A CIRCADIAN RHYTHM IN OVARIAN CHOLESTEROL

By

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ABSTRACT

Total ovarian cholesterol levels in the Wistar and Holtzman rat were found to exhibit a circadian (= diurnal) rhythm in the mature female and in the immature PMS-HCG treated animal although certain differences existed between the two strains. Cholesterol levels were determined at 1:30 p.m. and 8:30 p.m. on each day of the oestrous cycle in mature animals. A 19% depletion occurred at metoestrus in the Wistar rat but no significant change was noted in the Holtzman rat. During dioestrus a significant depletion occurred in both strains, i.e. 15% in the Wistar and 19% in the Holtzman strain. On the day of prooestrus the cholesterol levels in both strains showed a 50% depletion. During oestrus the values for the Wistar remained low while the levels in the Holtzman showed a significant increase of 25%. No diurnal rhythm occurred in mature animals kept in constant light or in animals which had been androgen-sterilized with 1.25 mg of testosterone propionate on day two of age. The cholesterol levels were also measured in immature animals of both strains that had been prepared for use in OCD assay for LH, i.e., 50 IU of PMS on day 22 and 24 and 25 IU of HCG on day 26. Measurements were taken at 8:30 a.m., 1:30 p.m., 8:30 p.m., and 2:30 a.m. on day 36 of age. The immature Wistar rat showed a highly significant diurnal rhythm with the highest value at 1:30 p.m. and the lowest value at 2:30 a.m. The immature rat of the Holtzman strain failed to show any rhythm but the cholesterol levels were twice as high as those noted in the Wistar rat. Androgen sterilization abolished the diurnal rhythm in the Wistar animals and significantly increased the ovarian cholesterol values in both strains. The results indicate that a diurnal rhythm in ovarian cholesterol is present. The cholesterol depletion on the day of dioestrus is discussed with regard to its role in the timing of ovulation.

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Changes in ovarian cholesterol during the oestrous cycle are of special interest because these changes are generally considered to reflect the cyclic influences of gonadotrophins. Studies on the ovaries of the rat using histochemical methods indicate that ovarian cholesterol increases during dioestrus and reaches a maximum level on the day of prooestrous. A rapid depletion occurs in the latter part of prooestrous followed by a gradual return to high levels during subsequent days of the oestrous cycle (Everett 1945; Beyler & Szego 1954; Franchimont & van Cauwenberge 1962). Claesson & Hillarp (1947) studying the interstitial tissue of the rat ovary reported two peaks in cholesterol concentration, one at prooestrous and one at metoestrus. With the exception of cholesterol determinations made on the day of prooestrous, all previous reports have been concerned with cholesterol determinations made at one time during each day of the cycle. Lawton & Schwartz (1965) reported a diurnal rhythm of ovarian ascorbic acid in PMS-HCG treated immature rats (animals prepared for use in OAAD assay for LH).

The present report is concerned with the possibility that ovarian cholesterol also might exhibit this diurnal rhythm. Both mature cycling rats and PMS-HCG treated immature rats prepared for the ovarian cholesterol depletion (OCD) assay for LH (Bell et al. 1964) were utilized. The OCD assay as originally reported by Bell et al. (1964) has not been useable in this laboratory and it was also hoped that the current study might shed some light on the reasons for its inadequacy. In addition, the effects of androgen sterilization and constant light on ovarian cholesterol levels were studied.

MATERIALS AND METHODS

Rats of the Purdue-Wistar and Holtzman strains were used in this study. All animals were kept in a controlled environment of about 22°C, with a relative humidity of 45–55 per cent. The light-dark cycle consisted of 13 hours of light and 11 hours of darkness with the light cycle starting at 7:30 a.m. Food and water were given ad lib.

The PMS-HCG* treatment in immature animals consisted of subcutaneous injections of 50 IU of PMS on days 22 and 24 of age followed by 25 IU of HCG on day 26. Androgen sterilization was accomplished by a single subcutaneous injection of 1.25 mg testosterone propionate on day 2 of age. This was followed by PMS-HCG injection procedure in the immature animals. On day 36 (10 days following HCG injection) the animals were killed and ovarian cholesterol determinations were made at 8:30 a.m., 1:30 p.m., 8:30 p.m., and 2:30 a.m.

Mature rats (3–4 months old) were housed four per cage and daily vaginal smears were taken for 3–4 cycles. The rats were killed and ovarian cholesterol determination made at 1:30 p.m. and 8:30 p.m. on each day of the oestrous cycle. Only rats showing

* Pregnant mares' serum gonadotrophin (PMS) and Human chorionic gonadotrophin (HCG) were obtained through the courtesy of Dr. J. B. Jewell, Ayerst Laboratories, New York.
regular four day cycles were used. Mature rats approximately two months old were also placed in constant light until the vaginal smears showed persistent oestrus for a two week period. These animals were then killed at 1:30 p.m. and 8:30 p.m. and the ovarian cholesterol levels determined. Mature rats which had received 1.25 mg testosterone propionate on day 2 of age were killed and cholesterol determinations made when the rats were 3–4 months old.

The ovaries of all rats were removed, quickly cleaned, and weighed to the nearest 0.1 mg on a torsion balance. Ovaries were then homogenized with 10 ml of glacial acetic acid, and the homogenate filtered through Whatman No. 1 filter paper. An aliquot of this filtrate was then analyzed by the Zlatkis' method for total cholesterol (Zlatkis et al. 1953). Cholesterol was expressed as µg per 100 mg of the ovarian weight. Statistical analysis was done using the Newman-Keuls and Student's t tests.

RESULTS

Ovarian Cholesterol in Mature Cycling Rats

Fig. 1 shows the results of this study. The cholesterol level in the Wistar rat decreased significantly ($P < .01$) between 1:30 p.m. (1.37 µg/100 mg) and

![Graph showing ovarian cholesterol concentrations during the oestrous cycle of the mature Wistar and Holtzman rat. Values were determined at 1:30 p.m. and 8:30 p.m. on each day of the cycle. Number in parenthesis indicates the number of rats used at each point.](fig1.jpg)

Fig. 1.

A comparison of ovarian cholesterol concentrations during the oestrous cycle of the mature Wistar and Holtzman rat. Values were determined at 1:30 p.m. and 8:30 p.m. on each day of the cycle. Number in parenthesis indicates the number of rats used at each point.
8:30 p.m. (1.08 μg/100 mg) on the day of metoestrus. This depletion did not occur in the Holtzman rat, instead a slight but not significant increase was observed during this period. During the afternoon of dioestrus both strains showed a significant ($P < .05$) depletion of ovarian cholesterol (Wistar 1:30 p.m. – 1.67 μg/100 mg, 8:30 p.m. – 1.42 μg/100 mg; Holtzman 1:30 p.m. – 2.05 μg/100 mg, 8:30 p.m. – 1.74 μg/100 mg).

The cholesterol levels increased significantly ($P < .05$) in both strains by 1:30 p.m. on the day of prooestrus (Wistar, 1.76 μg/100 mg; Holtzman, 2.14 μg/100 mg). During the afternoon of prooestrus there was a marked and significant ($P < .01$) drop in cholesterol in both strains (8:30 p.m. levels – Wistar, 1.15 μg/100 mg; Holtzman, 1.18 μg/100 mg). During the day of oestrus the cholesterol levels remained relatively low (avg. 1.25 μg/100 mg) in the Wistar rat and did not manifest any rhythm. In the Holtzman rats the 1:30 p.m. level on the day of oestrus was 1.07 μg/100 mg and the 8:30 p.m. level was 1.43 μg/100 mg (significant increase $P < .05$).

### Ovarian Cholesterol in Androgen Sterilized Mature Rats and in Mature Rats Exposed to Constant Light

Since both constant light and androgen sterilization are considered to inhibit the release of ovulatory amounts of LH, these treatments were used in an attempt to abolish the observed diurnal rhythm. Both androgen sterilization and constant light blocked the diurnal rhythm in mature animals (Table 1). The average cholesterol concentration for the combined 1:30 and 8:30 p.m.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cholesterol levels</th>
<th>Ovarian weight Avg. for both ovaries (mg)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1:30 p.m.</td>
<td>8:30 p.m.</td>
<td></td>
</tr>
<tr>
<td>Constant light</td>
<td>3.20±0.10</td>
<td>3.30±0.15 N.S.</td>
<td>38.7±3.0</td>
</tr>
<tr>
<td>Androgen sterilization</td>
<td>2.76±0.09</td>
<td>2.87±0.10 N.S.</td>
<td>31.5±3.2</td>
</tr>
<tr>
<td>Dioestrus in normal Wistar</td>
<td>1.67±0.04</td>
<td>1.42±0.13*</td>
<td>67.6±2.8</td>
</tr>
</tbody>
</table>

N.S. – not significant different from the 1:30 p.m. values.

* $P < .05$. 

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values was 2.81 μg/100 mg in the androgen sterilized rat and 3.25 μg/100 mg in the animals kept in constant light. These concentrations were significantly greater \( (P < .01) \) than dioestrus levels in the normal Wistar rat \( (1.67 \mu g/100 \text{ mg}) \). The ovarian weights in both groups were significantly smaller \( (P < .01) \) than in the normal Wistar rat.

**Ovarian Cholesterol in PMS-HCG Treated Immature Rats**

The ovarian cholesterol level in the Wistar rat pretreated with PMS and HCG increased significantly \( (P < .01) \) from 1.58 μg/100 mg at 8:30 a.m. to 2.16 μg/100 mg at 1:30 p.m. (Fig. 2). A significant depletion \( (P < .01) \) occurred

![Figure 2](image)

A comparison of ovarian cholesterol levels in PMS-HCG treated, immature Wistar and Holtzman rats. Values were determined at four times during the tenth day following PMS-HCG treatment in both non-androgenized and androgenized animals.

- **W-C** Wistar control: non-androgenized with PMS-HCG treatment.
- **W-A** Wistar androgenized: androgen sterilization followed by PMS-HCG treatment.
- **H-C** Holtzman control: non-androgenized with PMS-HCG treatment.
- **H-A** Holtzman androgenized: androgen sterilization followed by PMS-HCG treatment.
between 1:30 p.m. and 8:30 p.m. in that the levels fell from 2.16 μg/100 mg at 1:30 to 1.32 μg/100 mg at 8:30 p.m. The level at 2:30 a.m. (1.06 μg/100 mg) was less than the 8:30 p.m. level but this decrease was not significant. The Holtzman rats failed to show this diurnal rhythm but had much higher cholesterol levels than the Wistar rats (avg. 3.23 μg/100 mg).

**Ovarian Cholesterol in Androgen Sterilized, PMS-HCG Treated Immature Rats**

Neither of the two strains of rats manifested a diurnal rhythm following androgen sterilization (Fig. 2). The ovarian cholesterol concentration for each strain (Wistar, 4.19 μg/100 mg; Holtzman, 4.02 μg/100 mg) was significantly higher (P < .01) than the non-androgenized, PMS-HCG treated rats (Wistar, 2.16 μg/100 mg – 1:30 p.m. level; Holtzman, 3.24 μg/100 mg). The ovarian weights were significantly (P < .01) reduced in the androgen sterilized rats (Wistar, 100.3 mg; Holtzman, 124.7 mg) compared to non-androgen sterilized animals (Wistar, 122.5 mg; Holtzman, 132.1 mg).

**DISCUSSION**

The depletion of ovarian cholesterol that occurs during the afternoon of prooestrus and dioestrus in the adult Wistar rat and during dioestrus in the Holtzman rat is especially interesting. This time period during the afternoon on the day of prooestrus is generally assumed to be the time during which LH is released (*Everett & Sawyer* 1953; *Schwartz & Caldarelli* 1965). Since LH has been shown to mobilize cholesterol which acts as a precursor for ovarian steroids in the rat (*Armstrong et al.* 1964), rabbit (*Solod et al.* 1966), and bovine corpus luteum (*Hall & Koritz* 1965), it appears that the depletions observed during prooestrus and dioestrus might also reflect a release of LH on these days.

The presence of the ovary between 10:00 a.m. and 4:00 p.m. on the day preceding prooestrus is necessary to produce the large scale LH release on the day of prooestrus (*Schwartz* 1964). *Schwartz & Caldarelli* (1965) have also demonstrated increased blood levels of LH during the afternoon or evening of dioestrus. These observations plus the reports that ovariectomy (*Hemmingsen* 1933) or injections of LH antiserum (*Bourdel & Li* 1963) on the day before prooestrus, but not on the day of prooestrus, blocks vaginal cornification, indicate that the ovarian cholesterol depletion on the day of dioestrus may reflect steroid production which is necessary to cause ovulation on the day of prooestrus and vaginal cornification during oestrus. It is interesting to note that another hormone, prolactin, shows a diurnal rhythm in pituitary levels in the rat, reaching a peak between 2:00 p.m. and 4:00 p.m. and depleting to basal
levels by 10:00 p.m. on each day of the oestrous cycle (Clark & Baker 1964). The involvement of prolactin in ovarian steroidogenesis is not well understood but there is some indication that the presence of prolactin may be necessary for LH to bring about steroid production (Baird et al. 1961). The depletion in ovarian cholesterol noted in this study also show an interesting parallel to the fluctuations of LH-RF during the oestrous cycle. This appears in the data reported by Ramirez & Sawyer (1965) even though the authors made no mention of this. The Wistar rat showed an ovarian cholesterol depletion on the day of metaoestrus which was not observed in the Holtzman rat. The reason for this difference between the two strains is not apparent at this time.

A diurnal rhythm in ovarian cholesterol also exists in the PMS-HCG treated Wistar rat but was not found in the PMS-HCG treated Holtzman rat. This rhythm could influence the results of OCD assays for LH as proposed by Bell et al. (1964), and could account for some of the problems which have been encountered in this assay (Skosey & Goldstein 1966) if a strain comparable to the Wistar rat were used. It should be pointed out, however, that Bell et al. (1964) used a single PMS injection whereas two injections of PMS were used in our experiments. The reactions of the ovary in these two strains of rats to PMS-HCG treatment appear to be quite dissimilar. The ovarian cholesterol levels in the Holtzman rat were considerably greater than those of the Wistar, and the response of the Wistar rat in the OAAD test for LH is quite different from that of the Holtzman (Larsen & Hamburger 1963; Yokota et al. 1965; Koed & Hamburger 1965; Daniels 1966).

The complete abolition of a cholesterol diurnal rhythm by androgen sterilization is a strong indication that the control of the daily rhythm resides in the hypothalamic-hypophyseal LH releasing system. Injections of testosterone propionate in the neonatal rat produces a physiologic lesion in the hypothalamus (Barraclough 1963) and results in the production of a persistent oestrus condition which resembles the persistent oestrus produced by anterior hypothalamic lesions (Hillarp 1949; Woltius et al. 1962). Androgen sterilized rats do not have the capacity to release ovulatory amounts of LH (Woltius et al. 1962; Barraclough 1963).

The ovarian cholesterol diurnal rhythm was also blocked in the persistent oestrus rat produced by constant light. These animals are also considered to lack the ability to release ovulatory amounts of LH (Bradshaw & Critchlow 1966), which again indicates that the diurnal rhythm observed in the cycling rat is controlled via the release of LH.

REFERENCES

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