POST-COITAL CYTOLOGICAL CHANGES
IN THE ADENOHYPOPHYSIS OF THE
NON-PAROUS RABBIT

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SUMMARY

The basiphil ('mucoid') cells of the adenohypophysis of the non-parous female rabbit were studied at varying times after coitus. A progressive reduction in the number of granulated mucoid cells in the dorsolateral regions of the pars distalis proper was observed. No significant changes were noted either in the mucoid cells of the zona tuberalis or in the rather large angular and strongly AF-positive cells of the median and lateral regions of the pars distalis proper.

It is suggested that the decrease in cell numbers was due to a loss of specific granules, accompanied by the release of LH.

INTRODUCTION

In an earlier investigation (Allanson, Foster & Menzies, 1959), some aspects of the cytology of the adenohypophysis of the non-parous female rabbit were described. In the present study, an attempt has been made to elucidate the changes in the basiphil cells subsequent to coitus. Particular attention has been paid to the location of the cells involved, since an increasing body of evidence points to there being—at all events in some species—a fairly precise regional distribution of the different cell types.

The basiphil cells (referred to below as 'mucoid cells') were studied because the available histochemical evidence suggested (Pearse, 1952; Purves, 1961) that these cells were much more likely to be the source of the hormone (LH) principally involved in the post-coital ovarian changes culminating in ovulation than the carminophils, to which this function had previously been assigned by Friedgood & Dawson (1938).

MATERIAL AND METHODS

Twenty-one non-parous rabbits aged about 6 months were used. Of these, eight served as controls, while the pituitaries of the remainder were examined at various times from 30 min. to 5 hr. after coitus (Table 1). The ovaries of these animals were also removed and fixed.

The method of fixation by perfusion which was used has already been described (Allanson et al. 1957, 1959). In the present experiments, better control of the perfusion procedure was obtained by the use of an electrically driven pump.
As before, the fixative principally employed was 10% neutral formalin containing calcium and cadmium chlorides (‘FCC’), but formalin-corrosive sublimate (‘F\text{HgCl}_2’); 9 parts 10% neutral formalin and 1 part saturated HgCl\text{2} was also used occasionally.

Table 1. Experimental and control animals used

<table>
<thead>
<tr>
<th>Hr. post coitum</th>
<th>No. of animals</th>
</tr>
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<tbody>
<tr>
<td>Nil</td>
<td>8</td>
</tr>
<tr>
<td>0-5 - 1-75</td>
<td>5</td>
</tr>
<tr>
<td>2-0 - 3-0</td>
<td>5</td>
</tr>
<tr>
<td>3-5 - 5-0</td>
<td>3</td>
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</table>

Paraffin sections at 5\mu were cut serially in the coronal plane.

In addition to PAS-orange G, Gomori’s aldehyde fuchsir (AF) following oxidation with KMnO\text{4} (Halmi & Davies, 1953) was extensively used. The PAS performic acid-alcian blue technique (Adams & Swettenham, 1958) was also tried, but without success.

With the aid of a projection apparatus large sketches, to show the internal morphology and the general disposition of the cells, were made from sections regularly spaced through series obtained from one control gland and from five glands obtained 2\frac{1}{2} or more hr. post coitum.

RESULTS

The distribution of the cells in the adenohypophysis of the normal, virgin female rabbit has already been described in a previous paper (Allanson et al. 1959). Except for one additional piece of information which will be mentioned later, the description given there was fully supported by the study of the control glands used in the present work.

The examination of pituitaries removed up to 2 hr. post coitum (p.c.) showed no striking change in the frequency of mucoid cells, although an impression of a limited measure of degranulation and reduced staining with PAS in a particular area of the pars distalis was obtained. The changes became more apparent in a gland removed 2\frac{1}{2} hr. after mating and a representative part of the relevant region was examined in some detail, cell counts being made and compared with those from a suitable region in a control specimen. The sketches referred to above were of the greatest value in determining comparable areas of different glands.

The full extent of the area under consideration is shown in Pl. 1, fig. 1, and comprised the dorsolateral wings of the adenohypophysis, beginning caudally and extending rostrally to a position just short of the zona tuberalis. Estimates of the numbers of mucoid cells in samples from a representative part of this area, in both specimens, were made with the aid of a micrometer eyepiece square. In both cases, the number of mucoid cells in each of 8 unit squares was estimated for each of fifteen alternating serial sections. The total number of cells counted in the control gland was approximately 2400 and in the other approximately 1600; the average numbers per unit square were 39-6 (range: 35-47) and 26-4 (range: 20-32), respectively. A correction had to be made to allow for the fact that, owing to a slightly greater amount of shrinkage due to fixation, the total number of cells per unit square in the control specimen was roughly 7% more than in the other. After allowing for this, the average
unit count for the control was 39.6 and that for the gland removed 2¼ hr. p.c. was 28.2—representing a decrease of approximately 30% in the number of granulated mucoid cells (Pl. 1, figs. 2–4).

In glands examined at longer intervals after mating, the changes were detectable without recourse to making cell counts. Pl. 1, fig. 5, shows that the reduction in the number of PAS-positive cells was even more extensive at 3 hr. afterwards and essentially similar findings were obtained up to 5 hr.—the longest interval used in this investigation. These results are summarized diagrammatically in Text-fig. 1.

Examination of the whole post-coital series indicated that the decrease in number of mucoid cells was due to a degranulation of existing cells, since weakly staining cells with sparse granulation were always observed in the region concerned. The impression was formed that in the earlier stages there were ‘foci’ of degranulation, but that later on the process became more uniformly distributed and, at the same time, spread into the more medial region of the adenohypophysis.

Particularly in sections stained with AF after a preliminary oxidation with KMnO₄, some rather large, deeply staining and often angular cells with coarse granules were observed in glands obtained 3 hr. or more p.c. These cells, never very numerous, were more frequent in the medial region (Text-fig. 1), but were also scattered among the degranulating mucoid cells of the dorsolateral wings (Pl. 2, figs. 1, 2). They showed up particularly clearly because of the great reduction in numbers of the other mucoid cells. A careful examination of the corresponding region confirmed their presence in the glands of the control animals as well (Pl. 2, fig. 3). Here they were less conspicuous owing to numerous mucoid cells of the smaller kind and, although no counts were made, there was no reason to suspect them of being either more or less frequent than in the experimental animals.

As was pointed out in an earlier communication (Allanson et al. 1959) the mucoid cells of the zona tuberalis (Pl. 1, fig. 1) differed from those of the pars distalis proper
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(with the exception of the cells just described above) in being significantly larger and in being grouped in clusters (Pl. 2, fig. 4). When this region was studied in glands obtained 3 or more hours after mating, there was no evident difference from the appearance seen in control specimens (Pl. 2, fig. 5). No cell counts were made, since the extremely irregular distribution of the cells would have necessitated the counting of an inordinately large number for the results obtained to have had any significance. The absence of obvious signs of degranulation was also in accord with the view expressed above.

Reference has already been made (Allanson et al. 1959) to the fact that cells from the pars intermedia were to be found in the pars distalis proper. The regions of infiltration of intermedia cells were indicated on drawings of serial sections and an examination of these showed how extensive the intermingling was (see also Dawson, 1937). Islands of p. intermedia cells were found throughout almost the whole of the junction zone with the pars distalis, and individual cells, embedded quite deeply in the latter, could readily be identified (Pl. 2, fig. 6). These cells with their sharply defined but somewhat irregularly distributed granules were best seen after fixing in FCC and staining with AF, although, like the pars intermedia as a whole, they were also stained by PAS.

**DISCUSSION**

Ignoring the confusion in terminology, functional studies of the mammalian hypophysis during the last decade appear to have established the existence of at least two kinds of basophil ('mucoid') cell in a rather limited number of species—the TSH secreting thyrotrophs and the gonadotrophin-secreting gonadotrophs (Purves & Griesbach, 1951a, b; Halmi, 1952, in the rat; D’Angelo, 1955, in the guinea-pig; Racadot & Herlant, 1957; Racadot, 1959, in the cat; Herlant, 1956, 1962, in the bat; and Holmes, 1960, 1963, in the ferret). Further, in the rat (Purves & Griesbach, 1954; Purves, 1961), the cat (Racadot & Herlant, 1957), a species of bat (Herlant, 1956, 1962), the mole (Herlant, 1959) and the badger (Herlant & Canivenc, 1960), it seems likely that the gonadotrophs can be divided into those producing FSH and those producing LH. Finally, it can reasonably be concluded that in certain species the different cell types have, with varying degrees of precision, regional distributions within the adenohypophysis.

It is concluded from the results of the present study that the LH-producing cells are among those PAS-positive cells occupying the region delineated in Pl. 2, fig. 1, and Text-fig. 1, since it was here that an extensive degranulation and a significant reduction in numbers occurred within a few hours after coitus.

Pearse (1952), using rabbit material stained with a modified Mallory trichrome stain and using the preferential uptake of azocarmine by the nuclei of mucoid cells as a major criterion of their secretory state, concluded that these were the cells involved in gonadotrophin production. He did not, however, investigate their regional distribution. In this connexion Lison (1955), in a careful study of fixed liver cells, has thrown considerable doubt on the validity of using preferential nuclear staining as an index of cellular activity.

Racadot (1962), summarizing the distribution of basophil cells in certain mammalian species (not including the rat and rabbit), has described the LH-producing cells (the 'cellules gamma' of Herlant) as occupying a predominantly posterolateral
position. Thus, the situation in the rabbit (but less clearly so in the rat—see Purves & Griesbach, 1954) is seen to be in good agreement with this interpretation.

In conclusion, it may be said that the present work has provided evidence for the existence of four kinds of PAS-positive cells in the rabbit adenohypophysis:

1. Cells of the zona tuberalis, occupying a position roughly identical with Racadot's presumed FSH-producing β-cells in the cat and bat.

2. Fairly large, angular and strongly AF-positive cells thinly scattered through the pars distalis proper, but more concentrated in the median position. These had a distribution resembling that of Racadot’s presumed thyrotrophs or δ-cells.

3. Smaller cells, rather evenly scattered throughout the dorsolateral aspects of the pars distalis proper. These showed extensive degranulation p.c. and are, therefore, considered to produce LH.

4. Cells derived from and resembling those of the pars intermedia.

I wish to thank Miss J. Little and Miss S. Ruther for their technical assistance.

REFERENCES


DESCRIPTION OF PLATES

PLATE 1

Fig. 1. Sagittal section of pituitary gland of rabbit. The horizontal extent of the region showing cellular degranulation lies approximately between a—b and c—d. i.p. = infundibular process; i.s. = infundibular stem; z.t. = zona tuberalis. 20 µ celloidin section. Mallory. (× 16.)

Fig. 2. Basophil ('mucoid') cells from pars distalis proper of control specimen. Neutral formalin, calc., cad. chloride (FCC); PAS-fast green. (× 500.)

Fig. 3. As above. FCC; AF after KMnO₄ oxidation. (× 500.)

Fig. 4. Similar region to above, 2-5 hr. p.c., showing marked reduction in number of PAS-positive cells. FCC; PAS-orange G. (× 500.)

Fig. 5. Similar region to above, 3 hr. p.c., showing very occasional PAS-positive cells (marked by arrows). FCC; PAS-orange G. (× 500.)

PLATE 2

Fig. 1. Cells of pars distalis proper 3-5 hr. p.c. Formal.—corros.—sublim. ('FHgCl₂'); AF-staining. Extensive degranulation of mucoid cells, with the exception of sparsely scattered, rather large, angular and deeply staining cells (arrows). Note that the Golgi areas of acidophils and chromophobes are stained with AF. AF-orange G. (× 500.)

Fig. 2. Approximately similar region to above, 5 hr. p.c., showing AF-positive cells (arrows). FHgCl₂; AF-orange G. (× 500.)

Fig. 3. Similar region from a control specimen to that shown above. Rather large angular and strongly AF-positive cells are present (arrows) in addition to the smaller typical mucoid cells of this zone. FHgCl₂; AF-orange G. (× 500.)

Fig. 4. Clumped PAS-positive cells of the zona tuberalis of a control gland. FCC; PAS-orange G. (× 500.)

Fig. 5. Similar region to above, 3 hr. p.c. [There is no evidence of degranulation. FCC; PAS-orange G. (× 500.)

Fig. 6. Control specimen. Pars distalis proper showing cells of pars intermedia origin (arrows) approximately 100 µ from junction with pars intermedia. FCC; AF. (× 500.)