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Some Aspects of Hormonal Mechanism Involved in Persistent Estrus in the Rat

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In female rats, persistent cornification of the vaginal epithelium (constant vaginal estrus) may occur spontaneously in intact individuals[1–3], or can be induced experimentally by different procedures, such as transplantation of testis immediately after birth[4], injections of hormonic steroids during infancy (*vide infra*), parabiotic union with gonadectomized partners (*vide infra*), continued illumination[5–8], continuous auditory stimulation[9], ligation of oviducts[10] and hypothalamic lesions[11–15].

It is also well established that in orchidectomized rats bearing ovarian grafts outside the hepatic portal circulation, continued cornification takes place in the epithelium of subcutaneous vaginal transplants[16–18] like that observed in the *in situ* vagina of persistent-estrous rats.

In the majority of cases hitherto reported, the ovaries of persistent-estrous rats and ovarian grafts in orchidectomized rats contain follicles of varying sizes but lack corpora lutea. The interstitial tissue is sometimes hypertrophied.

In this paper, literature concerning hormonic conditions and mechanism involved in persistent estrus in rats, with special reference to that which is induced by post-natal administration of hormonic steroids and which takes place following parabiotic union with gonadectomized partners, will be reviewed and discussed.

Persistent estrus in rats given post-natal injections of hormonic steroids. It has been reported by a number of workers that female rats given injections of hormonic steroids for periods of varying lengths, beginning within a few days after birth, come to show continued cornification of the vaginal epithelium after they attain sexual maturity (estrogen[19–23], androgen[22,24,25], progesterone[26], desoxycorticosterone acetate[26].

TAKASUGI[22] found that, if testosterone propionate was injected to female rats for 10–30 days from the day of birth, it interfered with the differentiation of the lower vagina and consequently the vagina remained closed even after the animals attained puberty, but histological studies revealed that the epithelium of the upper part of the vagina had been cornified. On the other hand, SEGAL and JOHNSON[24] reported that in rats given injections of testosterone propionate on any 3 days during the first post-natal week, the vagina opened precociously and the epithelium exhibited continued cornification. BARRACLOUGH[25] also produced persistent-estrous rats with opened vagina by single injections of testosterone propionate at 2 or 5 days of age. The difference in response of the vagina to testosterone propionate between the rats treated with the steroid from the day of birth and those administered with it beginning a few days after birth was recently confirmed by KIKUYAMA[27]).

Besides hormonic steroids, TAKASUGI[26] showed that daily injections of 125 μg of cholesterol for the first 20

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days of post-natal life also induced the condition of persistent vaginal estrus in female rats after the attainment of puberty. Since injections of 2 mg daily of the same preparation of cholesterol for 20 consecutive days failed to elicit any estrogenic stimulation in the genital tract of ovariectomized adult rats, the results of administration during infancy are not attributable to estrogenic contaminations in the preparation.

A. Responsiveness of the ovaries of persistent-estrous rats to gonadotrophins. Although the ovaries of persistent-estrous rats lack corpora lutea, different experiments have shown that follicles in these ovaries can readily respond to both endogenous and exogenous gonadotrophins by transforming into corpora lutea.

If persistent-estrous rats are united in parabiosis with ovariectomized 'cyclic' rats, the ovaries become markedly increased in weight and highly luteinized, like the ovaries of 'cyclic' rats with gonadectomized parabionts[23,28]. It has been demonstrated by WITSCHI and LEVINE[29] that the strong luteinization in the ovaries of normal female after parabiotic union with gonadectomized co-twin is largely ascribable to the effect of LH produced by the anterior hypophysis of the normal parabiont. Therefore, it may be that the secretion of LH from the anterior hypophysis of persistent-estrous rats is increased after parabiotic union with gonadectomized partners and the ovaries of the rats respond to the LH by forming corpora lutea.

Vaginal cornification is interrupted in the persistent-estrous parabionts a few days after union, but eventually it is resumed and continues until the time of sacrifice. In the ovariectomized partners, vaginal estrus of a few days' duration takes place at irregular intervals.

The findings of SEGAL and JOHNSON[24], that ovulation occurred in persistent-estrous rats following the administration of small amounts of human chorionic gonadotrophin (HCG) or sheep hypophyseal LH, also show that the ovaries of these rats are reactive to exogenous gonadotrophins.

B. Gonadotrophic activity of the anterior hypophysis in persistent-estrous rats. From the above-mentioned results of SEGAL and JOHNSON[24], it seems likely that the hypophysis of persistent-estrous rats secretes a subnormal amount of gonadotrophins more or less at a constant rate.

NOUMURA[23], who assayed the gonadotrophic potency of the hypophysis from persistent-estrous rats, both intact and ovariectomized, on the weight of the ovaries and uteri of immature rats, reported that hypophyseal gonadotrophic potency in persistent-estrous rats was lower than that in normal cyclic rats. It was markedly increased after ovariectomy but was still lower than that in ovariectomized 'cyclic' rats.

Using the ovarian ascorbic acid depletion method, TALEISNIK and MCCANN[30] assayed LH in the hypophysis and blood plasma before and after ovariectomy in normal rats and persistent-estrous rats with hypothalamic lesions. Their results showed that, although there was no detectable LH in plasma from normal female rats, the hormone was found in assayable quantities 1 to 16 weeks following ovariectomy, the concentration of LH remaining unchanged during this period. On the other hand, in the blood plasma of rats with hypothalamic lesions no LH was detectable either before or after ovariectomy. Furthermore, in persistent-estrous rats, hypophyseal LH content was diminished to 33% of normal. Ovariectomy resulted in a highly significant increase in the content in one month, in both normal and persistent-estrous rats.

In contrast to these reports, SEGAL and JOHNSON[24] reported that the weaver-finch and rat tests revealed that the hypophysis of persistent-estrous rats contained larger quantities of both LH and total gonadotrophins than the gland of normal females. BARRACLOUGH and GORSKI[31] pointed out, however, that SEGAL and JOHNSON used for their assays the hypophyses of the rats which had been in persistent estrus for only short periods of time and, if assayed after prolonged estrogenic stimulation, the hypophyses would have been depleted of their stores of gonadotrophins.

TAKASUGI[26] and NOUMURA[23] have shown that the ovaries autografted into the spleen of ovariectomized 'persistent-estrous' rats become highly luteinized. This fact also indicates that the blood level of gonadotrophins, LH in particular, rises in persistent-estrous animals following withdrawal of circulating estrogen.

FLERKÓ and BÁRDOS[32,33] reported that in constant-estrous rats with lesioned anterior hypothalamus, formation of corpora lutea occurred if the blood level of estrogen was lowered by unilateral ovariectomy or unilateral ovariectomy plus removal of a half of the other ovary. These operations also elicited the interruption of persistent estrus. Luteinization was most pronounced in intrasplenic ovarian grafts in ovariectomized 'persistent-estrous' rats, but if the animals were injected with 4μg daily of estradiol during a 60-day post-operational period, grafts became atrophic and devoid of corpora lutea. Again these results have made it evident that the reduction or elimination of blood estrogen causes an increased secretion of LH from the anterior hypophysis of persistent-estrous rats.

On the other hand, it is well established that different kinds of hormonic steroids are effective in stimulating the anterior hypophysis of persistent-estrous rats to secrete enough LH to induce ovulation and luteinization.

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In spontaneous persistent-estrous rats[34,35] and persistent-estrous rats produced by hypothalamic lesions[36], ovulation and luteinization were induced by injections of progesterone. In an investigation on the effects of progesterone, corticoids and other hormonic substances on persistent estrus induced by post-natal administration of estrone, TAKASUGI[28] also observed that injections of progesterone (5 mg daily for 10 days) caused the formation of corpora lutea in 2 out of 6 animals. Luteinization also took place following injections of desoxycorticosterone acetate (5 mg daily for 10 days) in 3 out of 6 rats, but cortisone was ineffective in inducing luteinization. TAKEWAKI[37] elicited luteinization of follicles in subcutaneous ovarian grafts in orchidectomized rats by injections of progesterone or desoxycorticosterone acetate. FLERKÓ and BÁRDOS[33] suggested that progesterone antagonized the effect of endogenous estrogen and prevented its feed-back effect on the anterior hypophysis, as in the case of subtotal ovariectomy (*vide supra*).

Strong stressful stimuli also induce luteinization in persistent-estrous rats. TAKASUGI[38] injected 30 cm^3 of air under the dorsal skin of the rats and then 3 cm^3 of croton oil into the air chamber. He observed that the animals came into diestrus in a few days and vaginal cornification never took place until sacrifice performed 10 days later. At sacrifice, in 8 out of 12 rats the ovaries contained corpora lutea. TAKASUGI also reported that the content of total gonadotrophins of the hypophysis was significantly higher in stressed 'persistent-estrous' rats than in intact persistent-estrous individuals. TAKEWAKI[37] also observed that luteinization took place in subcutaneous ovarian grafts in orchidectomized rats after exposure to stressful stimuli (croton pouch, cold, adrenalin).

A number of persistent-estrous rats produced by continuous illumination ovulated within 24 h following electrical stimulation administered to either the amygdala or the septum pellucidum through permanent bipolar electrodes[7]. In anovulatory rats produced by post-natal administration of androgen, ovulation was elicited within 24 h after electrical stimulation of the hypothalamic areas extending just rostral and caudal to the ventral medial nucleus if it was applied following priming with progesterone[31].

C. Control of hypophyseal-ovarian system in persistent-estrous rats. It is now well established that the secretion of gonadotrophic hormones from the anterior hypophysis is controlled by the hypothalamus. GREEP[39], HARRIS and JACOBSOHN[40], and MARTINEZ and BITTNER[41] have demonstrated that the difference in the pattern of hypophyseal gonadotrophic activity between the male and female is caused by the difference in the controlling function of the hypothalamus between the two sexes, the hypophysis itself remaining indifferent. That persistent estrus is induced by placing lesions in the hypothalamus and ovulation is elicited in persistent-estrous rats by electrical stimulation of the hypothalamus shows that the hypothalamus plays an important part in the induction and maintenance of persistent estrus.

In this connection, the experiments with androgen-induced persistent-estrous rats reported by SEGAL and JOHNSON[24] is of interest. They transplanted the hypophysis from persistent-estrous rats into the emptied sella turcica of litter-mate normal females and observed that these animals resumed estrous cycles within 3 weeks after operation, eventually became pregnant, successfully delivered and suckled normal-sized litters. After the litters were weaned, the mothers resumed estrous cycles. This result clearly demonstrates that the maintenance of persistent estrus is dependent on the hypothalamus, the hypophysis itself being only of secondary importance, since if the hypophysis of persistent-estrous rats is placed under the control of the hypothalamus of normal cyclic rats, it produces gonadotrophins cyclically.

The experiments recently reported by KIKUYAMA[27] seem to show, however, that estrogen or androgen administered during infancy acts on certain sites at a level higher than the hypothalamus to exert permanent effects on the hypothalamus. He showed that if reserpine was injected concurrently with testosterone propionate or estradiol to infantile rats, the effects of the steroids inducing persistent estrus was attenuated or nullified and the ovaries of the treated rats developed both follicles and corpora lutea after the animals attained sexual maturity. In a control experiment, injections of the vehicle of reserpine together with androgen or estrogen did not interfere at all with the induction of persistent estrus by the steroid (Figures 1–4).

In KAWASHIMA's unpublished experiments, male rats were injected with reserpine for 10 days from the day of birth and orchidectomized on the 11th day. The animals were given subcutaneous ovarian grafts when reached 30 days of age and sacrificed 50 days later. Histological studies revealed that the ovarian grafts in these animals invariably contained both follicles and corpora lutea. In a control series of experiments, rats were orchidectomized at 10 days of age with no preceding treatment with reserpine and given subcutaneous ovarian grafts at 30 days of age. The grafts recovered 50 days later exhibited follicles of varying sizes but no corpora lutea. Finally, males orchidectomized on the day of birth and given ovarian grafts at 30 days of age

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also invariably developed both follicles and corpora lutea in the grafts. The last mentioned result is in good agreement with that of PFEIFFER[4] as well as those recently reported by YAZAKI[18] and KAWASHIMA[42], demonstrating that the male type of secretion of gonadotrophins in the rat is determined by the action of the testis within a short period after birth. The experiments with reserpine indicate that this effect of the testis is annihilated by the alkaloid.

As BARRACLOUGH and SAWYER[43] have shown, reserpine appears to inhibit afferent transmission into the reticular activating system in the ovulatory mechanism from the medullary brain stem and cerebellum. It seems likely, therefore, that the site of action of hormonic steroids injected into female rats soon after birth or of a substance, probably androgen, produced by the testes in infantile male rats is located in the brain stem *above* the hypothalamus.

FISKE and GREEP[44] reported that, in rats showing lengthened periods of estrus after 8 weeks' continuous lighting, the neurosecretory cells of the supraoptic nuclei were enlarged and large amounts of neurosecretory material appeared to be moving along the neurosecretory axons, while the pars nervosa was relatively depleted of Gomori-positive substance as compared with that of animals kept under usual laboratory conditions and those housed in darkness. The result suggests that the lighted rats both secrete and release increased amounts of neurosecretory material. The findings recently reported by OHASHI et al.[45] are of interest in this respect. They indicated that the gonadotrophic potency of the anterior hypophysis of lighted rats exhibited a temporary rise, but after a prolonged period of continuous illumination it became markedly lower than that of control animals. They also reported that, during the period of heightened gonadotrophic activity, the ovaries contained both large follicles and corpora lutea, while in the period of lowered activity the ovaries showed large follicles but lacked corpora lutea. The ovaries of the animals of FISKE and GREEP had large follicles and corpora lutea. Therefore, histological studies of the hypothalamus of rats after longer periods of illumination might prove of interest.

Persistent estrus in rats in parabiotic union with gonadectomized partner. As mentioned above, the ovaries of female rats parabiotically joined with gonadectomized males or females produce numerous corpora lutea soon after union. The whole ovary eventually transforms into a mass of corpora lutea, larger follicles being only seldom found. In a few weeks, however, a

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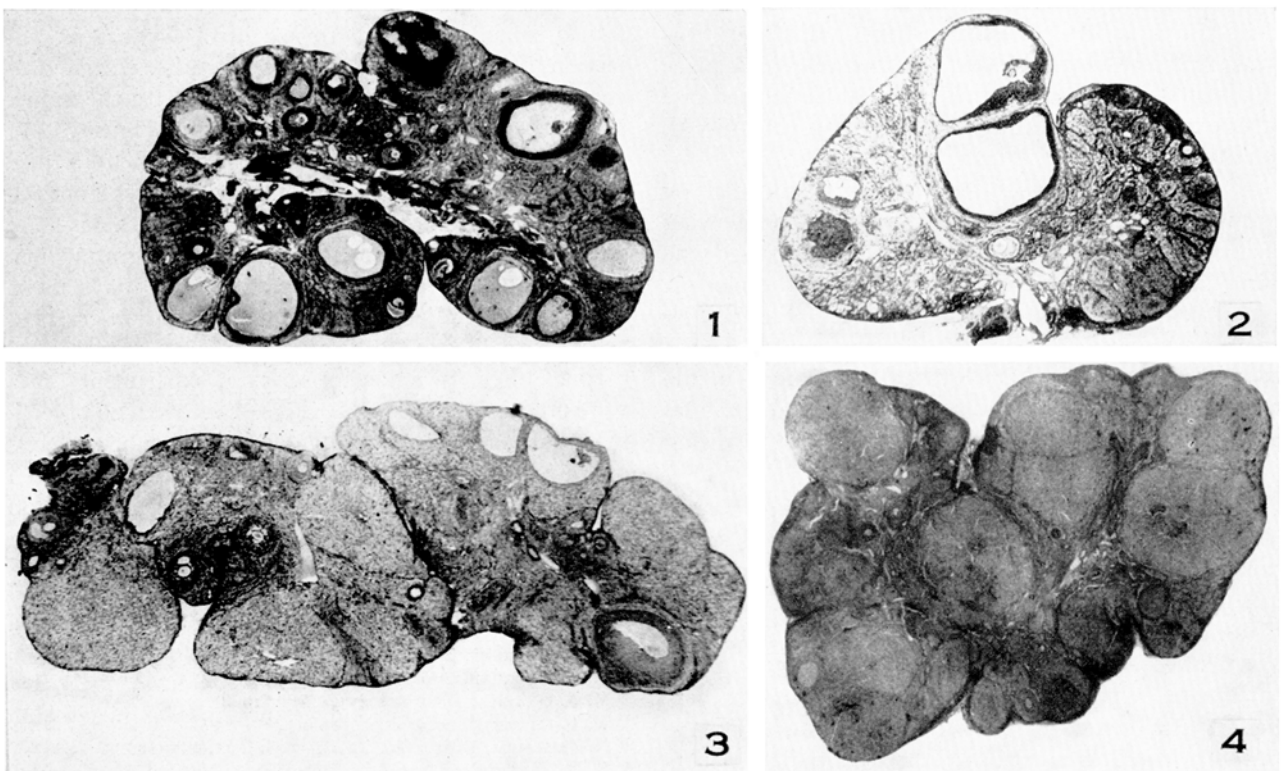

Fig. 1. Ovary of rat given injections of testosterone propionate together with vehicle of reserpine during infancy. Fig. 2. Ovary of rat given injections of estrone together with vehicle of reserpine during infancy. Fig. 3. Ovary of rat given injections of testosterone propionate plus reserpine during infancy. Fig. 4. Ovary of rat given injections of estrone plus reserpine during infancy (from KIKUYAMA[27]).

marked growth of follicles begins to occur, the corpora lutea disappear quickly and finally the ovaries become filled exclusively with large vesicular follicles and follicular cysts[46–48] (Figures 5, 6). The results are the same, regardless of the sex of the gonadectomized parabiont. HILL[49] reported that, during stage of corpus luteum formation, the females show irregular and prolonged estrous cycles, but when the stage of cyst formation sets in, they go into persistent estrus.

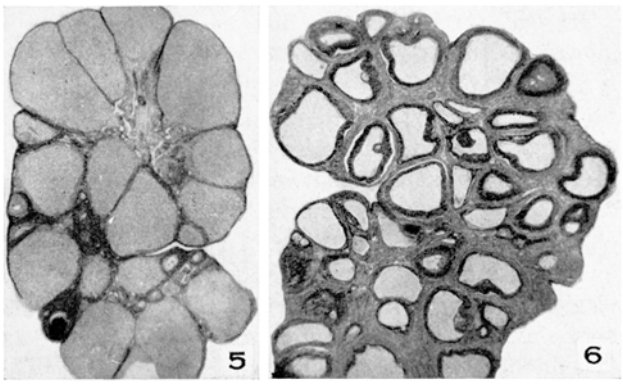

Fig. 5. Ovary of female rat 22 days after parabiotic union with orchidectomized male. Fig. 6. Ovary of female rat 55 days after parabiotic union with ovariectomized female.

WITSCHI and LEVINE[29], MØLLER-CHRISTENSEN[50], and DUSHANE et al.[51] demonstrated that if female parabionts in such pairs were hypophysectomized, persistent estrus accompanied by the growth of numerous follicles sets in within 4 or 5 days, without a preceding stage of corpus luteum formation. Moreover, once persistent estrus was established in female parabionts with gonadectomized partners, it was not interfered with by the removal of the animals' own hypophysis. On the basis of these results, WITSCHI and LEVINE[29] concluded (1) that the secretion of LH by the anterior hypophysis of female parabionts is responsible for the formation of numerous corpora lutea in their ovaries during the first few weeks after union with gonadectomized partners, (2) that the hypophysis of gonadectomized rats emits an increased quantity of FSH but no LH, and (3) that in the stage of cyst formation, the hypophysis of the female parabionts is almost inactive in secreting gonadotrophic hormones.

Some years after the appearance of the paper of WITSCHI and LEVINE[29], it was found that the ovaries transplanted into the spleen of gonadectomized male or female rats become highly luteinized[52–54]. It was also reported that the hormonic conditions in gonadectomized rats bearing successful intrasplenic ovarian grafts are very similar to those in gonadectomized rats without grafts.

The anterior hypophyses of gonadectomized rats with intrasplenic ovarian grafts exhibit many castration cells[53] and have an increased gonadotrophic potency almost equivalent to that of gonadectomized controls without ovarian grafts[55]. If vascularized adhesions of the graft-containing spleen to the abdominal wall have been established, the hypophyseal gonadotrophic potency is reduced.

Behaviour of the ovaries of female rats parabiotically joined with gonadectomized partners bearing intrasplenic ovarian grafts is approximately the same as that of females united with gonadectomized animals without ovarian transplants. In female parabionts, the estrous cycles become irregular and their ovaries develop numerous corpora lutea for the first few weeks after union, but eventually corpora lutea are replaced by large vesicular follicles and follicular cysts, and constant vaginal estrus stets in[56]. If the females in such pairs have been hypophysectomized, vaginal cornification begins to occur 5–6 days after parabiotic union and continues until sacrifice. The ovaries become rapidly hypertrophied, consisting of many large follicles. The stage of corpus luteum formation is obliterated[57].

Unpublished experiments recently carried out in this laboratory have shown that if hypophysectomized-ovariectomized rats bearing intrasplenic ovarian grafts are joined with ovariectomized partners with intrasplenic ovarian transplants, the grafts in the former animals develop large follicles and a few occasional luteinizing cysts, while those in the latter animals are highly luteinized.

Strong luteinization of intrasplenic ovarian grafts in gonadectomized rats cannot be accounted for by the assumption that the hypophysis of gonadectomized rats secretes an increased quantity of FSH but no LH. Adhering to this assumption, ACHILLES and STURGIS[58] conjectured that some hormones or hormone metabolites from intrasplenic ovarian grafts enter the systemic circulation and stimulate the hypophysis to release LH which induces luteinization in the ovarian grafts. ALLOITEAU[59], on the other hand, ascribed luteinization of intrasplenic ovarian grafts to a co-operation of FSH from the anterior hypophysis and estrogen from the grafts, since it is known that the luteinizing effect of

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gonadotrophic extracts may be enhanced by the simultaneous administration of estrogen.

It may be possible that to a certain extent these phenomena are involved in the luteinization of intrasplenic ovarian grafts. However, direct measurements of the blood level of gonadotrophins in the rat, which have recently been carried out by different techniques, demonstrate a definite increase in the content of LH in the blood after gonadectomy[30,60]. Therefore, the question which should be answered is not why intrasplenic ovarian grafts in gonadectomized rats become luteinized, but why the ovaries of hypophysectomized rats with gonadectomized parabionts fail to form corpora lutea.

In unpublished experiments carried out in this laboratory, if one member of two immature female rats in parabiosis was injected over 3 days with homogenate equivalent to one hypophysis from a male rat orchidectomized 2–3 months before, the ovaries of the injected rat became strongly luteinized while those of its cotwin exhibited the growth of follicles but sometimes no luteinization. In another series of experiments, purified FSH and LH, injected simultaneously in appropriate combinations into one member of the parabiotic pairs of two hypophysectomized immature rats, caused luteinization in the ovaries of the injected parabionts but only follicular development in their partners. Luteinization was elicited in both injected and non-injected members, if doses of LH were increased.

HILL[61] has demonstrated that, if a hormone is released by one of the two rats in parabiotic combination, the level of its concentration in the receiving member should be lower than in the producer. Therefore, it seems highly probable that, even if one parabiont produces enough quantities of FSH and LH to cause luteinization in its ovaries, the amounts of hormones transmitted to its hypophysectomized partner are so much reduced that LH can no longer reach the threshold level for inducing luteinization. However, the presence of some LH in addition to FSH in the blood of the hypophysectomized parabionts is demonstrated by the continued secretion of estrogen by their ovaries and the cystic growth of follicles.

The Strasbourg group (M. ARON, Cl. ARON et al.) has advanced the theory that there is only one gonadotrophin, of which large doses induce luteinization while small doses promote the growth of follicles without causing luteinization. (For summary of their papers, see ARON et al.[62]). This single-hormone theory may also account for the phenomena in question, but the present writer prefers the widely accepted double-hormone concept to this theory because the experiments with combinations of purified FSH and LH show that injections of the two hormones in sufficient amounts to induce luteinization in one animal cause the development of follicles but no luteinization in its parabiont.

Résumé. (1) Des Rattes blanches auxquelles on a injecté des stéroïdes hormonaux immédiatement après la naissance présentent après la puberté une kératinisation vaginale constante (oestrus).

Le contrôle hypophysaire de l'hypothalamus est presque acyclique, différent en cela de celui de la Ratte dont le cycle génital est normal. Ainsi les gonadotrophins d'hypophyse sont sécrétés acycliquement dans une proportion presque constante. L'élaboration et la sécrétion de gonadotrophins d'hypophyse sont subnormales, et l'on observe ni ovulation ni lutéinisation. Cependant, il est possible de stimuler l'hypophyse par diverses méthodes et d'accélérer d'autant plus la sécrétion de gonadotrophins. L'ovaire est facilement influencé par de tels gonadotrophins endogènes augmentés ou par les gonadotrophins exogènes administrés par injection, ce qui provoque l'ovulation et la lutéinisation. Chez la Ratte à oestrus constant, l'endroit où agissent les stéroïdes hormonaux injectés immediatement après la naissance n'est pas l'hypothalame, mais peut-être une partie de l'encéphale, située plus haut.

(2) Chez des Rats châtrés, l'élaboration et la libération de FSH et de LH dans les hypophyses s'accélèrent. Dans les ovaires des femelles hypophysectomisées unies en parabiose avec des animaux gonadectomisés, on observe la croissance de follicules, l'abolition de l'ovulation et l'absence de formation de corps jaunes. Ces Rattes présentent les symptomes d'oestrus constant. La concentration de LH ne peut atteindre le niveau du seuil chez les femelles hypophysectomisées, parce que le niveau de gonadotrophins transférés aux animaux est moins élevé que celui dans les sujets châtrés.

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