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The effects of age on the structure and porphyrin synthesis of the Harderian gland of the female golden hamster

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INTRODUCTION

The Harderian gland is a large orbital structure which occurs in most terrestrial vertebrates (Walls, 1942; Sakai, 1981). It is well developed in rodents where, in addition to producing a lipid secretion, the gland synthesises porphyrins which are stored as solid luminal accretions (Grafflin, 1942; Christensen & Dam, 1953; Johnston *et al.* 1983). The rodent Harderian gland is, therefore, used as a model of porphyrin biosynthesis and the most interesting species is the golden hamster in which the gland exhibits morphological sex differences in cell types (Woolley & Worley, 1954), in ultrastructural features (Bucana & Nadakavukaren, 1972; Payne *et al.* 1978) and in mast cell numbers (Payne *et al.* 1982). Furthermore, the female gland contains over a hundred times more porphyrin than the male and exhibits significantly greater activity of 5-aminolaevulinic acid synthase (ALA-S) (the rate-limiting enzyme of haem biosynthesis) (Lin & Nadakavukaren, 1982; Thompson *et al.* 1984).

In the hamster, it is well known that androgens suppress porphyrin biosynthesis and maintain a morphologically male gland (Hoffman, 1971; Payne, McGadey, Moore & Thompson, 1977; Sun & Nadakavukaren, 1980). It is also likely that ovarian hormones control the female gland since ovariectomy leads to degenerative changes in gland morphology and a reduction in porphyrin synthesis (Payne *et al.* 1982; Payne, McGadey & Johnston, 1985; Spike *et al.* 1985, 1986). It is surprising, therefore, since these dimorphisms are only pronounced after puberty, that no study has systematically examined changes in gland structure and activity in adult females over an age range that includes post-reproductive senescence (Bucana & Nadakavukaren, 1972, 1973; Payne *et al.* 1978). The present study examines the morphology of the Harderian gland, its porphyrin content and enzyme activity in adult female hamsters aged between 2 and 24 months.

MATERIALS AND METHODS

The animals used in this study came from a closed colony established in Glasgow University Anatomy Department in 1968. The female hamsters consisted of 5 groups of the following ages (*a*) 2 months ($n = 10$), (*b*) 6 months ($n = 10$), (*c*) 12 months ($n = 10$), (*d*) 18 months ($n = 10$) and (*e*) 24 months ($n = 9$). A long-day regime of 15 light:9 dark was used to minimise the effects of seasonal variation and animals were killed randomly throughout the year as the age groups became available.

The animals were killed with an overdose of sodium pentobarbitone (Sagatal). Blood was withdrawn by cardiac puncture using a heparinised syringe and blood porphyrin levels were subsequently determined using a Buchler Hemafluor TmZp

haematofluorimeter. Results were expressed as nmol/l. The liver was removed and half of each lobe assayed to determine the 5-ALA-S activity. Both Harderian glands were excised: one gland was halved longitudinally one half being assayed for its 5-ALA-S activity (Thompson *et al.* 1984). Protein concentrations of the liver and the gland were measured by the method of Lowry, Rosebrough, Farr & Randall (1951) and the results were expressed as nmol of 5-aminolaevulinic acid formed/g protein/h. The remaining half-gland was placed in 5 ml methanol (Analar) for porphyrin extraction and determination using the fluorimetric method of Rimington (1971). These methods have been described in detail in previous publications (Moore, Thompson, Payne & McGadey, 1980).

The other gland was placed in 2·5 % buffered glutaraldehyde (pH 7·4) and processed for light microscopy using amyl acetate as a clearing agent. Half of each gland was serially sectioned at 5 μm thickness and an interrupted series of 1 adjacent pair of sections in 10 was mounted. One section in each pair was stained with haematoxylin and eosin, the other with 1 % toluidine blue lightly counterstained with eosin. Each tissue block provided 8 sections per stain. The parameters measured here have been described and illustrated previously (Payne *et al.* 1982, 1985; Spike *et al.* 1985, 1986) and are shown diagrammatically in Figure 1. Briefly, they include:

(1) *Tubule degeneration*

Tubule degeneration is defined as the extreme reduction in epithelial height of all or part of a tubule profile, usually accompanied by a reduction in visible nuclei and frequently by tubule dilatation. The percentage area of degeneration was determined for each animal by photographing two sections (4 and 8) at low power and outlining the areas of degeneration on a photograph (printed to give a final magnification of approximately × 100) while re-examining the section at high power. A MOP-AMO$_2$ planimeter was used to measure the total area of the section and the area of tubule degeneration within that section, thus yielding a percentage degeneration score for each animal.

(2) *Neutrophils*

The number of tubules containing luminal neutrophils were counted in two sections (4 and 8).

(3) *Mitotic figures*

The number of epithelial cells showing mitotic figures was counted in one section (8). This method was used in preference to a conventional mitotic index because of the small numbers of figures observed.

(4) *Mast cells*

The number of mast cells was counted in each section. Mast cells were classified as interstitial (within the connective tissue of the gland) or peripheral (within the connective tissue capsule of the gland).

(5) *Porphyrin accretions*

Porphyrin accretions were counted in two sections (4 and 8) and classified as belonging to one of three groups:

(*a*) *Intraluminal.* Within the lumen of the tubule – the normal location for porphyrin stores.

(*b*) *Large interstitial.* Intraluminal accretions whose tubule walls have disappeared. These are often surrounded by foreign body giant cells.

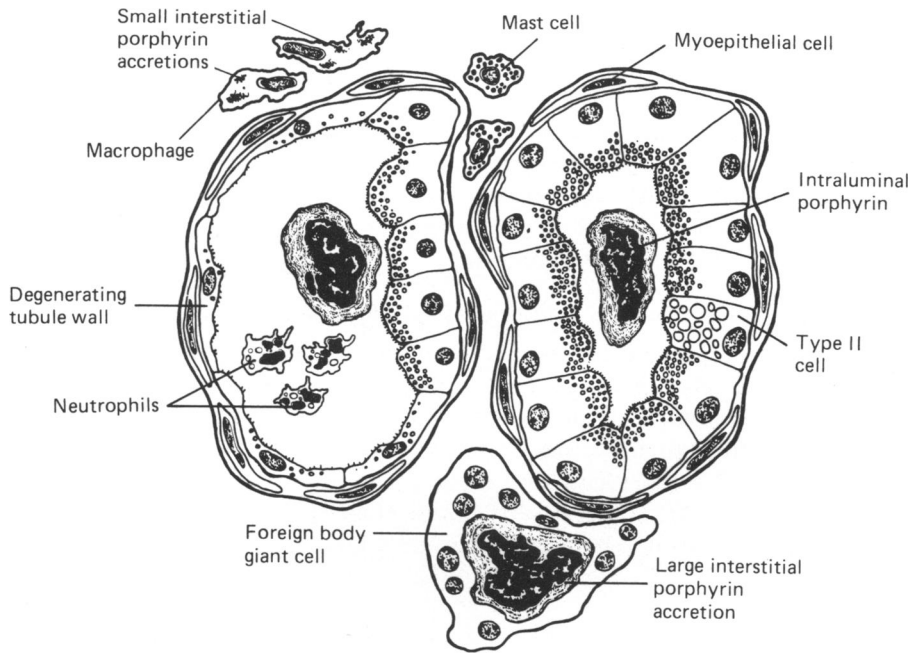

Fig. 1. A diagram based on transmission electron micrographs (Payne *et al.* 1985; Spike *et al.* 1985, 1986) showing degenerative changes which occur in the female hamster Harderian gland. For details, see Materials and Methods section.

(*c*) *Small interstitial.* Small deposits within individual free macrophages in the interstitial connective tissue of the gland.

As in (1), the areas and perimeters of sections were obtained using a MOP-AMO$_2$ planimeter to give derived values in terms of frequency per mm^2 of section (or, in the case of peripheral mast cells, frequency per mm of capsule).

(6) *Type II cells*

Tubule epithelial cells containing very large lipid vacuoles (Type II cells) are considered to be characteristic of male Harderian glands. This cell type was counted in 2 sections (4 and 8) using the midline intersection of a graticule. Only those tubules cut in transverse section 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.

Additional measures

The body weight, together with the weight of the uterus, was recorded for each animal at death. Females were observed for cyclicity for 2 weeks prior to death by looking for (*a*) the characteristic post-oestrous vaginal discharge (Orsini, 1961) and (*b*) sexual receptivity with a stud male. The presence or absence of mature ovarian follicles was noted at death. All cycling females were killed at oestrus.

Statistical analyses

Analysis of variance (*F*) was used to compare the various parameters measured in each group: Where this was significant, intergroup comparisons were made using Dunnett's test (1964).

RESULTS

Biochemical changes

(1) *Blood porphyrin levels* (nmol/l) (Fig. 2*a*)

There was a significant variance between the five age groups ($F = 4{\cdot}01$, $P < 0{\cdot}01$) with the levels at 18 months being significantly raised compared with levels at 2 months ($t = 4{\cdot}18$, $P < 0{\cdot}01$), 6 months ($t = 2{\cdot}53$, $P < 0{\cdot}05$) and 12 months ($t = 2{\cdot}67$, $P < 0{\cdot}05$). At 24 months, blood prophyrin levels were also raised compared with these three groups, but this was not significant.

(2) *Harderian gland porphyrin* (nmol/g) (Fig. 2*b*)

There was a highly significant variance between the different age groups ($F = 16{\cdot}07$, $P < 0{\cdot}001$), with levels being highest at 2 months and gradually decreasing thereafter. Values at 2 months were almost double those of the 12 months females ($t = 3{\cdot}43$, $P < 0{\cdot}01$) and more than three times those of the 18 months ($t = 6{\cdot}08$, $P < 0{\cdot}01$) and 24 months ($t = 6{\cdot}15$, $P < 0{\cdot}01$) females. Values at 6 months were also three times greater than in the 18 months ($t = 5{\cdot}12$, $P < 0{\cdot}01$) and 24 months ($t = 5{\cdot}26$, $P < 0{\cdot}01$) females, and these two latter groups both contained only half the value of the twelve months females ($t = 2{\cdot}60$, $P < 0{\cdot}05$ and $t = 2{\cdot}80$, $P < 0{\cdot}05$ respectively).

(3) *Harderian gland 5-aminolaevulinate synthase (ALA-S) activity* (nmol ALA formed/g protein/h) (Fig. 2*c*)

There was a significant variance between the five groups ($F = 4{\cdot}6$, $P < 0{\cdot}01$) with levels being lowest at 2 months, peaking at 6 months and decreasing gradually thereafter. Compared to the 6 months values, the 2 months ($t = 3{\cdot}83$, $P < 0{\cdot}01$), 18 months ($t = 3{\cdot}32$, $P < 0{\cdot}01$) and 24 months ($t = 2{\cdot}77$, $P < 0{\cdot}05$) levels were significantly reduced.

(4) *Liver 5-aminolaevulinate synthase (ALA-S) activity* (nmol ALA formed/g protein/h) (Fig. 2*d*)

There was no significant variance in liver enzyme activities between the various age groups.

Morphological changes (Table 1)

(*a*) *Interstitial mast cells*

There was significant variance in the number of interstitial mast cells/mm^2 gland section between the five age groups ($F = 4{\cdot}07$, $P < 0{\cdot}01$). The 2 and 6 months females had the highest numbers and these were significantly greater than the 18 months ($t = 2{\cdot}97$ and $2{\cdot}62$ respectively, $P < 0{\cdot}05$) and 24 months ($t = 3{\cdot}01$ and $2{\cdot}66$ respectively $P < 0{\cdot}05$) groups.

(*b*) *Peripheral mast cells*

Peripheral mast cells/mm capsule also showed significant variance ($F = 3{\cdot}86$, $P < 0{\cdot}01$) with the 6 months females having the greatest number, significantly higher than the 18 months ($t = 3{\cdot}41$, $P < 0{\cdot}01$), and 24 months ($t = 3{\cdot}27$, $P < 0{\cdot}01$) groups.

(*c*) *Intraluminal porphyrin accretions*

There was a highly significant difference between the age groups in the number of intraluminal deposits/mm^2 section ($F = 10{\cdot}43$, $P < 0{\cdot}001$). The greatest number occurred in the 2 months group and this was significantly greater than the 12 months

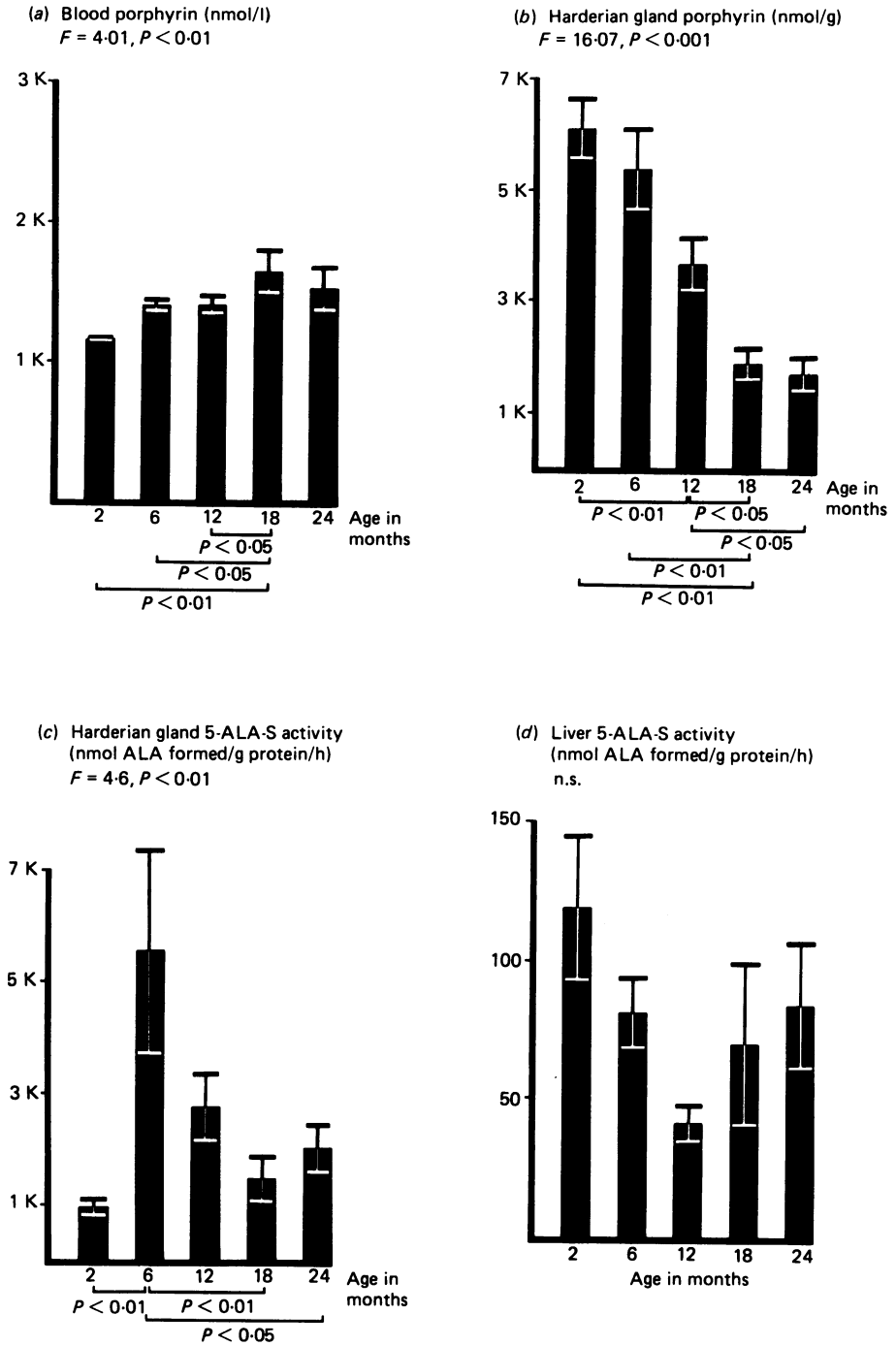

Fig. 2. (*a–d*). Changes with age in the female hamster of the porphyrin content of blood (*a*) and Harderian gland (*b*) together with changes in the activity of the rate-limiting enzyme 5-aminolaevulinate synthase (5-ALA-S) in the Harderian gland (*c*) and liver (*d*). All histograms show means ± S.E.M.

Table 1. *Morphological changes occurring in the Harderian gland of the female golden hamster from 2 to 24 months. All figures are means* ± S.E.M. *Intergroup comparisons and their significance levels are given in the text.*

	Age in months					
	2 ($n = 10$)	6 ($n = 10$)	12 ($n = 10$)	18 ($n = 10$)	24 ($n = 9$)	Analyses of of variance (F)
Interstitial mast cells/mm²	18·30 ± 2·73	18·5 ± 5·8	13·72 ± 5·38	3·22 ± 0·85	5·01 ± 1·29	4·07 $P < 0·01$
Peripheral mast cells/mm	1·00 ± 0·18	1·77 ± 0·66	0·66 ± 0·22	0·27 ± 0·05	0·33 ± 0·09	3.86 $P < 0·01$
Tubules containing neutrophils/mm²	0	0·07 ± 0·04	0·58 ± 0·19	0·37 ± 0·08	1·71 ± 0·40	12·19 $P < 0·001$
Intraluminal porphyrin accretions/mm²	28·58 ± 1·70	22·7 ± 5·7	16·94 ± 1·65	8·54 ± 1·43	8·37 ± 1·02	10·43 $P < 0·001$
Large interstitial porphyrin accretions/mm²	0·03 ± 0·02	0·04 ± 0·02	0·12 ± 0·05	0·10 ± 0·04	0·54 ± 0·16	7·84 $P < 0·001$
Small interstititial porphyrin accretions/mm²	0·01 ± 0·01	0·13 ± 0·09	1·04 ± 0·32	0·88 ± 0·18	4·30 ± 1·29	8·96 $P < 0·001$
Mitotic figures/mm²	0·48 ± 0·11	0·26 ± 0·08	0·42 ± 0·14	0·14 ± 0·05	0·33 ± 0·24	0·86 n.s.
% Area of tubule degeneration	1·03 ± 0·38	1·33 ± 0·39	1·63 ± 0·46	4·13 ± 1·33	5·33 ± 1·25	5·18 $P < 0·01$
% Tubules with Type II cells	0·18 ± 0·20	1·62 ± 0·76	0·32 ± 0·23	16·38 ± 2·38	18·16 ± 3·65	22·59 $P < 0·001$

($t = 2·52$, $P < 0·05$), 18 months ($t = 5·23$, $P < 0·01$) and 24 months ($t = 5·28$, $P < 0·01$) groups. Six months females also differed significantly from 18 months ($t = 3·69$, $P < 0·01$) and 24 months ($t = 3·74$, $P < 0·01$) females. The 12 months females had a value less than half that of the 2 months females, and this was also significantly higher than females at 18 months ($t = 2·71$, $P < 0·05$) and 24 months ($t = 2·75$, $P < 0·05$). This pattern resembles age changes in porphyrin concentrations as determined by assay (see above). In a previous study (Payne *et al.* 1982) major sex differences were observed in mast cell numbers and porphyrin content (both being higher in females), but there was no obvious quantitative relationship between these two parameters within the female group. In this experiment where porphyrin accretions and mast cell numbers appeared to co-vary it was decided to determine whether there was a significant correlation. Individual paired counts of mast cell and porphyrin accretions numbers were plotted and the correlation coefficient determined using basic statistics for two variables. A t test was then performed on this data. A correlation coefficient of $r = 0·43$ ($t = 3·25$, $P < 0·01$) was obtained. Assuming a linear relationship, the data fit a line $y = -0·28 + 0·63x$.

(d) Large interstitial porphyrin deposits

The number of large interstitial porphyrin deposits/mm² section increases with age and this variance is significant ($F = 7·84$, $P < 0·01$). The number of large interstitial deposits increased fivefold between 18 and 24 months and 24 months values are significantly greater than the 2 months ($t = 4·63$, $P < 0·01$), 6 months ($t = 4·54$, $P < 0·01$), 12 months ($t = 3·87$, $P < 0·01$) and 18 months ($t = 4·00$, $P < 0·01$) groups.

(*e*) *Small interstitial porphyrin deposits*

The age distribution pattern for the number of small interstitial porphyrin deposits/mm² section parallels that of the large interstitial deposits, and the variance is also significant ($F = 8{\cdot}96$, $P < 0{\cdot}01$). The 24 months females have significantly higher numbers of small interstitial deposits than the 2 months ($t = 4{\cdot}95$, $P < 0{\cdot}01$), 6 months ($t = 4{\cdot}94$, $P < 0{\cdot}01$), 12 months ($t = 3{\cdot}88$, $P < 0{\cdot}01$) and 18 months ($t = 3{\cdot}96$, $P < 0{\cdot}01$) females.

(*f*) *Mitotic figures*

There was no significant variance over the age series in the number of mitotic figures/mm² section ($F = 0{\cdot}86$, n.s.).

(*g*) *Tubules containing neutrophils*

There was a significant variance in the number of tubules containing neutrophils/mm² section, with the numbers increasing with age ($F = 11{\cdot}16$, $P < 0{\cdot}001$). The 24 months females had the greatest number of tubules containing neutrophils and this was significantly greater than the 2 months ($t = 5{\cdot}90$, $P < 0{\cdot}01$), 6 months ($t = 5{\cdot}86$, $P < 0{\cdot}01$), 12 months ($t = 4{\cdot}03$, $P < 0{\cdot}01$) and 18 months ($t = 4{\cdot}62$, $P < 0{\cdot}01$) groups.

(*h*) % *Area of tubule degeneration*

The % area of tubule degeneration increased as the age of the females increased and was significant ($F = 5{\cdot}18$, $P < 0{\cdot}01$). The 18 months group had a greater % of degeneration than the 2 months group ($t = 2{\cdot}56$, $P < 0{\cdot}05$) while the 24 months group had a greater % than the 2 months ($3{\cdot}55$, $P < 0{\cdot}01$), 6 months ($t = 3{\cdot}39$, $P < 0{\cdot}01$) and 12 months ($t = 3{\cdot}13$, $P < 0{\cdot}05$) groups.

(*i*) *Type II cells*

There was considerable variance between the age groups in the % of Type II cells which was highly significant ($F = 22{\cdot}89$, $P < 0{\cdot}001$). The 2 months and 12 months females had the lowest %, while the 18 and 24 months females had the highest %, with the 6 months figure being intermediate. The frequency at 2 months was significantly less than at 6 months ($t = 3{\cdot}45$, $P < 0{\cdot}01$), 18 months ($t = 5{\cdot}78$, $P < 0{\cdot}01$) and 24 months ($t = 6{\cdot}42$, $P < 0{\cdot}01$), while the frequency at 12 months was also significantly less than at 6 months ($t = 3{\cdot}92$, $P < 0{\cdot}01$), 18 months ($t = 6{\cdot}69$, $P < 0{\cdot}01$) and 24 months ($t = 7{\cdot}44$, $P < 0{\cdot}01$).

DISCUSSION

The female hamster Harderian gland is one of the most active sites of porphyrin biosynthesis known, although the function of the gland and its porphyrin stores remains unclear. In the present study there are changes in both gland activity and histology in the female hamster over the age range examined. Biochemically, the gland shows a rise in activity of the enzyme 5-aminolaevulinate synthase between 2 and 6 months of age, followed by a decline. Porphyrin content shows a continual decrease from 2 months until 24 months. Since the gland stores porphyrins (normally as solid intraluminal accretions) the content always reflects both current and past synthesis. Little is known in the female gland of the rate of deposition of porphyrin, although

visible stores change rapidly under experimental conditions. For example, they rise rapidly from zero in castrated males (Payne *et al.* 1977) and decrease markedly in ovariectomised females given androgens (Spike *et al.* 1985). Nor is anything known of the rate of movement of stored porphyrin along the duct system, although cholinergic stimulation can quickly result in porphyrin-rich 'blood tears' in rats (e.g. Tashiro, Smith, Badger & Kezur, 1940).

Histologically, some age changes appear to relate to decreases in porphyrin content and synthesis, e.g. the decrease in mast cell numbers with age. It is known that the porphyrin-producing female gland contains some 40 times more mast cells than the non-producing male gland while castration of the male results in both elevated porphyrin synthesis and mast cell numbers (Payne *et al.* 1982). Other changes, however, may relate to gland tubule integrity. For example, the area of gland showing thin-walled tubules increases with age, as does the number of tubules showing invasion by neutrophils. Furthermore, as tubule walls degenerate, so porphyrin stores occur increasingly within the gland interstitium, either as large accretions surrounded by foreign body giant cells or as small deposits within individual macrophages.

Little information has been available concerning age and porphyrin synthesis in Harderian glands. Figge & Davidheiser (1957) found that enzyme activity in mouse gland rose rapidly until 2 months of age but remained static thereafter until one year – the maximum age tested; gland structure was not examined. In the present study, those changes which occur after 12 months of age include a progressive decrease in gland porphyrin concentration together with a continued low level of enzyme activity and mast cell numbers. Furthermore, the number of Type II cells (characteristic of the non-synthesising male gland) increases, as does the incidence of tubule degeneration, neutrophilic invasion and abnormally located (i.e. interstitial) porphyrin. Similar changes occur after ovariectomy (Payne *et al.* 1985; Spike *et al.* 1985, 1986) so hormone insufficiency could explain degeneration in hamsters over 12 months of age. Reproductive failure in female hamsters can begin at 9 months, though 14-15 months is a more normal figure (e.g. Whitney, 1963; Hafez, 1970). However, ageing *per se* may be responsible for the changes found, as may increasing subjection of the glands to inflammatory processes. Thus tubule degeneration and invasion by lymphocytes, plasma cells and neutrophils occurs in senile atrophy of the lacrimal gland and may result from chronic inflammation of it or other body tissues (e.g. Damato, Allan, Murray & Lee, 1981; Nasu, Matsubara & Yamamoto, 1984). The Harderian gland is a major site of immunocompetent cells in many species (e.g. Albini, Wick, Rose & Orlans, 1974; Burns, 1975; Burns & Maxwell, 1979).

The chief characteristics of the hamster Harderian gland are major sex differences in porphyrin synthesis and gland structure (Bucana & Nadakavukaren, 1973; Thompson *et al.* 1984), together with hormonal control of both synthesis and structure in both sexes (Hoffman, 1971; Lin & Nadakavukaren, 1979; Spike *et al.* 1985, 1986). Indeed, it has been argued that the gland may be a useful model for clinical conditions such as acute intermittent porphyria which are rare before puberty, occur thereafter more frequently in women than in men, and are exacerbated by hormonal disturbances such as the menstrual cycle, pregnancy and the contraceptive pill (Brodie *et al.* 1977; McColl *et al.* 1982). These present data extend our knowledge of factors controlling porphyrin synthesis in this particular gland and confirm the links betweens productivity and gland structure.

SUMMARY

The effects of age on structure and porphyrin synthesis were examined in the Harderian gland of the female golden hamster. An age range of 2–24 months was examined. Porphyrin enzyme activity reached a peak at 6 months and then declined; the porphyrin content of the gland (as determined both by biochemical assay and by the number of visible porphyrin accretions) also declined from 6 months. Mast cells, found in large numbers in the actively synthesising female gland, declined with age. Conversely, tubule epithelial cells with large lipid vacuoles (Type II cells – characteristic of the non-synthesising male gland) increased in frequency. There was considerable evidence of degenerative changes in gland structure with age. These included thinning of the tubule walls, invasion of the tubule lumen by neutrophils and the appearance of porphyrin stores within the interstitium of the gland. The latter were either large accretions surrounded by foreign body giant cells, or smaller deposits within individual free macrophages. Changes may be the result of ageing itself, of hormone insufficiency in post-reproductive senescence or inflammatory processes.

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