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Influences of the Pineal and Adrenal Glands on the Proestrous Release of Hormones in Rats Maintained in a Reduced Photoperiod

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ABSTRACT

The influence of pinealectomy (Px) and/or adrenalectomy (Adx) on reproductive processes was examined in female rats maintained in a reduced photoperiod (8 h L:16 h D). Estrous cycle length, number of ova ovulated and organ weights (ovaries, uterus or pituitary gland) were not significantly altered by either surgical procedure. Moreover, the critical period for LH release as defined by Nembutal blockade was unaffected by these treatments. In contrast, serum LH levels on proestrus (third proestrus subsequent to surgery) were reduced overall by Px. In Px-Adx rats either the magnitude of the LH surge was subnormal or the onset of the LH rise was delayed. The time periods selected for blood sampling did not allow us to distinquish between these 2 effects. In any case, serum LH values were markedly depressed at 1700 h in Px-Adx animals. At this time serum progesterone concentrations were also reduced in this group of rats. Both Px and/or Adx significantly altered the time course of serum prolactin on proestrus and at 1700 h in Px-Adx animals, serum concentrations were significantly elevated above control levels. Our data demonstrate that both the pineal and adrenal glands influence the release of pituitary hormones in animals maintained in a reduced photoperiod.

INTRODUCTION

The timing of LH release and ovulation in cycling rats is controlled by a neurogenic mechanism which is subject to cueing by the light-dark cycle (Everett et al., 1949). When the time of onset of the light phase is altered, a comparable temporal change in the timing of the critical period for LH release occurs (Critchlow, 1963). Therefore, it appears that the environmental light-dark cycle acts as a *zeitgeber* for synchronizing the pituitary discharge of LH in the female rat.

The secretory activity of the pineal gland is influenced by the environmental lighting. Hydroxyindole-O-methyltransterase (HIOMT), which regulates the synthesis of pineal indoles, reaches maximum activity during the dark phase of the light-dark cycle and exhibits minimum activity during the light phase (Wurtman, et al., 1963). The pineal gland and its secretions have been reported to influence reproductive function in female rats. Pinealectomy caused ovarian hypertrophy which could be reversed by melatonin injections (Wurtman et al., 1959) and increased the incidence of estrous smears (Wurtman, 1968). Additionally, Kitay and Altschule (1954) reported that bovine pineal extracts inhibit ovulation in the rat. However, several laboratories have questioned the importance of pineal gland involvement in reproductive processes of the female rat. Pinealectomy did not alter cycle length (Alleva et al., 1970; Blake, 1976), time of ovulation (Alleva et al., 1970), number of ova ovulated (Alleva et al., 1970; Blake, 1976; Tigchelaar et al., 1975) or the magnitude and timing of the preovulatory rises of LH, FSH and prolactin (Blake, 1976). It must be noted that the animals utilized in these studies were all maintained in long photoperiods (12, 14 or 16 h light/day). To date no attempt has been made to study the effects of pinealectomy on the timing and synchronization of the preovulatory surge of gonadotropins and prolactin in rats maintained in a reduced photoperiod. In such a photoperiod, the possible role of the pineal might be more evident since the pineal would remain active for a longer duration.

Recent studies in our laboratory have suggested that the adrenal gland can also influence the timing of LH release on proestrous afternoon. We demonstrated that the time course of plasma LH concentrations was

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abnormal in adrenalectomized rats (Mann et al., 1975). Furthermore, the light-dark cycle and adrenal steroids may interact to synchronize the timing of LH release in female rats (Mann et al., 1976).

As a result, we thought it would be informative to study the effects of a reduced photoperiod (8 h light, 16 h darkness), pinealectomy and pinealectomy combined with adrenalectomy on reproductive processes of female rats. The objectives of this investigation were 2-fold: 1) to define a possible role for the pineal gland in female cyclicity and the proestrous release of LH, prolactin and progesterone in rats maintained in a reduced photoperiod and 2) to demonstrate any possible interaction between the adrenal glands and pineal in influencing these parameters.

MATERIALS AND METHODS

Sprague-Dawley female rats (50–55 days of age) which were bred in our colony (original stock was obtained from Russell Miller Farms, Cazenovia, NY) were maintained in a temperature and light controlled room (8 h light:16 h dark schedule, lights on 0800 h-1600 h) for a minimum of 2 weeks prior to experimentation. Another group of female rats were maintained in another vivarium in which the lighting schedule was 14 h light:10 h dark (lights on 0400 h). Only those animals that had demonstrated at least 2 consecutive 4-day estrous cycles were used in the described studies. Vaginal smears were examined between 0800–0900 h by vaginal lavage. Estrous cycles were composed of 1 day of nucleated epithelial cells (proestrus), 1 day of cornified epithelial cells (estrus) and 2 days of diestrus in which most cells were leucocytes with a few cornified epithelial cells.

Operating Procedures

On the afternoon (1200–1500 h) of the second day of diestrus, animals were either pinealectomized (Px), adrenalectomized (Adx) or pinealectomized and adrenalectomized (Px–Adx) under sodium pentobarbital anesthesia (30 mg/kg body weight, Nembutal, Abbott Labs). Sham pinealectomy (ShPx) and sham pinealectomy accompanied by adrenalectomy (ShPx-Adx) were also performed. Pinealectomy was performed according to the method of Bliss and Bates (1974). Sham pinealectomy involved the exact surgical procedure as pinealectomy except the forceps were inserted through the slit in the dura mater in the closed position. Adrenalectomized animals were maintained on physiological saline throughout the studies. Only rats which showed no detectable pineal and adrenal remnants upon sacrifice were included.

(Study 1)–Determination of Critical Period

On the third proestrus following surgery, groups of animals (8/group) were given Nembutal (30 mg/kg body weight) at either 1345, 1445, 1545 or 1645 h, in order to determine the critical period for LH release in each experimental group. On the following day (estrus), animals were killed at 1200 h and Fallopian tubes were examined for the presence of ova. The critical period for LH release in intact rats in an 8 h light-16 h dark schedule was also compared to rats maintained in 14 h of light.

(Study 2)–Hormone Levels

Once the critical period was established, another study was performed to determine serum changes of LH, prolactin and progesterone on proestrous afternoon. On the third proestrus following surgery, animals were rapidly decapitated at either 1100, 1400 or 1700 h. Animals were not sequentially bled since we have shown in a previous study (Mann et al., 1975) that this procedure alone blocks LH release and ovulation in Adx rats. The pituitary glands, ovaries and uteri were removed from all animals and placed in 10% formalin. Once the tissues were fixed each organ was weighed. Blood samples were allowed to clot at 4°C for 24 h, centrifuged and stored frozen. Serum prolactin and LH were determined according to the procedures of Niswender et al. (1968, 1969). The NIAMD kit was used to measure prolactin. All prolactin values are expressed in terms of the RP-1 standard. LH was measured using an antibody to ovine LH supplied by Dr. G. D. Niswender. The LH-RP-1 standard served as a reference preparation. This standard has a potency of .03 × NIH-LH-S1. All values have been converted to the NIH-LH-S1 standard. Serum progesterone concentrations were determined using an antibody prepared against progesterone-6-BSA and supplied by Dr. Niswender. The specificity of this antibody is such that progesterone measurement can be made without prior chromatography (Niswender, 1973). The validation of this assay has been reported previously (MacFarland and Mann, 1977).

During the course of these studies, 5 radioimmunoassays were conducted for progesterone, 2 for prolactin and 1 for LH. The within and between assay coefficients of variation for a plasma pool were 6.5% and 12.3% for progesterone, 6.0% and 8.8% for prolactin and 10.9% for LH. These analyses were based upon multiple replicates of different volumes of a plasma pool.

Statistics

Data were statistically analyzed using 2 × 3 × 3 factorial analysis of variance and the Newman-Keul's test for multiple comparisons (Keppel, 1973).

RESULTS

Critical Period

As can be seen from Table 1, there was no significant difference in length of the critical period for LH release ($P>0.05$) between intact animals maintained in 14:10 h and 8:16 h lighting. Moreover, the timing of the critical period was not significantly altered by surgical treatment.

TABLE 1. Critical period for LH release.

Treatment group[c]	Time of injection 1 h 45 min[a]	2 h 45 min	3 h 45 min	4 h 45 min
14:10 Controls	0/8[b]	1/8	4/8	8/8
8:16 Controls	0/8	2/8	3/8	8/8
ShPx	0/8	0/7	4/9	7/8
Px	1/8	1/8	4/8	6/8
Adx	0/8	0/8	2/8	8/8
ShPx–Adx	0/8	0/8	3/8	6/8
Px–Adx	0/8	1/8	2/8	7/8

[a]Each time of injection represents amount of time past midday colony time.

[b]No. of rats ovulating/total no. of rats/group.

[c]Control = intact female; ShPx = sham pinealectomy; Px = pinealectomy; Adx = Adrenalectomy; ShPx–Adx = sham pinealectomy and adrenalectomy; Px–Adx = pinealectomy and adrenalectomy.

Serum LH Data

Figure 1 summarizes serum LH concentrations observed in different surgical groups on the afternoon of proestrus. Analysis of the LH data demonstrated that: a) serum LH levels changed significantly with time ($P<0.001$); b) pinealectomy significantly reduced serum LH levels overall ($P<0.001$); c) Px-Adx rats had lower serum LH levels than Px, Adx or ShPx-Adx animals ($P<0.025$); d) the combination of Px and Adx significantly lowered LH values exhibited at 1700 h when compared to all other groups; e) LH concentrations in Adx rats at 1700 h were significantly elevated above control levels ($P<0.001$). The reduced levels of serum LH observed at 1700 h in Px-Adx rats suggest that either the amount of LH released was normal but delayed or the magnitude of the LH surge was subnormal. However, Px-Adx rats were found to ovulate a normal complement of ova when sacrificed on estrus. Sh-Px also lowered LH levels in Adx rats at 1700 h, but the effect observed was substantially less than with Px.

Progesterone data

Changes in serum progesterone were similar to those seen with LH (Fig. 2). Statistical analysis demonstrated that: a) serum progesterone levels changed significantly with time ($P<0.001$); b) when Px and Px-Adx animals were combined in order to perform the analysis of variance, it was found that this group had a reduced progesterone concentration at 1700

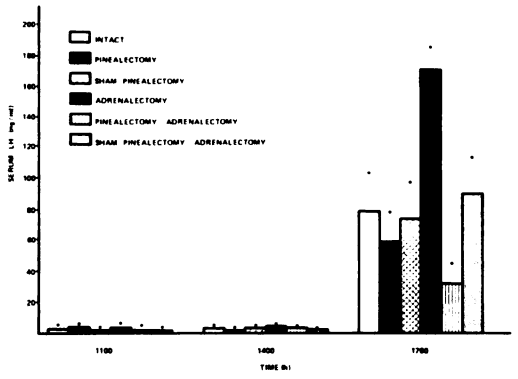

FIG. 1. Serum LH concentrations on the third proestrus following surgery. Each bar graph represents the mean of 6–8 rats. Dots above bar graphs represent standard error of the mean.

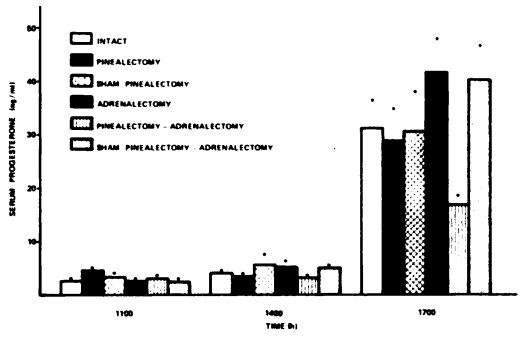

FIG. 2. Serum progesterone levels on the third proestrus following surgery. Each bar graph represents the mean of 6–8 rats. Dots above bar graphs represent standard error of the mean.

h when compared to the other groups ($P<0.05$). No other differences in serum progesterone were significant among the groups.

Prolactin Data

As illustrated in Fig. 3: a) serum prolactin levels among the groups changed with time ($P<0.001$); b) Px and/or Adx had a significant effect on the way serum prolactin values changed with time at the sampling periods examined (1100, 1400, 1700 h; $P<0.05$); c) at 1700 h, Px-Adx animals showed elevated values as compared to Px, Adx or ShPx-Adx animals ($P<0.05$).

Other Parameters

There was no significant alteration of cycle length or smear patterns as a result of any surgical treatment (Table 2). Moreover, regardless of surgical group, absolute or relative weights of the ovaries, uterus and pituitary gland were not significantly different from those of control animals.

DISCUSSION

In this study we examined the short term (9–10 days) effects of Px and/or Adx on a number of reproductive parameters in female rats maintained in an 8 h light:16 h dark schedule. We found that a) the pineal and adrenal glands were not essential for maintenance of estrous cycle length; b) removal of these glands did not alter ovarian, uterine or pituitary gland weights; c) the length of the critical period for LH release was not significantly affected in either Px and/or Adx rats; d) however, in Px-Adx animals serum levels of LH and progesterone were reduced at 1700 h on proestrous afternoon; e) conversely, serum LH concentrations were elevated at this time in Adx rats with intact pineal glands.

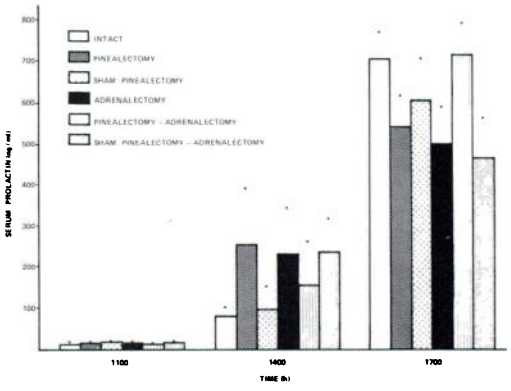

FIG. 3. Serum prolactin concentrations on the third proestrus following surgery. Each bar graph represents the mean of 6–8 rats. Dots above bar graphs represent standard error of the mean.

TABLE 2. Length of estrous cycles (days) following surgery.

Surgical group	First cycle	Second cycle
Control (56)[a]	4.00 ± 0.00[b]	4.00 ± 0.00
ShPx (42)	4.26 ± 0.09	4.07 ± 0.04
Px (43)	4.23 ± 0.07	4.09 ± 0.04
Adx (45)	4.27 ± 0.09	4.11 ± 0.06
ShPx–Adx (54)	4.33 ± 0.08	4.15 ± 0.06
Px–Adx (50)	4.44 ± 0.10	4.10 ± 0.05

[a]Number of rats/group.

[b]Mean estrous cycle length (days) ± S.E.

This study confirms the results of several other laboratories regarding the lack of influence of the pineal gland on the length of the estrous cycle (Alleva et al., 1970; Blake, 1976; Takeo et al., 1975). Conversely, Chu and coworkers (1964) pinealectomized immature rats and 2 months later observed an increase in the incidence of estrous smears. Hoffman (1973) found a shift in cycle length from 4 to 5 days following pinealectomy. Moreover, several studies indicated that pinealectomy caused an increase in ovarian, uterine and anterior pituitary weights (Wurtman et al., 1959 and 1968; Tigchelaar and Nalbandov, 1975, Takeo et al., 1975) whereas Bick et al. (1969) found that pinealectomy had no effect on sexual organ weights. Our results agree with the latter study. These discrepancies may be caused by differences in the strain of rat utilized or in photoperiod lengths, as well as by the amount of time elapsed between surgery and sacrifice. In our study all animals were maintained in a reduced photoperiod of 8 h. Therefore, the pineal's effects should have been magnified in the control animal. Also, all animals were sacrificed within 9 or 10 days following surgery. It is quite possible that if animals were maintained for a considerably longer period of time following surgery, changes in cycle length and organ weights may have been observed.

Our data demonstrate that pineal removal alone does not significantly alter the critical period for LH release, the magnitude of the

preovulatory LH surge or the number of eggs ovulated in female rats maintained in a short photoperiod (8 h light). The first 2 findings are comparable to those reported by Blake (1976) in pinealectomized rats maintained in a 14 h light:10 h dark schedule. In addition, the failure of Px to alter ova counts is in agreement with the work of Tigchelaar and Nalbandov (1975). In contrast, Dunaway (1968) studied PMS-induced ovulation in immature rats and found alterations in the time of ovulation following pinealectomy, but Alleva et al. (1970) could not detect a change in the time of ovulation following pinealectomy of mature rats.

We have presented evidence that in rats that were both Px and Adx, serum levels of LH were subnormal at 1700 h on proestrus. This reduction in circulating levels of LH could be interpreted in a number of ways. First, it may mean that the timing of LH release was normal although the amount released by the pituitary in these animals was reduced, but was sufficient to induce ovulation of a normal quota of ova. Several earlier studies have indicated that as little as 10–15% of the normal level of LH will cause full ovulation in rats (Wuttke et al., 1971; Turgeon and Barraclough, 1973) and in hamsters (de la Cruz et al., 1976). This interpretation is attractive in light of our other data that the critical period as defined by Nembutal blockade was unaltered in Px-Adx rats. Conversely, it is also possible that Px-Adx rats are more sensitive than intact animals to the blocking effects of Nembutal on ovulation and thus, any change that occurred in the timing of the critical period may have gone undetected. We are not aware of any studies where this latter possibility has been examined. An alternate interpretation of our data in Px-Adx rats is that the LH surge was normal in amount but delayed by several hours. Therefore, at 1700 h we may have been observing the early rising leg of the ovulatory gonadotropin surge. If the latter is true, it provides further evidence that the daily rhythms of pineal and adrenal hormones may serve as timing signals for LH release. Unfortunately our data do not allow us to ascertain which of the two explanations is the more probable. It should be noted that either ShPx or Px lowered plasma LH levels at 1700 h in Adx rats. We believe this effect is most likely related to the trauma of ShPx and/or to some disturbance of blood flow to or from the pineal gland as a result of this surgical procedure.

Adrenalectomy has been reported to have various effects on the events associated with ovulation in the female rat. Campbell et al. (1977) found that when surgery was performed on the morning of proestrus (0800 h), there were no significant deviations in the circulating patterns of LH, FSH and progesterone that afternoon, except that levels of all 3 hormones were attenuated at 1700 h. In a longer term study Peppler and Jacobs (1976) reported that adrenalectomized rats ovulated fewer ova during subsequent cycles, had reduced numbers of large ovarian follicles and, in animals adrenalectomized for 30 days, unilateral ovariectomy did not lead to compensatory ovarian hypertrophy or an increase in the number of ova shed from the remaining ovary. The authors suggest that either these animals were incapable of responding to elevated FSH levels or the amount of FSH being released was reduced. In a previous study we examined the effects of adrenalectomy on the temporal pattern of the LH surge on the third proestrus subsequent to surgery as well as on the number of eggs ovulated the following morning (Mann et al., 1975). While we failed to demonstrate a significant effect of adrenalectomy on the number of eggs ovulated in animals maintained on either saline or corticosterone, we did find that the temporal pattern of serum LH concentrations was abnormal on proestrous afternoon. In a subsequent study we found that in long term ovariectomized (21 days) rats in which the adrenals had also been removed, the time course of their response to the positive feedback effects of estrogen and progesterone was different from that of ovariectomized animals with intact adrenal glands (Mann et al., 1976). Of even greater interest, the adrenalectomized rats appeared to be more sensitive to this positive feedback action as judged by a greater than normal plasma LH surge. Thus, it would appear that the adrenal glands secrete a hormone or hormones which controls the amount of LH being released as well as influences, to a degree, its timing. The data in the present study bear out these conclusions. Serum LH concentrations at 1700 h in Adx rats were more than 2-fold greater than in intact animals. It is impossible to say whether there was any influence on the timing of the LH rise since samples were only taken at 1100 h, 1400 h and 1700 h, al-

though it should be noted that the Nembutal defined critical period was not significantly altered in these animals. In any case, it is apparent from these data that one or more adrenal hormones have an effect on the regulation of pituitary gonadotropin secretion. One site of possible action may be at the level of the pituitary gland where it has been reported that adrenal progesterone secretion can influence the level of an estrogen-inducible progesterone receptor (Evans et al., 1977). We also have preliminary data that indicate that estrogen-primed adrenalectomized rats are more sensitive to exogenously administered luteinizing hormone releasing hormone than are animals with intact adrenal glands. However, further studies are needed to define the adrenal hormones involved in this process as well as their specific site(s) of action.

It is especially interesting and worth emphasizing that Px-Adx rats failed to exhibit the elevated levels of plasma LH at 1700 h that were shown by animals which were only adrenalectomized. In fact, the combination of surgical treatments reduced rather than enhanced plasma LH concentrations. It is obvious that the pineal and adrenal glands somehow interact in the regulation of LH secretion, but the exact nature of this interaction is unclear.

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REFERENCES

Alleva, J. J., Waleski, M. V. and Alleva, F. R. (1970). The Zeitgeber for ovulation in rats: non-participation of the pineal gland. Life Sciences 9, 241–246.

Bick, H., Giolli, R. A., Dearden, L. C. and Stuart, R. R. (1969). The effect of pinealectomy and environmental lighting on gonadal, thyroid and total body weight of female Long-Evans rats. Experientia 25, 531–532.

Blake, C. A. (1976). Effects of pinealectomy on the rat estrous cycle and pituitary gonadotropin release. J. Endocrinol. 69, 67–75.

Bliss, D. K. and Bates, P. L. (1973). A rapid and reliable technique for pinealectomizing rats. Physiol. and Behav. 11, 111–112.

Campbell, C. S., Schwartz, N. B. and Firlit, M. G. (1977). The role of adrenal and ovarian steroids in the control of serum LH and FSH. Endocrinology 101, 162–172.

Chu, E. W., Wurtman, R. J. and Axelrod, J. (1964). An inhibitory effect of melatonin on the estrous phase of the estrous cycle of the rodent. Endocrinology 75, 238–242.

Critchlow, B. V. (1963). In: Advances in Neuroendocrinology. (A. V. Nalbandov, ed.) University of Illinois Press, Urbana, Illinois.

de la Cruz, A., Arimura, A., de la Cruz, K. and Schally, A. V. (1976). Effect of administration of antiserum to luteinizing hormone-releasing hormone on gonadal function during the estrous cycle in the hamster. Endocrinology 98, 490–497.

Dunaway, J. E. (1968). Effects of pinealectomy on the timing of PMS-induced ovulation. Anat. Record. 160, 342.

Evans, R. W., Sholitin, L. J. and Leavitt, W. W. (1977). Progesterone receptor in the rat anterior pituitary: effect of estrogen priming and adrenalectomy. Program for the 10th annual meeting of the Society for the Study of Reproduction, Austin, Texas, August 14–17.

Everett, J. W., Sawyer, C. H. and Markee, J. E. (1949). A neurogenic timing factor in control of the ovulatory discharge of luteinizing hormone in the cyclic rat. Endocrinology 44, 234–250.

Hoffmann, J. C. (1973). The influence of photoperiods on reproductive functions in female mammals. In: Handbook of Physiology, Section 7, Volume 2, Part I. (R. O. Greep and E. B. Astwood, eds.). American Physiological Society, Washington, D.C. pp. 57–77.

Keppel, G. (1973). Design and Analysis. Prentice-Hall Inc., Englewood Cliffs, New Jersey.

Kitay, J. I. and Altschule, M. D. (1954). Effects of pineal extract administration on ovary weight in rats. Endocrinology 55, 782–784.

MacFarland, L. A. and Mann, D. R. (1977). The inhibitory effects of ACTH and adrenalectomy on reproductive maturation in female rats. Biol. Reprod. 16, 306–314.

Mann, D. R., Korowitz, C. D. and Barraclough, C. A. (1975). Adrenal gland involvement in synchronizing the preovulatory release of LH in rats. Proc. Soc. Exp. Biol. Med. 150, 115–120.

Mann, D. R., Korowitz, C. D., MacFarland, L. A. and Cost, M. G. (1976). Interactions of the light-dark cycle, adrenal glands and time of steroid administration in determining the temporal sequence of LH and prolactin release in female rats. Endocrinology 99, 1252–1262.

Niswender, G. D., Midgley, A. R., Jr., Monroe, S. E. and Reichert, L. E., Jr. (1968). Radioimmunoassay of rat luteinizing hormone with antiovine LH serum and ovine LH^{131}. Proc. Soc. Exp. Bio. Med. 128, 807–811.

Niswender, G. D., Chen, C. L., Midgley, A. R., Jr., Meites, J. and Ellis, S. (1969). Radioimmunoassay for rat prolactin. Proc. Soc. Exp. Biol. Med. 130, 793–797.

Niswender, G. D. (1973). Influence of the site of conjunction on the specificity of antibodies to progesterone. Steroids 22, 413–424.

Peppler, R. D. and Jacobs, J. J. (1976). The effect of adrenalectomy on ovulation and follicular development in the rat. Biol. Reprod. 15, 173–178.

Takeo, Y., Anazawa, M., Shirama, K., Shimizu, K. and Maekawa, K. (1975). Pinealectomy and sexual rhythm in female rats. Endocrinol. Japan, 22(3), 219–224.

Tigchelaar, P. V. and Nalbandov, A. V. (1975). The effect of the pineal gland on ovulation and pregnancy in the rat. Biol. Reprod. 13, 461–469.

Turgeon, J. and Barraclough, C. A. (1973). Temporal patterns of LH release following graded preoptic electrochemical stimulation in proestrous rats. Endocrinology 92, 755–761.

Wurtman, R. J., Altschule, M. D. and Holmgren, U. (1959). Effects of pinealectomy and of a bovine pineal extract in rats. Amer. J. Physiol. 197, 108–110.

Wurtman, R. J., Axelrod, J. and Phillips, L. S. (1963). Melatonin synthesis in the pineal gland control by light. Science 142, 1071–1073.

Wurtman, R. J., Axelrod, J. and Kelly, D. E. (1968). The Pineal. Academic Press, New York.

Wuttke, W., Cassell, E. and Meites, J. (1971). Effects of ergocornine on serum prolactin and LH and on hypothalamic content of PIF and LRF. Endocrinology 88, 737–741.

RECOMMENDED REVIEWS

Ellis, L. C. (ed.) (1976). Endocrine role of the pineal. American Zoologist 16, 3–101.