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Quantitative studies on the effects of hormones on structure and porphyrin biosynthesis in the Harderian gland of the female golden hamster. 1. The effects of ovariectomy and androgen administration

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INTRODUCTION

The Harderian gland lies within the bony orbit of many vertebrates. Its functions remain unclear: it is usually thought of as a source of lubrication for the nictitating membrane (Cohn, 1955; Kennedy, 1970), but more recently it has been proposed as a link in the retinal–pineal–gonadal system (Wetterberg, Geller & Yuwiler, 1970; Reiter & Klein, 1971; Clabough & Norvell, 1973), as a source of pheromones (Payne, 1977, 1979; Thiessen, Clancy & Goodwin, 1976; Thiessen & Yahr, 1977) and as a site of immune response (Wight, Burns, Rothwell & Mackenzie, 1971; Mueller, Sato & Glick, 1971; Bang & Bang, 1968; Sundick, Albini & Wick, 1973; Albini, Wick, Rose & Orlans, 1974; Burns, 1979).

In rodents, the Harderian gland is used increasingly as a model of porphyrin biosynthesis, and that of the golden hamster is of particular value as it exhibits major sex differences. The female gland contains appreciably more porphyrin than the male (Christensen & Dam, 1953; Bucana & Nadakavukaren, 1972*a*; Payne, McGadey, Moore & Thompson, 1979) and this is normally laid down as solid intraluminal accretions. Moreover, many porphyrinogenic enzymes, including δ-aminolaevulinic acid synthetase (the rate-limiting enzyme for porphyrin production), are considerably more active in female than in male glands (Thompson *et al.* 1984). These biochemical sex differences in the hamster are accompanied by a number of structural differences (Bucana & Nadakavukaren, 1972*b*, 1973). Briefly, female glands possess only one cell type, whereas male glands possess two. All male cells contain a unique ultrastructural feature, the polytubular complexes. There is also a difference in interstitial mast cell numbers, with the female gland containing forty times more than the male (Payne *et al.* 1982).

In the male gland, there are well proven links between gonadal hormones and Harderian gland structure and activity. Thus, castration results in all male gland characteristics assuming a female pattern while androgen administration prevents these changes (Hoffman, 1971; Payne, McGadey, Moore & Thompson, 1977; Payne *et al.* 1982). In the female gland, such links are less well documented. It is known that porphyrin content and enzyme activity alter with the season (Moore, McGadey, Payne & Thompson, 1980) and over various reproductive states (Payne, McGadey, Moore, & Thompson, 1978), but the exact physiological basis of these observations

is unclear, nor have they been correlated with morphological changes. Again, androgen administration (Sun & Nadakavukaren, 1980) or exposure to extremely short periods of daylight (Nadakavukaren & Lin, 1983) may produce structural virilisation of the female gland, while ovariectomy may result in a series of degenerative changes in gland structure (Payne *et al.* 1982; Payne, McGadey & Johnston, 1985). However, these changes have not been quantified, nor has there been any attempt to correlate them with changes in porphyrin content or porphyrinogenic enzyme activity.

The present study was undertaken to quantify the effects of ovariectomy and/or androgen administration on the structure, porphyrin content and enzyme activity of the Harderian gland of the female hamster by comparing the glands of intact males, intact females, females which had been ovariectomised for five months and females which had been ovariectomised for five months but had received androgen administration during that period.

MATERIALS AND METHODS

The animals used in this study came from a closed colony established in 1968. The hamsters were (*a*) intact males, $n = 11$, (*b*) intact females, $n = 20$, (*c*) females bilaterally ovariectomised for five months, $n = 22$ and (*d*) females bilaterally ovariectomised for five months during which time they received androgen administration (4·5 mg testosterone propionate per week in three separate 1·5 mg doses), $n = 11$. All animals were one year old at death and were killed at the same time of year with an overdose of sodium pentobarbitone (Sagatal).

Both Harderian glands were removed and weighed, one gland being fixed in 3% buffered glutaraldehyde for light microscopy. Half of the other gland was placed in 5 ml of methanol (Analar) and refrigerated until required for the determination of the porphyrin content. Protoporphyrin constitutes 95% of the total porphyrin content, and all data were expressed as total porphyrin content (nmol/g tissue). The extraction and determination procedures (Rimington, 1971) have been described previously (Moore *et al.* 1980). The remaining half of the gland was homogenised (Polytron) immediately after killing the animal, sonicated in a small volume of ice cold saline, and the activity of δ-aminoaevulinic acid synthetase was measured by a modification of the method of Freshney & Paul (1970) as described by Thompson *et al.* (1984). Protein was measured by the method of Lowry, Rosebrough, Farr & Randall (1951) and the results were expressed as nmol δ-aminoaevulinic acid formed/g protein/hour.

Tissue for light microscopy was fixed in 3% buffered glutaraldehyde, pH 7·4, for 48 hours and was then dehydrated in an alcohol series and cleared in amyl acetate before being embedded in wax. Half of each gland was serially sectioned at a thickness of 5 μm and an interrupted series of 1 section in 10 was mounted. The sections

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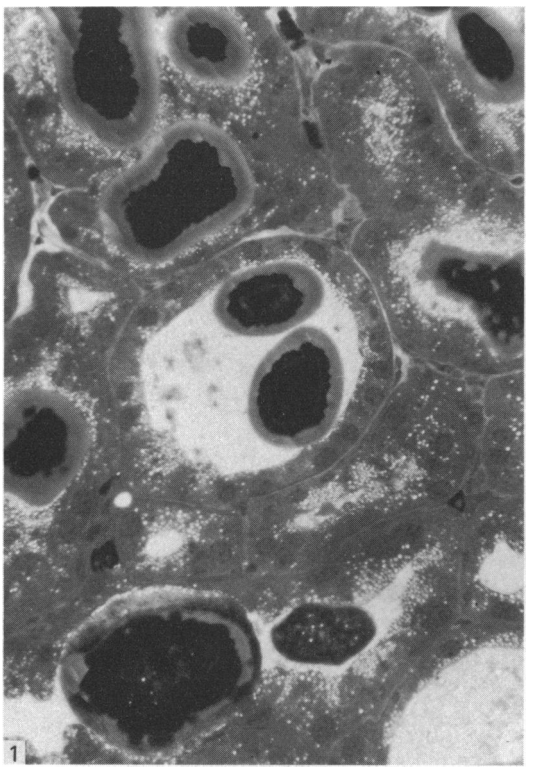
Fig. 1. An area of female hamster Harderian gland. Several tubule profiles can be seen containing solid intraluminal porphyrin accretions. × 300.

Fig. 2. An area of interstitial tissue from a female hamster Harderian gland showing a large interstitial porphyrin accretion (*P*) and several small interstitial porphyrin accretions (arrows) located within macrophages. × 575.

Fig. 3. An interstitial macrophage containing small crystalline porphyrin deposits (arrows) within its cytoplasm. × 9500.

Fig. 4. An area of macrophage cytoplasm showing porphyrin deposits ranging from dispersed needle-like crystals (*A*) to well-demarcated dense aggregates (*B*). × 11200.



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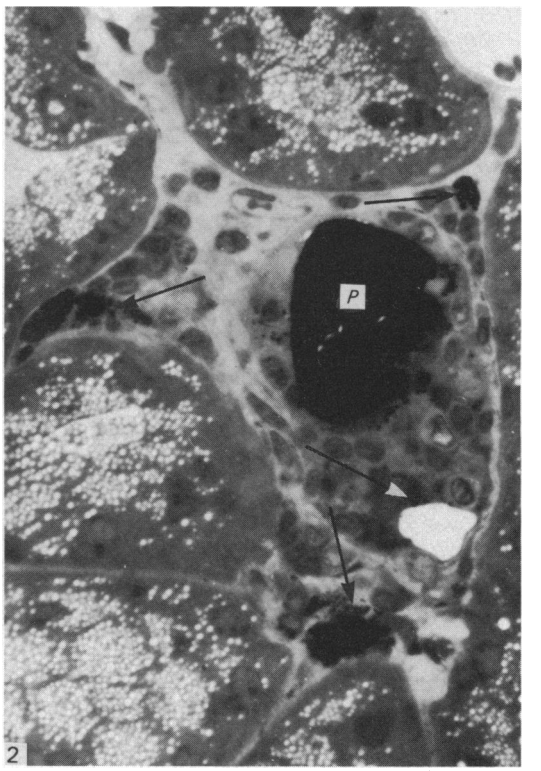

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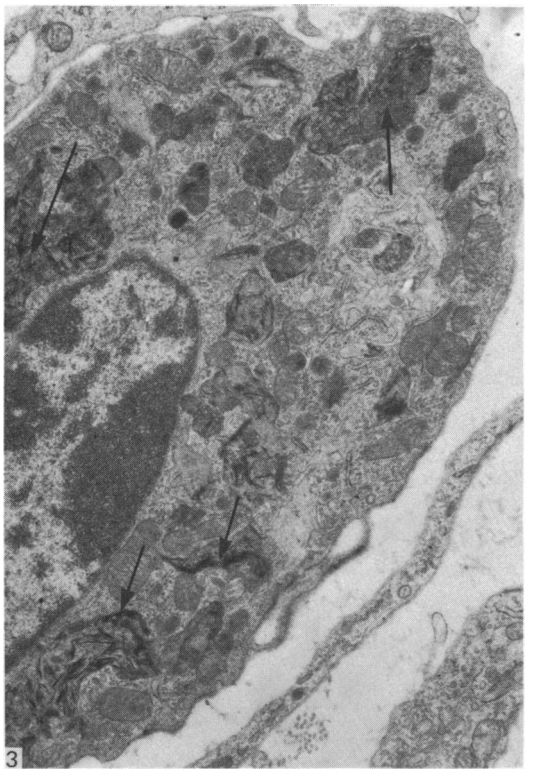

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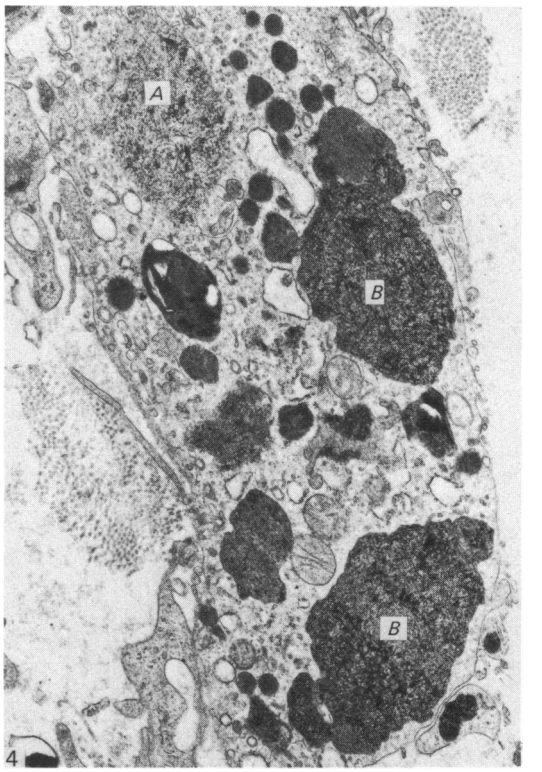

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were stained with haematoxylin and eosin, or 1% toluidine blue lightly counterstained with eosin.

Each tissue block provided 8–10 sections per stain. The following parameters were carried out for each animal:

(1) Mast cells were counted in each section.

(2) Intraluminal porphyrin accretions (Fig. 1) were counted in each section.

(3) Large interstitial porphyrin deposits (Fig. 2) were counted in each section.

(4) Small interstitial porphyrin deposits (such as occurred within free macrophages, Figs. 2–4) were counted in two sections (sections 4 and 8).

(5) The number of tubules containing intraluminal neutrophils (Figs. 5–6) were counted in two sections (sections 4 and 8).

The area and perimeter of each section were measured using a MOP-AMO_2 planimeter to give derived values in terms of frequency per mm^2 of section.

(6) The percentage area of tubule degeneration was determined for each animal by photographing two sections (sections 4 and 8) at low magnification and then outlining areas of degeneration on the photograph (printed to give a final magnification of approximately $\times 100$), while re-examining the sections at high magnification. The MOP-AMO_2 planimeter was then used to measure the total area of each section and the area of degenerating tubules within that section, yielding a percentage degeneration score for each animal. Degeneration was defined as an extreme reduction in epithelium height involving all or part of a tubule profile, usually accompanied by a marked reduction in visible nuclei (Figs. 7–8).

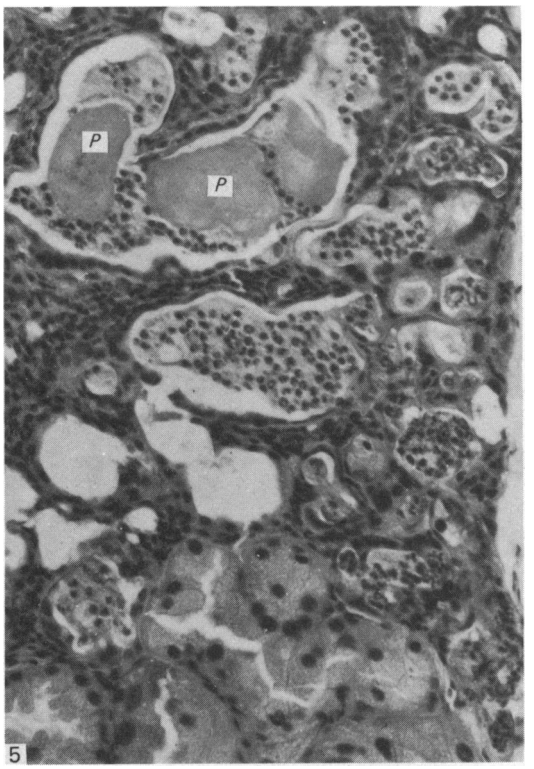
(7) Type II cells (Fig. 9) were counted in two sections (sections 4 and 8) out of the eight available, using the midline intersection of a graticule. Only those tubules cut transversely and showing a definite lumen were counted; the number of profiles containing Type II cells was expressed as a percentage of the total number of tubule profiles counted. Three tissue blocks from each group, chosen at random, were re-embedded for electron microscopy to determine whether polytubular complexes were present (Figs. 10–11). The residue of the block was dewaxed in xylene, cleared for several hours in propylene oxide and embedded in a series of propylene oxide/resin mixtures until finally being embedded in resin, in flat capsules with the cut face downwards. The block was then retrimmed and thin sections were cut. Fifty cells from each animal were examined with the electron microscope for the presence of polytubular complexes.

Fig. 5. An area of degenerating tubules from the Harderian gland of an ovariectomised female hamster showing massive neutrophil invasion. Some pale porphyrin accretions (*P*) are present. Normal tubules can be seen at the bottom of the micrograph. $\times 225$.

Fig. 6. Neutrophils in the lumen of a degenerating tubule. They possess filopodial extensions and their cytoplasm contains large vacuoles. $\times 6700$.

Fig. 7. An area of the Harderian gland of an ovariectomised female hamster showing normal tubules (on the right) and degenerating tubules (on the left) with highly attenuated epithelial walls. $\times 312$.

Fig. 8. This shows the walls of two adjacent tubules. That on the left is composed of normal epithelial cells with numerous apical lipid vacuoles. That on the right is degenerate and composed of highly attenuated epithelial cells; the tubule lumen (*L*) is filled with lipid vacuoles and cytoplasmic debris. $\times 2700$.



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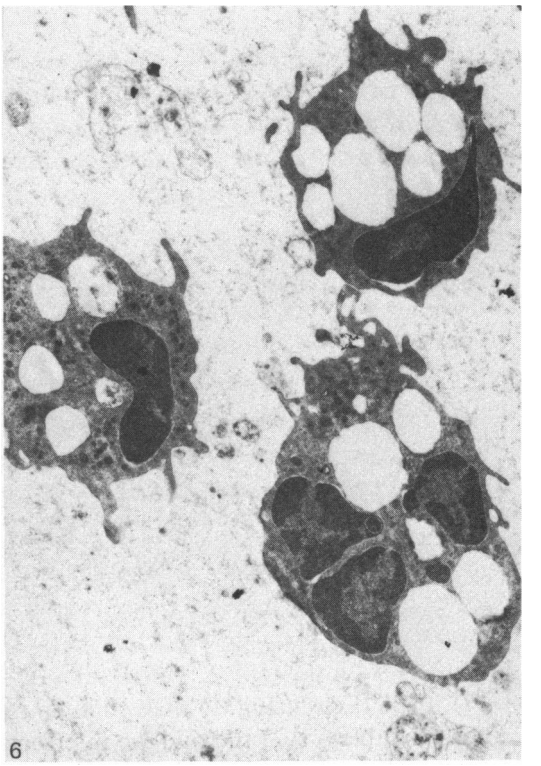
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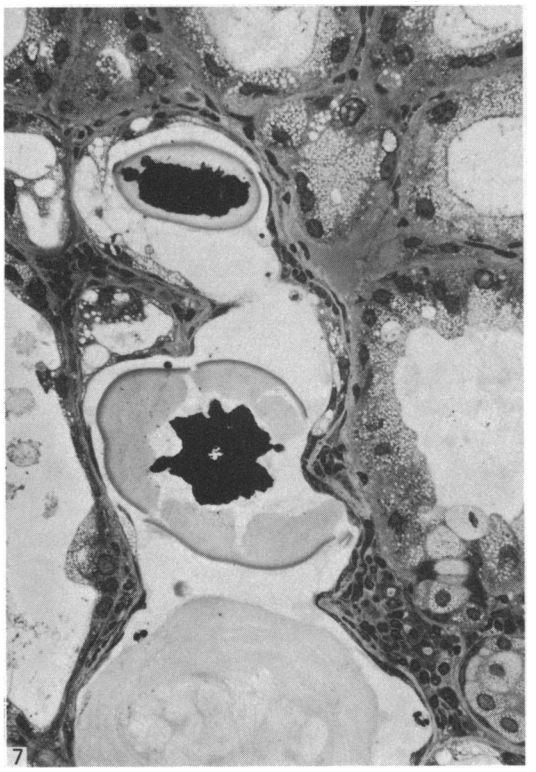
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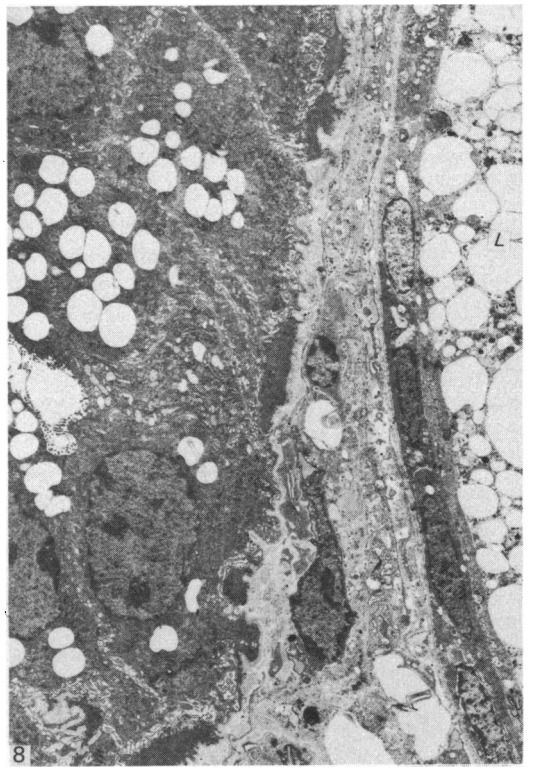

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Statistical analysis

For each parameter, the three groups of females were compared using one-way analyses of variance (F test). Where this proved significant, individual inter-group comparisons were made using Dunnett's test (Dunnett, 1964). Individual female groups were compared with male groups using Student's t test.

RESULTS

Two broad patterns of change were observed when the experimental groups were compared with control animals. These were a pattern of masculinisation changes occurring predominantly in the testosterone-injected ovariectomised females and a pattern of degenerative changes occurring predominantly in the ovariectomised females.

Masculinisation changes (Table 1)

Total porphyrin content of Harderian glands

Fluorometric assay revealed that the porphyrin content of the Harderian glands of ovariectomised females was similar to that of intact ones. Conversely, testosterone administration resulted in a highly significant reduction (80 %) in porphyrin content to about 700 nmol/g, although this was still significantly higher ($t = 8{\cdot}04$, $P < 0{\cdot}001$) than male values (approximately 40 nmol/g). These data were supported by the results of counting the number of solid intraluminal porphyrin accretions (Fig. 1) which were similar in control and ovariectomised females, but which were significantly reduced (60 %) in adrogen-treated females. Nevertheless, the low number found in androgen-treated females was still significantly higher than the zero level found in control males ($t = 6{\cdot}47$, $P < 0{\cdot}001$).

Δ-aminolaevulinic acid synthetase activity in Harderian glands

The activity of this rate-limiting enzyme was reduced significantly from control levels both in ovariectomised (by 65 %) and androgenised (by 92 %) females. The low level of enzyme activity in androgenised females did not differ significantly from the level of activity in male glands.

Type II cells

Tubule epithelial cells containing very large lipid vacuoles (Type II cells) were considered to be characteristic of male Harderian glands (Fig. 9). In the present study, 99 % of tubule profiles in male glands contained Type II cells while less than

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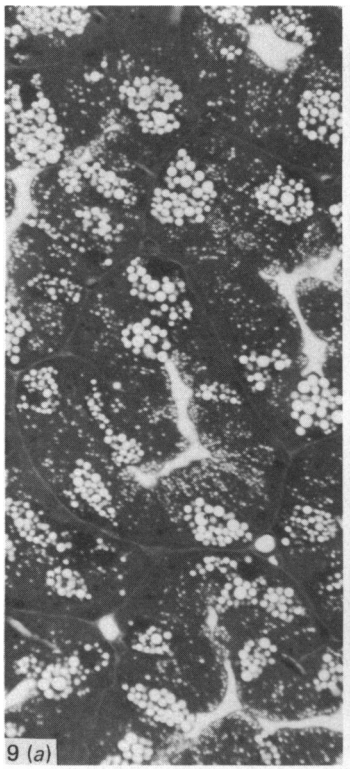
Fig. 9 (*a–c*). (*a*) An area of male hamster Harderian gland. The tubule walls are composed of epithelial cells with small lipid vacuoles (Type I) and cells with extremely large vacuoles (Type II). × 312. (*b*) An area of female hamster Harderian gland. The tubule walls are composed of one cell type only. × 400. (*c*) An area of Harderian gland from an androgen-treated female hamster. Type II cells with large lipid vacuoles are clearly present. × 312.

Fig. 10. An area of apical cytoplasm from an epithelial cell of the Harderian gland of an androgen-treated female hamster. Several polytubular complexes (arrows), characteristic of male gland cells, can be seen. An intraluminal porphyrin accretion (*P*) is present. × 27000.

Fig. 11. Polytubular complexes from the Harderian gland of an androgen-treated female hamster. × 80000.



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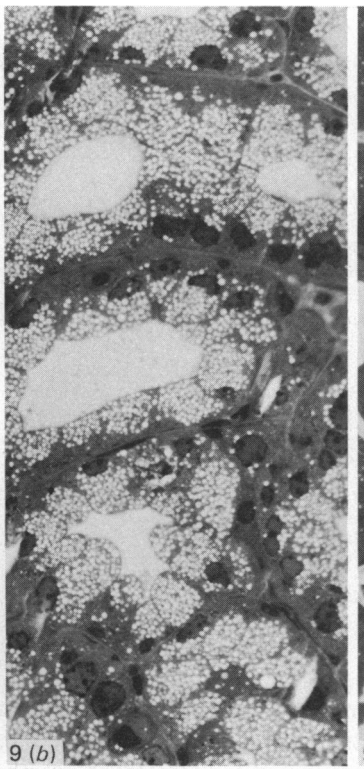

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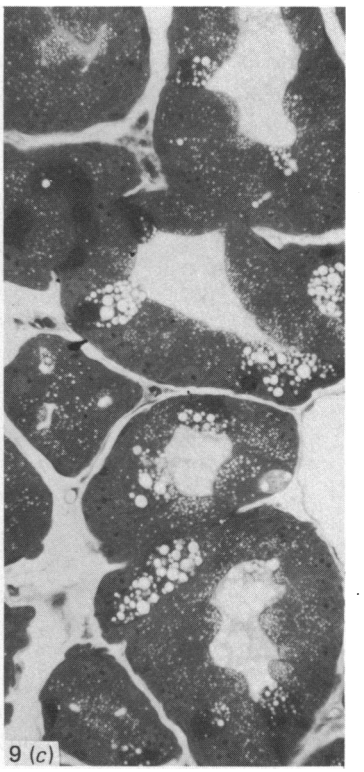

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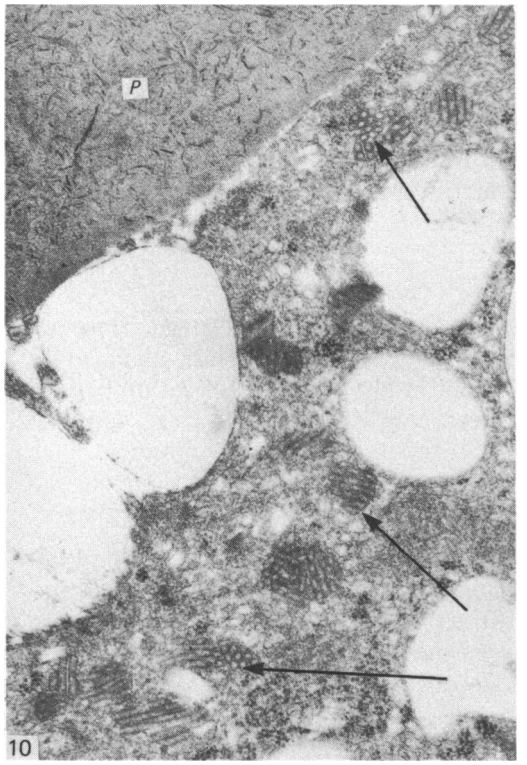

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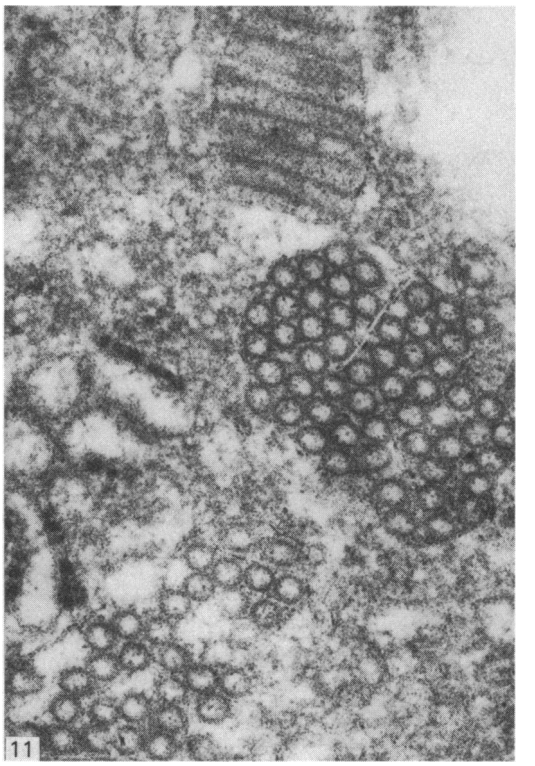

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Table 1. *The porphyrin content and porphyrinogenic enzyme activity of the hamster Harderian gland, together with a number of morphological parameters usually associated with the male gland, in intact female hamsters* (n = 20), *ovariectomised females* (n = 22), *androgen-treated females* (4·5 *mg testosterone/week*, n = 11) *and intact males* (n = 11)

This Table shows (i) the frequency of intraluminal porphyrin accretions/mm² in a 5 μm section; (ii) the total porphyrin content (nmol/g tissue); (iii) the activity of the porphyrinogenic enzyme δ-aminolaevulinic acid synthetase (ALA-S; nmol ALA formed/g protein/hour); (iv) the % tubules containing Type II cells; (v) the % animals (n = 3 per group) and the % cells (50 per animal) containing polytubular complexes. All values are means ± S.E.M.

	Intact females (n = 20)	Ovariectomised females (n = 22)	Androgen-treated females (n = 11)	Analysis of variance (F)	Intact males (n = 11)
Intraluminal porphyrin accretions/mm² section	8·49 ± 0·92	8·60 ± 1·31	3·52 ± 0·57**	5·01, $P < 0·05$	—
Total porphyrin content	3300 ± 302	2947 × 236	706 ± 82**	18·63, $P < 0·001$	43 ± 4
ALA-S activity	5176 ± 1492	1826 ± 442*	430 ± 112**	9·62, $P < 0·001$	169 ± 20
% Tubules with Type II cells	0·6 ± 0·3	8·7 ± 1·1**	38·0 ± 5·1**	80·21, $P < 0·001$	98·8 ± 1·0
	(n = 3)	(n = 3)	(n = 3)	—	(n = 3)
Polytubular complexes present					
(i) % animals	—	—	100	—	100
(ii) % cells	—	—	93 ± 2	—	100

Differs significantly from intact (control) females; * $P < 0·05$, ** $P < 0·01$.

1 % of tubule profiles in female glands did so. Compared with intact control females, the frequency of Type II cells was significantly higher in both ovariectomised (occurring in 8·7 % of tubules) and androgen-treated (38 %) female glands, although the latter still differed significantly from male values (t = 12·19, $P < 0·001$).

Polytubular complexes

There was further ultrastructural evidence of gland masculinisation, since all androgen-treated females examined possessed polytubular complexes identical to those in male cells (Figs. 10, 11). They occurred in 93 % of the cells of androgen-treated females, and in 100 % of the cells of intact control males. They were not seen in either control or ovariectomised females.

Degenerative changes (*Table 2*)

Normal female hamster Harderian gland tubules consisted of a single layer of tall columnar epithelial cells, which contained small lipid vacuoles, and surrounded a lumen which might contain solid porphyrin accretions. The epithelial cells were supported by myoepithelial cells located within the basal lamina.

In ovariectomised females, a number of degenerative events (Payne *et al.* 1982; 1985) may form a sequence. Firstly, the epithelium becomes attenuated and the enlarged lumen is invaded by neutrophils. As the tubules degenerate further, large

Table 2. *A number of morphological parameters usually associated with gland degeneration in the Harderian glands of intact female hamsters* (n = 20), *ovariectomised females* (n = 22), *androgen-treated females* (*4·5 mg testosterone/week*, (n = 11) *and intact males* (n = 11)

This Table shows (i) the percentage area of tubule degeneration in 5 μm sections; (ii) the frequency of tubules containing luminal neutrophils/mm^2 in a 5 μm section; the frequencies of (iii) large and (iv) small (or phagocytosed) interstitial porphyrin accretions/mm^2 in a 5 μm section; (v) the frequency of interstitial mast cells/mm^2 in a 5 μm section; (vi) the frequency of mast cells/mm of gland capsule. All values are means ± S.E.M.

	Intact females (n = 20)	Ovariectomised females (n = 22)	Androgen-treated females (n = 11)	Analysis of variance (F)	Intact males (n = 11)
% Area of tubule degeneration	3·57 ± 0·77	5·43 ± 0·91	4·51 ± 0·92	1·36 n.s.	—
Tubules with luminal neutrophils/mm^2 section	1·36 ± 0·24	2·30 ± 0·35*	0·76 ± 0·13	6·35 < 0·01	—
Large interstitial porphyrin accretions/mm^2 section	0·10 ± 0·03	0·42 ± 0·09	0·82 ± 0·32**	6·87 < 0·01	—
Small interstitial porphyrin accretions/mm^2 section	1·78 ± 0·43	6·93 ± 1·8**	4·67 ± 1·03	8·86 < 0·001	—
Mast cells/mm^2 section	6·71 ± 1·51	25·73 ± 4·30**	11·19 ± 2·75	10·41 < 0·001	0·31 ± 0·14
Mast cells/mm capsule	0·73 ± 0·12	1·82 ± 0·27**	0·49 ± 0·12	15·98 < 0·001	0·06 ± 0·01

Differs significantly from intact (control) females; *P < 0·05, **P < 0·01.

intraluminal porphyrin accretions are found within the interstitium where they often become surrounded by macrophages, including foreign body giant cells. These large interstitial accretions would appear to be broken down into progressively smaller deposits found within individual macrophages.

Tubule degeneration (Table 2; Figs. 6, 7)

This was defined as tubule profiles whose epithelium was (wholly or in part) highly attenuated; these tubules were also often dilated but this feature was not used as a criterion. The area of tubule degeneration was similar in all three groups of females (Table 2), which presumably reflected current change in the gland epithelium. Other features such as invasion by neutrophils (which may precede degeneration) or interstitial porphyrin deposits (which may be the result of previous degeneration) showed considerable changes in females with experimentally changed hormone levels compared with controls.

Tubules containing neutrophils (Table 2; Figs. 4, 5)

The number of tubules containing neutrophils was raised significantly in ovariectomised (but not androgenised) females compared with controls. Male glands did not exhibit this tubule invasion.

Interstitial porphyrin accretions (Table 2; Figs. 2–4)

The total porphyrin content and the number of visible intraluminal accretions were similar in intact and ovariectomised females, but both were greatly reduced in androgen-treated females (Table 1). However, compared with intact control females, the number of large interstitial accretions was raised in ovariectomised and androgen-treated females, significantly so in the latter group ($t = 3{\cdot}60$, $P < 0{\cdot}01$). Similarly, the number of small, phagocytosed, interstitial porphyrin accretions was raised in ovariectomised and androgen-treated females, significantly so in the former group ($t = 4{\cdot}06$, $P < 0{\cdot}01$). Male glands possessed neither intraluminal nor interstitial porphyrin stores.

Mast cells (Table 2)

As previously reported (Payne *et al.* 1982) there was a large sex difference in mast cell numbers in the hamster Harderian gland, both in the interstitial tissue ($t = 3{\cdot}29$, $P < 0{\cdot}01$) and in the connective tissue capsule ($t = 4{\cdot}58$, $P < 0{\cdot}001$), with females possessing significantly higher numbers than males. Ovariectomy resulted in a further rise in mast cell numbers, both in the interstitium ($t = 4{\cdot}26$, $P < 0{\cdot}01$) and capsule ($t = 4{\cdot}60$, $P < 0{\cdot}01$). Mast cell numbers in androgen-treated females were intermediate between intact and ovariectomised females, differing significantly from the latter (interstitium, $t = 2{\cdot}82$, $P < 0{\cdot}05$; capsule, $t = 4{\cdot}79$, $P < 0{\cdot}01$).

DISCUSSION

This quantitative assessment of the effects of ovariectomy, alone, or coupled with androgen administration, on the structure, porphyrin content and porphyrinogenic enzyme activity of the Harderian gland of the female golden hamster confirms the link between gonadal hormones and gland structure and activity. The results indicate that, in this species, ovarian hormones are necessary to maintain the structure and activity of the normal female Harderian gland and that androgen administration results in the masculinisation of gland characteristics.

Ovariectomy alone results in a number of apparently degenerative changes in gland structure and a reduction in the activity of δ-aminolaevulinate synthetase, the rate-limiting enzyme for porphyrin production. As the epithelium of the tubules becomes attenuated, the lumen is invaded by neutrophils which may be phagocytosing necrotic epithelial debris in general (Vethamanay, Vethamanay & Bessis, 1975) or lipid droplets to which neutrophils may be specifically attracted (Tainer, Turner & Lynn, 1975) and which are produced in quantity by the epithelial cells (Lin & Nadakavukaren, 1981). Strum & Shear (1982) reported leucocytes phagocytosing lipid droplets produced by epithelial cells of mouse Harderian gland. Following presumably complete degeneration of tubules, porphyrin accretions are found within the interstitial tissue. Interstitial porphyrins occasionally occur in normal female hamsters and have also been reported in the rat (Grafflin, 1942) and the Australian murid *Pseudomys australis* (Johnston, McGadey, Payne & Breed, 1983). These large interstitial deposits are often surrounded by macrophages (Payne *et al.* 1985). While these macrophages may be individual cells held together by interdigitations, more frequently they have become fused forming a foreign body giant cell capable of sequestering particulate matter (Sutton & Weiss, 1966). Small interstitial deposits occur within individual macrophages where they are seen as characteristic needle-like

porphyrin crystals, either densely compacted or loosely arrayed. These may represent stages in the digestion of porphyrin, as may pale areas which have been described within the cytoplasm of these macrophages (Payne *et al.* 1985) and which may represent solubilised porphyrin. Blood porphyrin levels are elevated in ovariectomised females. This may be a general systemic effect of ovariectomy, or it may be that stores of interstitial Harderian porphyrin enter interstitial blood vessels, lymphatics or the retro-orbital venous sinus which surrounds the gland (Sakai & Yohro, 1981), but there is no direct evidence that this occurs, nor of the mechanism by which it might do so. As well as these degenerative changes involving gland tubules and their porphyrin stores, there are also marked changes in mast cell numbers. The present paper confirms that the Harderian glands of female hamsters contain substantially higher numbers of mast cells than do the glands of males, both in the interstitium and in the connective tissue capsule of the gland (Payne *et al.* 1982). It is also clear in the present experiment that mast cell numbers are further increased in ovariectomised females compared with intact controls. The reasons for this sex difference and the hormonal control of mast cell numbers are unclear. It may be that mast cell numbers increase in response to interstitial porphyrin since these cells frequently occur near such deposits; furthermore, androgen-treated females, which also exhibit increased interstitial porphyrin deposits, also show raised numbers of mast cells.

Ovariectomy alone does not result in masculinisation of the female hamster Harderian gland, but when it is accompanied by androgen administration the gland soon assumes male characteristics. The activity of δ-aminolaevulinic acid synthetase falls to levels not significantly different from those of male glands, the total porphyrin content of the glands (both in terms of biochemical assay and the number of visible porphyrin accretions) is greatly reduced, and there is an increase in the number of interstitial deposits of porphyrin, particularly in the number of large deposits. It is of interest that in ovariectomised females the significant increase is in the number of small, phagocytosed, interstitial porphyrin deposits, suggesting that degeneration proceeds further in ovariectomised than in androgen-treated females where the process stops with the sequestration of large interstitial porphyrin deposits. Again, it is of interest that androgen-treated females do not display elevated blood porphyrin levels (R. C. Spike, unpublished data). The marked increase in Type II cells and the appearance of polytubular complexes are further evidence of the masculinising effects of androgens on the female hamster Harderian gland. It is well established that castration of male hamsters results in the gland assuming female characteristics of porphyrin production and structure, a change which can be prevented by androgen administration (Hoffman 1971; Payne *et al.* 1977). The way in which androgens appear to alter gland structure and porphyrinogenic capacity simultaneously has led authors to suggest functional links between the two. Thus, Bucana & Nadakavukaren (1973) suggested that polytubular complexes in the male gland may break down or prevent the formation of solid intraluminal porphyrin stores, while Jones & Hoffman (1976) suggested that polytubular complexes may prevent porphyrin biosynthesis by inhibiting the condensation of δ-aminolaevulinic acid to porphobilinogen. There is no direct evidence for this at present.

While it seems clear from the present study that ovariectomy influences the female hamster Harderian gland, it is not established which particular consequence of ovariectomy is important. Some authors have suggested that gonadotrophins, particularly luteinising hormone, act directly on the gland, so that both castration

and ovariectomy affect the gland as a consequence of elevated gonadotrophin levels (Hoffman, 1971; Clabough & Norvell, 1973). This seems unlikely, since while castration leads to markedly raised porphyrin levels (Payne *et al.* 1977) and porphyrinogenic enzyme activities (G. G. Thompson, unpublished data) in male glands, the present study indicates that ovariectomy results in decreased enzyme activity and no rise in porphyrin content. Furthermore, the findings that Harderian glands possess receptors for testosterone (Gustafsson & Pousette, 1975) and oestradiol (Weaker, Villareal & Sheridan, 1983) would suggest that gonadal hormones themselves are the more likely controllers of gland structure and activity. This, in turn, may be consistent with the observed effects of ovarian steroids which enhance porphyrin biosynthesis in rats and which are raised in clinical cases of acute porphyria (Moore, Paxton, Beattie & Goldberg, 1973; Paxton, Moore, Beattie & Goldberg, 1974).

SUMMARY

The ovary is necessary to maintain the morphology and porphyrin biosynthesis of the female hamster Harderian gland. Ovariectomy results in a series of degenerative changes which may form a sequence. Tubule walls become attenuated and the lumen invaded by neutrophils. The subsequent elimination of the tubule walls results in previously intraluminal porphyrin stores coming to lie within the interstitium where they may be surrounded by macrophages, often forming foreign body giant cells; individual macrophages containing small porphyrin stores are also numerous within the interstitium. Mast cell numbers rise in the Harderian glands of ovariectomised females. When ovariectomy is accompanied by androgen administration, virilisation of the gland occurs, with decreased porphyrin content and porphyrinogenic enzyme activity and the appearance of male structural features such as Type II cells and polytubular complexes.

The role of the testis in regulating the structure and porphyrin biosynthesis of the male hamster Harderian gland is well known. This present (quantitative) investigation suggests a similar role for the ovary in the female.

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