AGE-DEPENDENT CHANGES IN THE PITUITARY-GONADAL RELATIONSHIP

II. A STUDY OF PITUITARY FSH AND LH CONTENT IN THE FEMALE RAT*

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Summary. Pituitary stores of FSH and LH were determined in female rats of ages ranging from 12 to 14 days (24 g body weight) to an estimated 280 days (>375 g body weight). The aim was to study the endocrine basis for lack of ovarian compensatory hypertrophy in the aged rat. Both the concentration (μg/mg) and total content (μg/gland) of FSH in the pituitary glands of aged rats were two to three times higher than those in the glands from the adult rats (60 to 180 days old and weighing 200 to 300 g). A similar but less marked tendency was present for LH.

These results support the view that lack of ovarian compensatory hypertrophy in the aged rat is due to a decline in the output of FSH and LH from, but not due to deficiency of gonadotrophins in, the pituitary gland of the aged rat.

INTRODUCTION

An earlier study (Labhsetwar, 1967a) indicated that unilateral ovariotomy in the aged rat (body weight >375 g) does not result in the compensatory hypertrophy of the remaining ovary when examined 10 days later. It was further shown that ovaries of the aged rat remain capable of responding to PMSG. It was therefore postulated that lack of ovarian compensatory hypertrophy in the aged rat may be related to a decrease in the output of gonadotrophic hormones from the pituitary gland. Such a decrease could result either from the deficiency of gonadotrophins in the pituitary gland or it might be secondary to interference with the release of these hormones. To examine these alternatives, the pituitary content of follicle stimulating hormone (FSH) and luteinizing hormone (LH) was assessed throughout the life cycle of the female rat with a particular emphasis on the aged animal.

* The first paper in this series (Labhsetwar, 1967a) was unnumbered.
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MATERIALS AND METHODS

Rats

All rats, both experimental as well as assay animals, were of the Holtzman strain. They were housed in air-conditioned quarters under 14 hr of artificial illumination and 10 hr of darkness and permitted free access to Purina chow and tap water. The female rats of known ages were purchased. The exact age of the aged rats was not known, but was calculated from the regression line of body weight on age as described earlier (Labhsetwar, 1967a). The 12- to 14-day-old rats were born in the laboratory to parents of the Holtzman strain. Both males and females were included in this age group. Vaginal smears were taken from rats in different age groups. The rats were killed when the smears consisted predominantly of leucocytes.

Autopsy

Rats were killed between 08.00 and 11.30 hours as described elsewhere (Labhsetwar, 1967b). The anterior pituitary glands were pooled within each age group and stored at −20°C until bio-assayed. Because of the smaller size, the glands of 12- to 14-day-old rats were weighed in groups of five.

Bio-assays

FSH. The hCG augmentation method of Steelman & Pohley (1953) described earlier (Labhsetwar, 1967b) was used. Each assay included two doses of a standard and one dose of an unknown preparation (four assay rats/dose). The augmenting dose of hCG was 40 i.u./assay rat. The assay data were analysed by employing the multiple assay design analysis of Borth (1960). The indices of precision on two occasions, when unknowns were tested, were 0.078 and 0.090.

LH. The ovarian ascorbic acid depletion method of Parlow (1961) employing one ovary and a 4-hr interval and incorporating the recent modification of Bogdanove & Gay (1967) was used. The assay rats (55 to 60 g when 24 days old) were primed sequentially with 50 i.u. each of rFSH (Equinex, Ayerst) and hCG (A.P.L., Ayerst) beginning on the 26th day of life with an interval of approximately 65 to 72 hr between injections. In addition, each assay rat received a daily injection of oestradiol-17β (10 μg/day, 0.2 ml corn oil, subcutaneously) beginning 5 days after hCG administration. The right ovary of each rat was used for assay 10 days after hCG injection (or after five daily oestradiol injections). The left ovary was used 14 days after hCG injection. The daily oestradiol injections were continued until 24 hr before removal of the second ovary. Throughout this study a four-point symmetrical assay design with two doses of standard LH and two doses of each unknown preparation was used, several such preparations being tested at each time. A minimum of four assay rats was assigned to each dose level. The assay data were analysed according to the method of Gaddum (1953) as adapted by Borth (1960) for multiple assay design. In all instances, 'g' values were smaller than 0.1 and therefore ignored in the calculation of 95% confidence limits as recommended by Gaddum (1953). The mean index of precision (λ) for the initial ovary was 0.176 and for the second ovary, 0.156.
Pituitary FSH and LH in aged rats

Two different, but reputedly equipotent, reference standards of LH (NIH s-11 and s-12) were used during the course of these studies.

RESULTS

Organ weights
With the exception of 12- to 14-day-old and 33-day-old animals, rats in all the remaining age groups, including those in the oldest, had corpora lutea in the ovaries. The relative ovarian weight (mg/100 g body weight) decreased with age, confirming an earlier observation (Labhsetwar, 1967a). The absolute (mg/animal) uterine weights increased with age ($P<0.01$) but the relative weight declined (Text-fig. 1). On the other hand, while the absolute pituitary weight increased with age, the relative weight remained remarkably stable ($P>0.05$) with the exception of the 12- to 14-day group, which also included males (Text-fig. 1).

Vaginal smears
The various groups of rats from which vaginal smears were taken are shown by an asterisk in Text-figs. 2 and 3. While regular 4- or 5-day cycles were discernible in adult rats (60 to 180 days old), there were fewer regular cycles in aged rats. It is to be emphasized that none of the aged rats had persistent

\[ \text{Text-fig. 1. Changes in absolute (○) and relative (●) uterine weights and absolute (■) and relative (□) pituitary weights as a function of age. Each value, except relative pituitary weights, expressed as mean ± S.E. The standard errors of the relative pituitary weights are not shown because they were less than 5% of the mean in all groups. The mean uterine weight includes only those rats with solid uteri.} \]
vaginal cornification during 2 to 3 weeks before autopsy. All females in the two youngest groups (12- to 14-day and 33-day) had closed vaginae.

**Histology**

Sections of ovaries (7 µ) from a few aged and adult (80-day-old) animals were stained with a tetrachrome method. Upon microscopic examination, a marked increase in the interstitial tissue and an increased incidence of cystic

![Diagram](https://via.placeholder.com/150)

**Text-fig. 2.** Changes in FSH (NIH-s-4) concentration (A), total content (B) and content/100 g body weight (C) in female rats as a function of age. The bar at the top of each column in A corresponds to 95% confidence limits while the asterisk at the base denotes groups killed when in vaginal di-oestrus. The number at the base of the column in B indicates number of rats/group. The 12- to 14-day group included males.

follicles were noted in the ovaries of aged rats when compared with those from the adult animals. These observations are essentially similar to those made by Wolfe (1943) on an extensive series of rats.

**FSH**

The pituitary FSH concentration (µg/mg) was high in the youngest rats aged 12 to 14 days but by Day 33 it had dropped significantly. All rats in this group had fluid-filled uteri but closed vaginae. In adult rats ranging in age from 76 to
136 days, the FSH concentration remained remarkably constant ($P>0.05$) fluctuating between a narrow range of 2 to 4 $\mu$g/mg (Text-fig. 2). In aged animals, on the other hand, the hormone concentration rose sharply ($P<0.05$). This high FSH level in the aged animals was confirmed when repeated (Group II, Text-fig. 2, A). The total FSH content ($\mu$g/gland) and the relative FSH content ($\mu$g/pituitary/100 g body weight) followed essentially the same patterns (Text-fig. 2, B and C).

**LH**

The concentration of LH ($\mu$g/mg) was significantly higher in 33-day-old rats than in adult rats aged 76 days. It should be noted that all 33-day-old rats had closed vaginae but, unlike those used for FSH determination, they also had solid uteri. In the adult rats of three different ages (Text-fig. 3), LH concentration varied to a considerable degree. Part of the variability could be attributed to the stage of the oestrous cycle when the rats were killed. In aged rats, LH concentration was relatively high although the increase was not as sharp as that for FSH. This tendency for a relatively high level of LH to be found in aged animals was confirmed when the experiment was repeated on two different occasions (Text-fig. 3) and was particularly marked when expressed
as μg/gland (Text-fig. 3, B). The LH levels in the two youngest groups were as high as those in the aged animals when expressed as μg/pituitary/100 g body weight (Text-fig. 3, C).

Ovarian compensatory hypertrophy

A group of six animals was randomly selected from a batch of aged animals in which pituitary LH and FSH were to be determined (Group II, Text-figs. 2 and 3). These were unilaterally spayed, as was also a group of 62-day-old rats for comparison. At autopsy 10 days later, the remaining ovary was removed. Ovarian compensatory hypertrophy failed to occur in the aged rats (Table 1), confirming the earlier observation (Labhsetwar, 1967a). Similarly, increase in the luteal count seen in the adult animals was not observed in the aged rats (Table 1).

Table 1

<table>
<thead>
<tr>
<th>Agea (days)</th>
<th>No. of rats</th>
<th>Body wtb (g)</th>
<th>Ovarian wc (mg)</th>
<th>% Increase</th>
<th>No. of corpora lutea</th>
<th>% Increase</th>
</tr>
</thead>
<tbody>
<tr>
<td>62</td>
<td>7</td>
<td>248±7</td>
<td>38.7±1.5</td>
<td>41.6</td>
<td>6.0±0.7</td>
<td>61±7</td>
</tr>
<tr>
<td>&gt; 280c</td>
<td>6</td>
<td>396±7</td>
<td>50.3±5.0</td>
<td>49</td>
<td>6.0±0.5</td>
<td>6.0±1.1</td>
</tr>
</tbody>
</table>

*** P < 0.005 when compared to respective control.
* Not significant (P > 0.05) when compared to respective control.
b Removed and weighed 10 days after the left ovary.
c Age estimated from regression line of body weight on age as described earlier (Labhsetwar, 1967a).
d At unilateral spaying.

DISCUSSION

The results demonstrate that the pituitary gland of the aged rat is not deficient in FSH and LH. Lack of ovarian compensatory hypertrophy in the aged animal does not appear to be the consequence of an inability of the pituitary to synthesize FSH and LH. It appears, rather, to be due to a decline in the output of these hormones, resulting in their marked accumulation in the pituitary gland. A significant decrease in relative uterine weight in the aged animal may be a reflection of a decline in oestrogen secretion resulting from such a decrease in LH output.

Some degree of FSH and LH secretion, however, continues in the aged rat. This is attested by the fact that ovaries of the aged rat contained vesicular and cystic follicles of varying sizes and numerous corpora lutea. Wolfe (1943), in his extensive study, found a decrease both in follicular and luteal counts in the ovaries of the aged animals.

Originally, Lauson, Golden & Sevringhaus (1939) determined the ‘total’ gonadotrophin content of the pituitary throughout the life cycle of the female rat by employing uterine and ovarian weights of immature rats as assay endpoints. They found a considerable increase in pituitary gonadotrophin
content of the aged rats (>2.5 years old). The present study confirms this observation and further shows that the increase involves both LH and FSH and that it probably occurs at a much earlier age than reported by these authors. The finding indicating high levels of LH and FSH in pituitary glands of the prepuberal rats is in accord with the earlier observations of many workers (Hoogstra & Paesi, 1955; Ramirez & McCann, 1963; Barraclough, 1966; Moore, 1965/1966; Matsuyama, Weisz & Lloyd, 1966, for LH; and Corbin & Daniels, 1967; Kragt & Ganong, 1968, for FSH).

Matsuyama, Weisz & Lloyd (1966) reported a decrease in pituitary LH content and some elevation in the total pituitary gonadotrophins (mouse uterine weight method) in aged rats (343 to 352 days old and weighing 352 g). But unlike the rats in the present experiments, their rats showed a persistent vaginal cornification for at least 25 days before autopsy. It is presumed that differences in LH content between the two studies are at least partially related to this condition.

The alterations in the ovaries, pituitary and uterus are obviously involved in a decline in the reproductive capacity with advancing age, but the chronological relationship between these factors is incompletely understood (Lipschutz, Igiesias & Salinas, 1963; Verzar, 1966). It appears that in the aged rat pituitary, FSH and LH levels are elevated at a time when ovaries are capable of responding to PMSG (Labhsetwar, 1967a) and the uterus retains the capacity to form deciduomata (Labhsetwar, unpublished observations). Therefore, alterations in the gonadotrophin release from the pituitary gland seem to occur earlier than the detectable changes in the sensitivity of ovaries and uterus. It is well established that release of gonadotrophins from the pituitary gland is regulated by the hypothalamus (see review by Harris, Reed & Fawcett, 1966). It is tempting to postulate that impaired hypothalamic control of the pituitary rather than intrinsic alterations in the pituitary, ovaries or uterus is the primary factor involved in initiating gerontological changes in the pituitary–ovarian axis.

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REFERENCES


