THE ONSET OF FIRST OESTRUS IN THE GUINEA-PIG AND THE EFFECTS OF GONADOTROPHINS AND OESTRADIOL IN THE IMMATURE ANIMAL

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SUMMARY

The immature female guinea-pig approaching first oestrus and ovulation has been studied. A clear relationship between the rate of growth of the animal and the age at puberty has been found. The 'crucial period', a time just before first oestrus, when external signs of endogenous hormonal activity give an indication of the presence of ripening follicles in the ovary, has been defined. At this time premature ovulation can be regularly induced by a single injection of gonadotrophin (guinea-pig anterior pituitary homogenate, purified luteinizing hormone or follicle-stimulating hormone). Injection of oestrogen advanced vaginal opening but, in contrast to the rat, did not cause premature ovulation.

INTRODUCTION

The hormonal mechanisms involved in the onset of puberty are as yet not completely defined. Early observations by Foà (1900) indicated that the maturation of the reproductive system is influenced by some factor other than the gonads and the classical work by Smith & Engle (1927) showed the importance of the pituitary gland in influencing sexual maturation in the rat. These later workers suggested that the immature anterior pituitary gland failed to release the gonadotrophins which it contained in 'adult amounts'. This hypothesis was confirmed by Harris & Jacobsohn (1952) who transplanted anterior pituitary tissue from newborn rats to the median eminence of the hypophysectomized mother and found that the resumption of normal oestrous cycles and ovulation occurred. Donovan & van der Werff ten Bosch (1956, 1959) showed that electrolytic lesions placed in the anterior hypothalamus of immature rats could cause premature opening of the vaginas, ovulation and pregnancy – indicating that the onset of puberty might be controlled by the hypothalamus. They suggested that the lesions had removed an inhibitory action of the hypothalamus on the pituitary, causing the premature release of gonadotrophins and that the onset of puberty was due to a decrease in sensitivity of the hypothalamic receptors to oestrogen (Donovan & van der Werff ten Bosch, 1965). Ramirez & McCann (1963) showed that ovariectomized immature rats required a lower concentration of
oestradiol benzoate than the castrated adult, to reduce plasma luteinizing hormone (LH) to normal levels – indicating the higher sensitivity of the immature rat hypothalamus to the inhibitory action of oestrogens. Recently it has been suggested that in the rat, exogenous oestrogen advances sexual maturity (Ramirez & Sawyer, 1965) and that implants of oestradiol benzoate in the anterior hypothalamus advance vaginal opening (Smith & Davidson, 1968) and in the median hypothalamus advance ovulation (Motta, Fraschini, Giuliani & Martini, 1968).

Little work has been done in the guinea-pig on the factors controlling puberty. In 1911 Loeb described the ovarian histology of the untreated guinea-pig approaching first oestrus, and Ford & Young (1953) studied the length of the first oestrus and time of ovulation. Several workers have reported varying results from the injection of gonadotrophins into the immature guinea-pig; guinea-pig anterior pituitary gland (Loeb, 1932; Schmidt, 1937; Hamburger & Pederson-Bjergaard, 1946) or purified sheep LH (Deanesly, 1966). The aim of the present work was to study the onset of first oestrus in the untreated guinea-pig and to examine the effects of exogenous gonadotrophins or oestrogen on the reproductive system of the immature animal.

**MATERIALS AND METHODS**

Immature female albino guinea-pigs of the Hartley strain (Animal Virus Research Institute, Pirbright, Surrey) were used. They were weaned on collection from the breeder and were of known age (12–19 days old). They were fed on Diet S.G.1 (Messrs Dixon and Sons, Ware, Herts) and hay, supplemented with cabbage six times a week and ascorbic acid (0.5 g/l) added to their drinking water three times a week. From the day when the animals were received they were observed once every 24 h when the state of the vaginal membrane and the size and vascularity of the nipples were noted. Each animal was weighed regularly three times a week. Groups of animals were killed on each day of first oestrus and 15 animals were killed 1–5 days after the end of oestrus. One group of ten animals weighing 240–280 g, termed ‘immature controls’ was killed when the vagina was closed and there were no signs of nipple growth or increased vascularity.

**Guinea-pig gonadotrophins**

Anterior pituitary glands from adult male and female guinea-pigs (weighing about 600 g) and young females (about 320 g) were dissected as quickly as possible after death and stored at −15 °C. No gland was stored for longer than 4 months. When required, the glands were thawed, homogenized singly in 0.75 ml 0.9 % NaCl solution and the homogenate injected immediately into the experimental animal.

**Purified sheep gonadotrophins**

Luteinizing hormone and follicle-stimulating hormone (NIH-LH-S7 and FSH-S9) were dissolved in 0.9 % NaCl solution and stored as stock solutions in a concentration of 1 mg/ml at −15 °C. When required the solutions were thawed, and 200 µg or 50 µg injected immediately.

**Oestradiol-17β**

A stock solution of oestradiol-17β in arachis oil (Searle and Co. Ltd., 0.5 mg/ml) was diluted and stored at room temperature. Three groups of six animals weighing
190–230 g were injected with 0·5, 2·0 or 5·0 µg/day for 5 days and killed 5 days after the last injection. A further eight guinea-pigs were injected daily with 2·0 µg for 5 days; 4 days after the last injection the animals were injected with 200 µg LH and killed 48 h later. All injections were s.c. into the flank of the animal.

**Autopsy**

All animals were killed with chloroform. The ovaries were fixed in formol–saline and processed for histological examination, serial sections were cut at 7 µm and every 10th section mounted and stained with haematoxylin and eosin. After dissection, the uterus was gently pressed between two sheets of filter paper to remove the fluid in the lumen, and then weighed.

**RESULTS**

One hundred and eighty-five paired ovaries were examined histologically, but in no instance was there any evidence of corpora lutea (c.L.) from a previous ovulation. At all times in the untreated animals, atretic as well as maturing follicles were seen.

**Untreated animals**

Various criteria were assessed as indications of the onset of puberty.

**Body weight and age**

The coefficient of variation for the distribution of the weight of the guinea-pigs at puberty was 8·0, whereas the coefficient of variation of the ages was 24·8, confirming that the weight of the animal at puberty is a more constant parameter than its age.
The weight of each animal at 24 days of age was taken as an indication of rate of growth and was plotted against the age on the day before vaginal opening (Text-fig. 1). The correlation coefficient was 0.79, suggesting a close relationship between the rate of growth and age at puberty.

First oestrus and ovulation

It was found that there was a period, before the vaginal membrane ruptured for the first time, when the nipples increased rapidly in size and vascularity and the external genitalia became swollen. This period varied between 0 and 6 days, but in most animals two of these three external signs of endocrine activity were observed within 3–4 days of the vaginal membrane beginning to rupture. Since it was intended to induce premature ovulation it was necessary to establish a period near the beginning of the first oestrus during which ovulation did not occur in the untreated animal. Table 1(a) summarizes the ovarian histology of groups of untreated animals killed at this time. Ovulation had not occurred in any animal killed on or before the first day the vagina showed signs of opening. All 15 animals in the group killed after the first oestrus (which varied in length from 4 to 13 days) had ovulated. Table (1b) shows a significant difference between uterine weights of animals killed before external signs of hormonal stimulation were seen (‘immature controls’) and animals killed during first oestrus when the ovaries contained ripening follicles.

Table 1. First oestrus in the guinea-pig

(a) Relationship between state of vagina and ovarian histology

<table>
<thead>
<tr>
<th>State of vagina*</th>
<th>No. of animals</th>
<th>Maturing or mature follicles</th>
<th>Ripe follicles</th>
<th>Corpora lutea</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>10</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>17</td>
<td>12</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>13</td>
<td>5</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>7</td>
<td>3</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>12</td>
<td>1</td>
<td>5</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>8</td>
</tr>
</tbody>
</table>

(b) Uterine weights†

<table>
<thead>
<tr>
<th></th>
<th>Animals killed during first oestrus</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>'Immature controls'</td>
</tr>
<tr>
<td></td>
<td>(250–280 g) (group 1)</td>
</tr>
<tr>
<td>No. of animals</td>
<td></td>
</tr>
<tr>
<td>Mean uterine wt (g)</td>
<td></td>
</tr>
</tbody>
</table>

* C = vagina closed; 0 = first day vaginal membrane showed signs of rupture; 1, 2, 3 and 4 = days after vaginal membrane first showed signs of rupture.
† P > 0.001 for Groups 1 v. 2 and 2 v. 3.

Experimental studies

In the adult guinea-pig, Reed (1967, 1968) has shown that there is a limited period of a few days before spontaneous ovulation occurs, when the ovarian follicles can be prematurely ruptured by exogenous gonadotrophins. It was therefore planned to inject the gonadotrophin as near as possible to the beginning of first oestrus. Since it
was found that spontaneous ovulation did not occur within 24 h of vaginal opening, and ideally 48 h were to elapse between the injection and autopsy, the aim was to predict the 72 h period before the vagina would begin to open. This period was called the ‘crucial period’ – the beginning of this time being the first or second day that two of the signs of endocrine activity were observed. In a few animals 24 h after the injection, the vaginae were found to be opening. These animals were killed immediately so that the possibility of spontaneous ovulation occurring could still be excluded.

**Gonadotrophin injected during the ‘crucial period’**

In the groups of animals injected with guinea-pig anterior pituitary homogenates ovulation was induced in 18/33 animals as judged by the presence of freshly ruptured follicles or corpora lutea seen on histological examination of serial sections of the ovaries. In addition to the induced ovulation, most of the remaining follicles in the ovaries were luteinized. In the untreated animals, killed after ovulation had occurred, only four luteinized follicles were seen in 29 pairs of ovaries examined. Similar results were obtained with single injections of 200 µg purified LH when ovulation was induced in 11/14 animals. In some animals, although luteal tissue was present in some follicles, the ova were retained and ovulation had not occurred (Table 2). In animals injected with 50 µg LH, ovulation occurred in 4/6 animals and again luteinized follicles were found in all ovaries of this group. There appeared to be no reduction in the overall amount of luteal tissue with the smaller dose of LH. Purified FSH (200 µg) was found to be as effective as LH in inducing ovulation and luteinizing follicles. There did not appear to be any increase in size or number of the Graafian follicles.

**Table 2. The effects on the immature guinea-pig ovary of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) injected before or during the ‘crucial period’**

<table>
<thead>
<tr>
<th>Gonadotrophins injected during ‘crucial period’</th>
<th>No. of animals</th>
<th>Induced ovulation</th>
<th>Induced luteinized follicles only</th>
<th>No effect seen</th>
</tr>
</thead>
<tbody>
<tr>
<td>A.P. (single gland)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (adult)</td>
<td>7</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Female (adult)</td>
<td>9</td>
<td>5</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Female (young)</td>
<td>9</td>
<td>3</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>A.P. (two glands)</td>
<td>8</td>
<td>5</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Female (young)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Purified LH (ovine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 µg</td>
<td>14</td>
<td>11</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>50 µg</td>
<td>6</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Purified FSH (ovine)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 µg</td>
<td>10</td>
<td>8</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Gonadotrophins injected before ‘crucial period’**

| ‘Immature controls’                           |                |                  |                                  |                |
| A.P. (adult female)                           | 6              | 2                | 4                                | 0              |
| LH, 200 µg                                    | 4              | 0                | 2                                | 2              |
| LH, 50 µg                                     | 4              | 0                | 4                                | 0              |

A.P. = guinea-pig anterior pituitary gland homogenate.
‘Immature controls’ injected with gonadotrophins

Single injections of gonadotrophin into ‘immature controls’ induced ovulation in 2/14 only, while luteinized follicles without ovulation were seen in 10/14 animals, in contrast to animals injected during the ‘crucial period’ (Table 2). The remaining follicles were atretic (Plate, fig. 1). In six ‘immature controls’ which received four daily injections of 50 µg FSH, the vaginae remained closed and no increase in growth or vascularity of the nipples was observed. At autopsy 24 h after the last injection there was little difference between the mean uterine weight (0.47 g) compared with that of the untreated ‘immature controls’ (0.40 g). Histologically, 5/6 of the guinea-pigs showed two or three follicles containing cells of the luteal type but the remaining follicles were normal.

![Text-fig. 2. Pattern of vaginal opening in two groups of immature guinea-pigs (body weight at start of injections, 190-230 g) when: (i) 2 µg oestradiol-17β were injected daily for 5 days, and (ii) when one injection of 200 µg luteinizing hormone (LH) was given after treatment with oestradiol-17β. Each division represents one day. Open divisions = vagina closed; hatched division = vagina half open; solid division = vagina open.]

**Oestradiol-17β**

The pattern of vaginal opening after injecting oestradiol-17β is shown in Text-fig. 2. Growth and increased vascularity of the nipples were observed in all animals and vaginal opening occurred on the 3rd to 5th day after the first injection – the three doses used being equally effective. However, there were no ruptured follicles or c.L. in the ovaries (Plate, fig. 2). In the eight guinea-pigs injected with LH after oestradiol-17β the appearance of the ovarian follicles was indistinguishable from those of the group of ‘immature controls’ injected with gonadotrophins (see Plate, fig. 1) and ovulation had not occurred.

**DISCUSSION**

The results indicate that, in the immature guinea-pig, ovulation can be regularly induced by a single injection of gonadotrophin (guinea-pig anterior pituitary homogenate, ovine LH or FSH) provided the injection is given at a time when the follicles have reached the appropriate stage of maturity. At this time (the ‘crucial period’) external signs of endogenous hormonal activity – increase in vascularity and growth of nipples and external genitalia – are observed. These results are comparable with findings in the adult animal in which ovulation can be induced by a single injection of gonadotrophin given 3-4 days before spontaneous ovulation is due (Reed, 1970). Deanesly (1966) found that LH injected into immature guinea-pigs weighing 270-
200 g caused ovulation in only one animal. It is probable that most of these animals had not reached the crucial period and the findings of 'luteinized or disorganised follicles with retained eggs' are very similar to those obtained in the present study when 'immature controls' (240–280 g) were injected with LH. Follicular maturation could not be detected after injecting FSH into immature animals. However, the finding that ovulation and luteinization of follicles occurred when 200 µg FSH had been injected during the 'crucial period', was similar to results obtained in the adult guinea-pig (Reed & Hounslow, 1971). It is unlikely that the slight contamination of FSH with LH (0.0008 µg LH-NIH-S1/200 µg FSH) could have been responsible for this effect. Zarrow & Gallo (1966) and Meyer & McCormack (1967) have suggested that FSH injected into immature rats releases LH or 'ovulating' hormones from the pituitary, causing ovulation and this mechanism could explain our results. Alternatively, the luteinizing effect of exogenous FSH may be due to a synergistic action with circulating endogenous LH (Greep, 1961). The increase in uterine weight in the immature guinea-pig at the beginning of first oestrus, when the ovaries contained ripening follicles and there were external signs of hormonal stimulation, is of interest. A rise in uterine weight has been shown in the mature cyclic animal before ovulation (Reed, 1966). These observations may be indications that ripening follicles are responsible for a preovulatory rise in oestrogen.

The observation that the weight of the guinea-pig is a more constant feature at the time of puberty than the chronological age is in accord with the observations of Widdowson & McCance (1960) in the rat. Text-figure 1 shows that there is a clear relationship between rate of growth of the guinea-pig and the age at puberty, suggesting that there may be an interaction between hypothalamic centres controlling body growth and the onset of puberty.

Ramírez & Sawyer (1965) have reported that oestradiol benzoate advances the first oestrous cycle in the rat, causes premature release of endogenous LH and ovulation. Our results show that although oestradiol-17β advanced vaginal opening in the guinea-pig this was not associated with ovulation and can be explained as a direct effect of exogenous oestrogen on the vagina. There was no morphological difference between the structure of the follicles in oestrogen-treated animals and 'immature controls' and the subsequent injection of LH did not cause ovulation, indicating that the ovaries of these animals had not yet reached the state of maturity associated with the 'crucial period'. The weight of the eight guinea-pigs in this group at 24 days of age ranged from 245 to 266 g. As indicated in Text-fig. 1, since these animals were 28 days old when killed, some should have been near the 'crucial period' at which time exogenous LH had previously induced ovulation in untreated animals. Since ovulation was not induced by LH in this group of animals treated with oestrogen, there was no evidence that oestradiol-17β had advanced sexual maturity: it may even have had a delaying effect. An explanation could be that the oestrogen had been given before the sensitivity of the hypothalamus to the inhibitory effects of oestrogen had decreased. The contrast between the present findings in the guinea-pig and those in the rat suggests that the mechanisms controlling the onset of puberty may differ among species.
We should like to thank the Endocrine Study Group, National Institutes of Health, Bethesda, U.S.A., for the gift of purified LH-S₂ and FSH-S₃ and Dr G. R. Venning at Messrs G. D. Searle, High Wycombe, Bucks, for the gift of oestradiol-17β. The work was aided in part by grants to P.G.M. which are gratefully acknowledged, from the Medical Research Council, the Cross Trust and the Population Council (from a grant to Professor G. W. Harris).

The results presented here formed part of a Thesis submitted by P.G.M. for the degree of B.Sc. in the University of Oxford, 1969.

REFERENCES


DESCRIPTION OF PLATE

Guinea-pig ovaries. Staining: haematoxylin and eosin; magnification, × 25.

Fig. 1. 'Immature controls' injected with 200 µg luteinizing hormone, showing luteinization or atresia of all follicles.

Fig. 2. 'Immature controls' after injection of oestradiol-17β showing maturing and atretic follicles.