were nurtured by the mother until weaned at 3 weeks of age. One group of control animals was ‘mock-operated’ on the day of birth.

Testosterone injection. The ovariectomized rats were given a subcutaneous injection of testosterone propionate (TP) in 40 % ethyl oleate and 60 % arachis oil on either the fifth, tenth or twentieth day from birth. The day of birth was counted as the first day, so that the fifth day covered the period 96–120 h after birth. Within these three groups different subgroups were given 50 μg, 100 μg, or 500 μg TP and an additional subgroup of the animals injected on the twentieth day was given 1000 μg TP. The required dose was administered in an injection of either 0·05 ml. or 0·1 ml. of the solution. In order to minimize leakage the tip of the needle was inserted at the nape of the neck and slid down under the dorsal skin as far as the upper lumbar region before making the injection. One control group of ovariectomized females was injected with 0·1 ml. of 40 % ethyl oleate and 60 % arachis oil on the fifth day from birth and another control group of ovariectomized females was not injected.

Ovarian graft to the eye. At the age of 3 months the rats, both testosterone-treated and controls, had immature ovarian tissue from 18- to 23-day-old donors placed in the anterior chamber of one eye, using the technique described by Goodman (1934). In a further small control group two normal rats had one ovary removed at 3 weeks of age and grafted to the anterior chamber of the eye in a one-stage operation, and one rat, whose own ovaries were left intact, also received a similar graft when 3 weeks old. The animals in this group were killed when 6 weeks old and the grafts, which had been observed in the living animals for 3 weeks, were examined histologically.

Post-operatively all the animals, apart from this last control group, had a vaginal smear taken daily and the development and activity of the ovarian grafts were observed. The animals were killed at 5 months of age (or sooner where the grafts had herniated) and at post-mortem examination a search was made for ovarian remnants. The ovarian grafts were removed, fixed in Bouin’s fluid and embedded in paraffin wax. Serial sections, cut at 6 μ and stained with haematoxylin and eosin, were studied microscopically.

RESULTS

Experimental animals

The number of animals in each subgroup and the dosage of TP administered are shown in Table 1.
**Ovary and brain sex**

Group I

These animals had received 50–500 μg TP at 5 days of age.

*Vaginal smear.* Until the ovarian graft became established the smear was dioestrous, but when the graft became vascularized the smear was persistently cornified, interrupted very occasionally by 1 or 2 days on which nucleated epithelial cells were present or on which there was a large quantity of stringy mucus.

Table 1. *Vaginal and ovarian response of rats, ovariectomized at birth, treated with graded doses of testosterone propionate at different stages of post-natal development*

<table>
<thead>
<tr>
<th>No. of animals</th>
<th>Dose of testosterone propionate (μg)</th>
<th>Vaginal smear</th>
<th>Ovarian graft function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (testosterone given on fifth day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a 8</td>
<td>50</td>
<td>Constant oestrus</td>
<td>Follicles</td>
</tr>
<tr>
<td>b 7</td>
<td>100</td>
<td>Constant oestrus</td>
<td>Follicles</td>
</tr>
<tr>
<td>c 5</td>
<td>500</td>
<td>Constant oestrus</td>
<td>Follicles</td>
</tr>
<tr>
<td>Group II (testosterone given on tenth day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a 5</td>
<td>50</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
<tr>
<td>b 7</td>
<td>100</td>
<td>Cycling (6 rats)</td>
<td>Follicles and corpora lutea (6 rats)</td>
</tr>
<tr>
<td>c 8</td>
<td>500</td>
<td>Constant oestrus (1 rat)</td>
<td>Follicles (1 rat)</td>
</tr>
<tr>
<td>Group III (testosterone given on twentieth day)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>a 8</td>
<td>50</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
<tr>
<td>b 7</td>
<td>100</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
<tr>
<td>c 8</td>
<td>500</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
<tr>
<td>d 7</td>
<td>1,000</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
</tbody>
</table>

*Ovarian grafts.* For the first few days after implantation of the graft the anterior chamber of the eye was cloudy and in some cases, where there had been damage to iridial vessels at the time of operation, contained blood. Within 4–6 days the eye cleared and blood vessels were seen to be growing into the graft. The interstitial tissue of the grafts increased in amount and the follicles enlarged, so that those on the surface of the graft were seen as vesicles filled with clear fluid whose daily enlargement could be readily followed. The follicles predominated over the interstitial tissue and some became very large and projected prominently from the surface of the graft. During the 2 months that the grafts were observed in the living animals no areas of haemorrhage were seen, nor were any corpora lutea apparent. One graft, in a rat given 500 μg TP, developed like the others for 5 weeks and then began to form many solid bodies. These bodies lacked the salmon coloration of normal corpora lutea and were
smaller and less well vascularized. No areas of fresh haemorrhage were seen prior to the formation of these bodies.

The histological appearance of all but one of the grafts was of numerous follicles at all stages of development. Most of the follicles seen were well developed and many were much larger than normal and contained so much liquor folliculi that the grafts presented a cystic appearance, with only a small amount of interstitial tissue lying between the follicles (Plate 1, fig. 1). No typical corpora lutea were seen, but several of the grafts contained one or two circumscribed aggregations of luteinized cells. These bodies were smaller than corpora lutea and had a much less striking vascularity. The luteinized cells appeared to be derived from the theca interna of degenerating follicles, which had matured without ovulating and then begun to regress (Plate 1, figs. 2, 3).

Very occasionally follicles were seen in which the ovum had degenerated, but was still discernible, while the cells of the theca interna had become luteinized and formed a prominent layer surrounding a thinner layer of more basophilic non-luteinized granulosa cells which appeared to be degenerating. Sections of the graft in which many solid bodies had formed displayed fewer follicles than the other grafts and many aggregations of luteinized cells, some forming solid bodies and others surrounding what appeared to be follicular antra. Two of the luteinized follicles contained a little old blood in the cavity, which was immediately surrounded by apparently degenerating granulosa cells outside which the thick layer of luteinized cells lay. No lymphocyte invasion of the graft was seen.

**Group II**

These animals had received 50–500 µg TP at 10 days of age.

*Vaginal smear.* Once the grafts became established all but one rat showed a 4- to 5-day cyclical activity in the smear, although the pattern was irregular at

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**Plate 1**

Fig. 1. Ovarian eye graft from an ovariectomized rat injected with 50 µg of testosterone propionate on the fifth day from birth. Many large, vesicular follicles are visible, with only a small amount of interstitial tissue present, giving the section a cystic appearance. Two thecal lutein bodies are visible. Scale mark represents 0.5 mm.

Fig. 2. Ovarian graft from the eye of an ovariectomized rat injected with 50 µg of testosterone propionate at 5 days of age. A number of large follicles, but no corpora lutea, are visible and in the area demarcated there is thecal luteinization occurring in the wall of a follicle in which the oocyte has degenerated and the stratum granulosum is degenerating. Scale mark represents 0.5 mm.

Fig. 3. A view at higher magnification of the luteinization of theca interna cells occurring in the area demarcated in fig. 2. The granulosa cells lining the follicular antrum and extending into the cumulus have lost their regular arrangement and the nuclei appear pyknotic. Scale mark represents 0.05 mm.

Fig. 4. Ovarian eye graft from an ovariectomized rat injected with 500 µg of testosterone propionate on the tenth day from birth, in which follicles and fresh corpora lutea can be seen. There are more follicles and fewer corpora lutea than in the normal ovary. Scale mark represents 0.5 mm.
first. The other rat (injected with 100 \( \mu g \) TP) showed persistent cornification of the vaginal smear in the same way as the rats in group I. This rat was one of four litter-mates which all received the same treatment.

**Ovarian grafts.** The grafts showed rapid follicular development and at about 10 days after operation many of the larger follicles were replaced by corpora lutea. During the succeeding weeks new follicles appeared, enlarged and were replaced by corpora lutea, so that the grafts appeared to be composed mainly of salmon-coloured corpora lutea with occasional follicles among them. The blood vessels ramifying over the grafts were particularly striking. The graft borne by the rat with a constantly oestrous vaginal smear did not follow this pattern but developed large follicles without forming corpora lutea, in the same way as the grafts described in group I.

Histological sections of the grafts contained a preponderance of apparently normal corpora lutea. These were in general larger than the ‘lutein bodies’ observed in group I, with a radial pattern of blood vessels between the cords of luteinized cells. No luteinization was seen around follicular antra. All the grafts showed some follicles in various stages of development, but there were usually fewer large follicles although four of the eight grafts in rats given 500 \( \mu g \) TP contained fewer corpora lutea than usual and a higher proportion of large follicles (Plate 1, fig. 4). The graft from the ‘constant oestrous’ rat contained many follicles, some of which were larger than normal, but no corpora lutea. It also contained one follicle in which the theca interna had begun to undergo slight luteinization.

**Group III**

These animals had received 50–1000 \( \mu g \) TP at 20 days of age.

**Vaginal smear.** All the smears quickly settled into a normal, cyclical pattern of 4–5 days duration.

**Ovarian grafts.** The grafts all developed mature follicles which were replaced by corpora lutea, and subsequently further follicles matured and formed corpora lutea in cyclical waves.

The histological appearance of all of the grafts was similar to that of a normal, mature ovary, with the major part of the graft occupied by corpora lutea and with a few maturing follicles present.

**General observations**

**Ovarian grafts.** The establishment of the graft appeared to depend on its early vascularization by the iridial vessels; the ultimate size of the graft, which usually occupied almost all of the anterior chamber of the eye by the time the animal was killed, did not appear to be closely related to the amount of ovarian tissue placed in the eye at operation. Herniation of the developing graft through the cornea was rare.

**Post-mortem examination.** No ovarian remnants were found in any of the animals which had been ovariectomized on the day of birth. In the rats in which
the ovarian graft had become established the uteri were of normal size and appearance and were well vascularized, whereas those rats in which the graft had regressed possessed small, atrophic uteri.

Control animals

Control rats (groups A–C in Table 2) which were ovariectomized at birth, with or without an injection of arachis oil and ethyl oleate at 5 days of age, or which were ‘mock-operated’ at birth behaved in a similar way. In all cases the vaginal smear was of a normal, cyclical pattern after the grafting of immature ovarian tissue to one eye. Follicles and corpora lutea developed in a cyclical fashion in the grafts in all of these control animals. The histological appearance of the grafts was similar to that seen in normal ovaries.

Table 2. Vaginal and ovarian response of various groups of control animals

<table>
<thead>
<tr>
<th>Procedure</th>
<th>No. of animals</th>
<th>Vaginal smear</th>
<th>Ovarian graft function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A: Ovariectomized at birth</td>
<td>7</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
<tr>
<td>0.1 ml. 60% arachis oil and 40% ethyl oleate s.c. at 5 days of age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20-day ovarian graft at 3 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed at 5 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group B: Ovariectomized at birth</td>
<td>7</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
<tr>
<td>20-day ovarian graft at 3 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed at 5 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group C: ‘Mock-operated’ at birth</td>
<td>3</td>
<td>Cycling</td>
<td>Follicles and corpora lutea</td>
</tr>
<tr>
<td>20-day ovarian graft at 3 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovariectomized at 5 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Killed at 6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group D: (a) One ovary removed and transplanted to eye at 3 weeks</td>
<td>2</td>
<td>—</td>
<td>Immature follicles</td>
</tr>
<tr>
<td>Killed at 6 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(b) Ovarian tissue from 3-week-old donor transplanted to eye at 3 weeks</td>
<td>1</td>
<td>—</td>
<td>Immature follicles (few)—graft regressing</td>
</tr>
<tr>
<td>Killed at 6 weeks</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The other control group (group D in Table 2) contained two animals with one ovary removed and one animal with its ovaries intact. The grafts were only present from 3 until 6 weeks of age. The grafts in rats with one ovary removed appeared healthy and well established, but they did not grow nor did their follicles mature. The graft in the rat with intact ovaries presented a similar appearance at first, but later seemed to be in the early stages of regression.
Histological examination showed the grafts from hosts with one ovary to contain healthy immature follicles but no mature follicles or corpora lutea. No ‘lutein bodies’ were seen. The graft in the rat with intact ovaries contained a few immature follicles but was largely regressing.

**General observations**

At post-mortem no ovarian remnants were found in ovariectomized animals. The uteri were similar to those in the experimental animals.

**DISCUSSION**

The assessment of ovarian function on the basis of the development of ovarian tissue transplanted to the eye has been discussed in another publication (Adams Smith & Peng, 1967). Follicular development in the transplants of gonadectomized female hosts treated with TP at 20 days of age, and of all but one of the hosts treated with TP at 10 days of age, followed the same pattern as in the ovary of the normal, mature female, whereas transplants in those animals treated with TP at 5 days of age and in one animal treated with TP at 10 days of age did not show this cyclical activity, but displayed follicular growth and formation of liquor folliculi without corpus luteum formation in a similar way to that described by Goodman (1934) in adult male rats.

In experimental animals reported upon in this study the cyclical activity of the ovarian transplants was dependent upon the time of TP administration. In animals in which TP was administered at 5 days of age the ovarian grafts displayed no cyclical activity and in those hosts treated with TP at 20 days of age the grafts displayed a normal cyclical pattern of activity, while the grafts in the eyes of rats treated with TP at 10 days of age functioned in either a cyclical or a non-cyclical way. These findings indicate that the female rat’s brain is susceptible to the masculinizing effect of TP for at least the first 5 days after birth, but that this susceptibility may be lost by 10 days of age and is certainly no longer present by 20 days of age, and are in agreement with the findings reported by Barraclough (1961). The female rats treated by Barraclough in the report referred to were not deprived of their ovaries, whereas the animals in the present study were ovariectomized on the day of birth. There does not appear to be any difference in the susceptibility to androgenic masculinization of the brain of the animals in these two experimental studies. This suggests that the presence of the ovary in the newborn female rat does not influence the sensitivity of the brain to the masculinizing effect of TP.

The dose range of TP employed was planned to cover the doses generally employed in masculinizing experiments, without the lowest dose being delayed in its effect beyond puberty. The largest dose (1000 μg) was used to determine whether masculinization would be induced at an age when the brain was no longer sensitive to lower dosages. It is of interest to note that a single dose of as
little as 50 µg will cause masculinization when administered at 5 days of age while 1000 µg is ineffective at 20 days of age. In fact, a single dose of 10 µg administered at 5 days of age to female rats has been reported to induce permanent sterility (Gorski & Barraclough, 1961). However, the results reported by Swanson & van der Werff ten Bosch (1964a, b) indicate that very small doses of TP (5 or 10 µg) may not bring about an anovulatory state until some time after puberty. It is possible that during the period that cells of the central nervous system are sensitive to testosterone the dosage of testosterone administered may influence the rate of cytodifferentiation towards the male pattern of sexual activity.

When the results in group II of the experimental animals are examined it can be seen that 10 days of age is an equivocal time in the sensitivity of the brain to the masculinizing effects of administered TP. The one animal in this group whose ovarian graft and vaginal smear indicated a masculinization of the brain had been given 100 µg TP, while its three litter-mates, given the same dosage, and eight animals given 500 µg TP, at 10 days of age, were not masculinized. Four of the eight grafts from the animals given 500 µg TP at 10 days of age (without any of them becoming masculinized) displayed a high proportion of large follicles and fewer corpora lutea than usual. A possible explanation of the finding is that the brains of these 10-day-old rats contained some cells which were still sensitive to the masculinizing influence of TP while other cells had matured beyond this sensitive stage. If this were so, there could be a reduced stimulus from the brain to cyclical release of gonadotrophin from the pituitary, resulting in less frequent ovulation. It should be noted that the apparent reduction in cyclical activity in the grafts of some of the rats in group II was not reflected in their vaginal smear cytology. Since the one masculinized animal in the group treated with TP at 10 days of age was given a dose of 100 µg, and the animals given 500 µg at the same age were not masculinized, it would appear that the dosage of TP administered cannot influence the brain towards masculinization once the originally sensitive cells of the nervous system have matured beyond a certain age.

The masculinizing influence of TP in rats with intact ovaries from the same colony as those used in the present experiment has been reported elsewhere (Adams Smith & Peng, 1966). Such a control group was not used in the present series as the presence of the host's ovaries will prevent the development of an ovarian graft and will thus limit observation on the activity of ovarian tissue to post-mortem findings. The observations of the functioning of ovarian grafts in animals of experimental group III and control groups A and B show that the sexual maturation of the female rat brain will go on in the normal way even in the absence of the ovaries, and indicate that this sexual maturation of the central nervous system in the female pattern is independent of the ovary. These results, taken in conjunction with the report of Harris (1964) that the male rat castrated on the day of birth undergoes sexual maturation of the brain in the female
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pattern, indicate that the brain of the rat is predisposed to undergo sexual maturation of the female type and that this maturation is not influenced by female gonadal hormones, but only by male gonadal hormones for a limited period of time. This is in agreement with Pfeiffer's (1935, 1936) findings. This is also the conclusion reached by Gorski & Wagner from recent experiments on infant rats subjected to gonadectomy or steroid injection, or both, or to gonadal transplant, and bearing subcutaneous ovarian grafts (Gorski & Wagner, 1965).

The failure of the ovarian grafts in the animals of control groups C and D to show any obvious signs of growth and function during such time as the hosts retained their own ovaries is of interest. This well-known phenomenon suggests that in both mature and immature rats there is insufficient gonadotrophin secreted to activate the ovarian grafts, but that with the oestrogen withdrawal brought about by ovariectomy there is an increased stimulus to release gonadotrophins. This increase in gonadotrophin release is able to stimulate full function of the ovarian graft in the mature animal, but not in the immature animal, suggesting that there is less gonadotrophin released by the immature host, possibly as a result of a greater sensitivity to oestrogen feedback in the immature rat (see Harris, 1961). The findings reported here are in agreement with those of Foà (1900), who found that mature rabbit ovaries transplanted to a pre-pubertal host become quiescent and undergo atrophy.

SUMMARY

1. The influence of the ovary upon the sexual maturation of the female rat’s brain, with or without the masculinizing influence of graded doses of testosterone propionate, has been investigated.

2. The results of this study indicate that the sexual maturation of the female rat’s brain is independent of the ovary and will follow its normal course, even in the absence of the ovary, unless the animal is subjected to the influence of androgen during a limited period of time.

3. The rat is sensitive to the masculinizing effect of testosterone propionate until it is 5 days old, but this sensitivity falls off rapidly and is largely lost by 10 days of age.

4. The dosage of testosterone propionate administered and the presence or absence of the ovary do not appear to influence the duration of the sensitive period.

RÉSUMÉ

L’ovaire et la maturation sexuelle du cerveau

1. L’influence de l’ovaire sur la maturation sexuelle du cerveau a été étudiée chez la ratte, soumise ou non à l’influence masculinisante du propionate de testostérone.

2. Les résultats obtenus montrent que la maturation sexuelle du cerveau de
la ratte est indépendante de l’ovaire et s’accomplit de façon normale même en l’absence d’ovaires, à moins que l’animal ne soit soumis à l’action d’androgènes pendant un temps déterminé.

3. La ratte est sensible à l’action masculinisante du propionate de testostérone jusqu’à l’âge de 5 jours, puis la sensibilité décroît rapidement et est pratiquement nulle vers l’âge de 10 jours.

4. La dose de propionate de testostérone administrée, ainsi que la présence ou l’absence des ovaires ne semblent pas influer sur la durée de la période sensible.

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REFERENCES


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