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Changes in Harderian Gland Activity in the Female Golden Hamster during the Oestrous Cycle, Pregnancy and Lactation

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The Harderian gland, which is situated within the bony orbit, is usually thought of as a source of lubrication for the eye. However, recent studies have suggested links with reproductive function. In the male golden hamster, both gland histology and activity are known to be under hormonal influence, and the present experiment was undertaken to examine gland weight and activity (as measured by the production of porphyrins) over the oestrous cycle and during pregnancy and early lactation in the female hamster. Gland weight, the number of solid intraluminal porphyrin accretions, and concentrations of copro- and proto-porphyrin were all maximal on day 1 of the cycle (oestrus) and at their lowest on day 2 (or jointly on days 2 and 3), rising gradually thereafter. Porphyrin concentrations are considerably higher during pregnancy and early lactation than during the cycle, and the solid porphyrin accretions, although diminished in number, are larger. Although there is no indication of either the function or the physiological basis of these changes during the cycle or pregnancy, these findings do suggest that in the female golden hamster, as in the male, there is a link between Harderian gland activity and reproductive function.

The Harderian gland is a compound tubulo-alveolar gland situated within the bony orbit of most terrestrial vertebrates. It is usually thought of as a source of lubrication for the eye, and is particularly associated with a well-developed nictitating membrane (Cohn, 1955; Kennedy, 1970). However, in many species its characteristics seem too elaborate for this to be its sole function. For example, in the golden hamster it exhibits a number of sex differences (for details, see Woolley & Worley, 1954; Paule *et al.*, 1955; Clabough & Norvell, 1973). Firstly, the gland is heavier in the male than in the female. Secondly, two cell types are described within the gland in the male, whereas there is only one cell type in the female. Thirdly, large amounts of porphyrin are produced by the gland in the female, to such an extent that they are deposited as solid accretions within the lumina of the gland tubules; the male gland produces very small amounts, which are not deposited in this way. Moreover, castration of the male hamster results in the loss of one cell type (so that the gland resembles that of the female) together with greatly increased porphyrin production, these changes being prevented by a variety of androgens (Hoffman, 1971; Payne *et al.*, 1975, 1977*b*).

More recently it has been suggested that the gland may be part of a retinal–pineal–gonadal axis (Wetterberg *et al.*, 1970; Reiter & Klein, 1971; Clabough & Norvell, 1973) and/or a source of pheromones (Thiessen & Rice, 1976; Payne, 1977). If there is any link between the Harderian gland and reproductive function, then it might be expected that gland activity in the female will alter over the oestrous cycle and pregnancy. These present experiments were undertaken in order to establish whether such changes do occur, by using porphyrin production by the gland as a measure of activity.

Materials and Methods

Changes in gland activity during the oestrous cycle (Expt. 1)

Experimental design. Data were obtained for each 12h period of the 4-day oestrous cycle, that is, from four light and four dark periods. Two groups of females were used, one housed since birth under conditions of 12h white light (06:00–18:00h) and 12h of darkness, and the other on a reversed lighting regimen of 12h of white light (22:00–10:00h) and 12h of dull red lighting. The cycles of both groups were verified by daily examination for the characteristic post-oestrus vaginal discharge, occurring on day 2 of the cycle (Orsini, 1961), and females under reversed lighting were also checked for behavioural oestrus by placing them with stud males for a few minutes 2–5h into the dark period. Only females with at least three consecutive normal cycles were used.

A total of 80 females were examined, ten for each 12h period. Data for the light period of each day were obtained by killing females housed under the normal lighting regimen between 09:00 and 12:00h (i.e. 3–6h into the light period), and data for the dark period of each day were obtained from females under the reversed lighting regimen killed at between 13:00 and 16:00h (i.e. 3–6h into the dark period). Animals were killed by sodium pentobarbitone (Nembutal) overdose, weighed and the Harderian glands weighed immediately after excision.

Porphyrin analysis. The methods for porphyrin analysis (and the initial verification of porphyrin types produced by the golden hamster) have been described in detail (Payne *et al.*, 1977*b*). Briefly, two methods for porphyrin estimation are used.

(*a*) Quantification of solid intraluminal porphyrin accretions. For each female, one of the identical Harderian glands (standardized as the left gland) was snap-frozen on solid CO_2 for frozen sectioning. The glands were sectioned at 20μm on a cryostat maintained at −25°C and one section was selected from four different areas, at least 200μm apart. These sections were placed on clean dry coverslips by allowing them to thaw momentarily and then dry, and were subsequently treated with 1% Bismark Brown to provide background staining. The sections were dehydrated through graded alcohols, cleared in xylene and mounted in DPX ('distrene/dibutyl phthalate/xylol'). Low-power micrographs of constant magnification (×25) were obtained for each section by using a Leitz Ultraphot, and the number of porphyrin accretions present was counted.

(*b*) Porphyrin content of the Harderian glands. Each right Harderian gland was placed in approx. 5ml of methanol and refrigerated until required for measurement of the porphyrin content.

Samples were homogenized (Polytron) in ethanol/1M-HCl (19:1, v/v) until no further fluorescence was observed in the supernatant under u.v. light. Conc. HCl was added to the combined supernatant solution to give a final concentration of 4.5M. Distilled water (25ml) was immediately added, and the pH of the resulting solution adjusted to neutrality by the addition of sodium acetate (solid) until the solution was neutral to Congo Red.

Total porphyrins were extracted with two washes of 25ml of diethyl ether, and the ethanol solution was discarded. The ether was washed once with 25ml of distilled water which was also discarded. Coproporphyrin and protoporphyrin were then extracted with 5ml quantities of 0.1M- and 1.5M-HCl respectively, and the concentrations of porphyrin measured on a fluorimeter standardized with a coproporphyrin solution. Uroporphyrin in the solid residue was extracted with aq. 1M-NH_3 until no more fluorescence could be observed. These solutions were combined with the saturated sodium acetate washes from the previous ether extractions, and the pH was altered to 1.5 with conc. HCl. This solution was extracted with 0.5vol. of redistilled cyclohexanone, allowed to separate for 20min, and re-extracted with a further 0.5vol. of cyclohexanone. The cyclohexanone washes were combined, and 2vol. of diethyl ether was added, mixed and extracted with 1.5M-HCl. The resulting extract was read at the Soret maximum on a spectrophotometer, and quantified by the method of Rimington (1971).

Changes in gland activity during pregnancy and early lactation (Expt. 2)

The observations on gland activity during the oestrous cycle were extended to cover pregnancy and early lactation. Vaginal cycles were followed for a large number of females, and regularly cycling females that were also showing behavioural oestrus on the expected day were placed into cages of stud males overnight and separated the next day.

Ten females were examined on the day of oestrus (these were different females from those examined in Expt. 1) and ten females on days 4, 8, 12 and 16 of the 16-day gestation period. Owing to slight variations in gestation length, females examined on day 16 included some immediately *pre-* and some immediately *post partum.* In addition, six females were examined on days 4 and 8 of lactation.

Each female was killed with sodium pentobarbitone (Nembutal) overdose, weighed, and the weights of the paired Harderian glands were obtained after excision. Porphyrin determinations were carried out exactly as described in Expt. 1.

Seasonal considerations

The data reported in Expts. 1 and 2 were obtained over 3 consecutive years (1975–1977), but all data were obtained during March and April for two reasons. Firstly, records for 10 consecutive years showed that colony fertility is at its height at this time, with over 70% of random matings resulting in offspring. Secondly, it is essential to obtain all data from a specific period of the year, since there is a marked seasonal variation in porphyrin content of the female hamster Harderian gland (Payne *et al.*, 1977*a*) as well as δ-aminolaevulinate synthase, the rate-limiting enzyme for haem biosynthesis (M. R. Moore & G. G. Thompson, unpublished results).

Statistical analysis

In Expt. 1 (changes over the oestrous cycle) relative gland weight and the number of porphyrin accretions per unit area were compared over the 4 days of the oestrous cycle by analyses of variance (F). Where these proved significant, comparisons between individual days were made by using Dunnett's (1964) test.

Because of the high intra-group ranges and variances of the fluorimetric assays of porphyrin (see the Results section), concentrations of copro-, proto- and total porphyrin were analysed from the dark part of each day only (i.e. in females whose vaginal cycles could be independently ratified by the cyclic occur-

Table 1. *Mean* (±S.E.M.) *relative weights, the median* (+*range*) *concentrations of copro-, proto- and total porphyrins, and the mean* (±S.E.M.) *numbers of accretions of porphyrin/unit area of the Harderian glands of female golden hamsters over the* 4 *days of the oestrous cycle*

Day 1 is the day of oestrus, and day 2 is characterized by the occurrence of the vaginal post-oestrus discharge. Also shown are data on the size of porphyrin accretions, characterized as large (>0.12mm) or small (<0.12mm), present within the glands of females sampled during the dark period of each of the 4 days of the cycle. N.S., Not significant.

Day of oestrous cycle ...	1	2	3	4	Analysis of variance
Paired gland weight (mg/100g)	155.6 (±6.5)	141.8 (±3.5)	150.4 (±5.0)	155.0 (±6.0)	$F=3.04$ $P<0.05$
Coproporphyrin (nmol/g)	32.5 (3–88)	3 (2–40)	10 (3–32)	19 (3–80)	$H=10.21$ $P<0.05$
Protoporphyrin (nmol/g)	1197 (259–2496)	210 (73–1823)	789 (224–1970)	1068 (265–1985)	$H=9.73$ $P<0.05$
Total porphyrin (nmol/g)	1230 (262–2546)	222 (75–1845)	799 (230–2002)	1099 (260–2010)	$H=9.75$ $P<0.05$
Porphyrin accretions/unit area	182 (±16)	121 (±14)	118 (±11)	133 (±15)	$F=4.23$ $P<0.01$
Average frequency of small accretions (<0.12mm)	195.67	120.90	108.87	123.32	$F=4.20$ $P<0.01$
Average frequency of large accretions (>0.12mm)	2.67	2.60	3.53	4.10	$F=0.62$, N.S.
Percentages of large accretions with largest dimension					
0.12–0.20mm	88	92	93	92	
0.20–0.28mm	12	8	7	8	

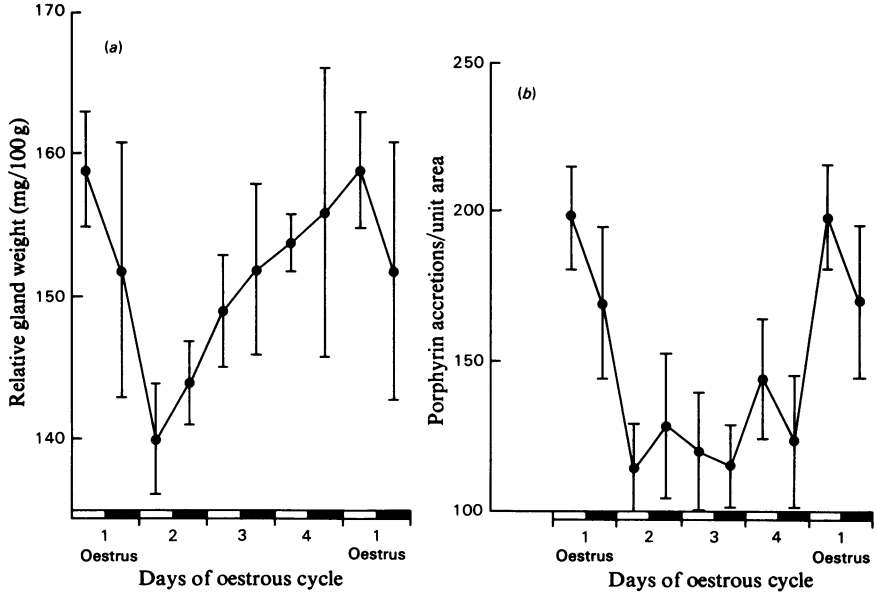

Fig. 1. *Relative weight*/100*g* (*a*) *of, and the average number of porphyrin accretions per unit area* (*b*) *within, the Harderian gland of the female golden hamster over the oestrous cycle*

All data are based on 10 females from each of the 8×12h light stages of the cycle. The data for day 1 of the cycle (oestrus) are depicted twice.

rence of behavioural oestrus), and were compared by non-parametric analyses of variance [Kruskal–Wallis one-way analysis of variance (H)]. Where these proved significant, individual days were compared by the Mann–Whitney test (U).

In Expt. 2 (changes over pregnancy and early lactation) relative gland weight and the number of porphyrin accretions per unit area were compared over the 7 days sampled by analyses of variance (F) and, as in Expt. 1, where these proved significant, individual comparisons were made by Dunnett's test. As in Expt. 1, concentrations of copro-, proto- and total porphyrin were analysed by the Kruskal–Wallis one-way analysis of variance (H) followed by individual comparisons by the Mann–Whitney test (U).

Results

Changes in gland activity during the oestrous cycle (Expt. 1)

Paired gland weight (see Table 1, Fig. 1). The weight of paired glands/100g body wt. showed a significant variance over the oestrous cycle, being highest on day 1 (oestrus) and lowest on day 2 (the day of the post-oestrus discharge), rising gradually thereafter. Individual comparisons showed that relative weight on day 2 of the cycle was significantly lower than on day 1 or 4 (by Dunnett's test, $t = 2.70$ and 2.61 respectively, $P < 0.05$).

Porphyrin accretions (see Tables 1 and 2). Like relative gland weight, the average number of solid intraluminal porphyrin accretions per unit area of a standard section of gland showed significant variance over the oestrous cycle, being highest on the day of oestrus. Individual comparisons showed that accretion counts on day 1 were significantly higher than on day 2 or 3 (by Dunnett's test, $t = 2.91$, $P < 0.05$, and $t = 3.14$, $P < 0.01$, respectively).

Although it was not possible to quantify the area per unit area of the gland occupied by the porphyrin accretions, considerable variation in the size of these accretions is apparent. Glands were analysed from the dark period of each day (i.e. from those females in which vaginal cycles were verified by behavioural oestrus) for the 4 days of the cycle, and all accretions measured in which the largest dimension was greater than an arbitrarily chosen 0.12mm [i.e. 3mm on the standard magnification (×25) used throughout the experiment]. The results of this analysis are shown in Table 1. Although the total number of porphyrin accretions alters significantly over the cycle, this is due to changes in the very small (<0.12mm) accretions; the number of large accretions (>0.12mm) remains relatively constant. For example, although the number of small accretions differs significantly between day 1 and 2 (as does the total number), not only are the total number of large accretions comparable between the two days, but, moreover, the number of large accretions within the various size ranges is remarkably similar.

Table 2. *Comparison of the weight and porphyrin content of the Harderian gland of female golden hamsters examined during the dark period of day 1 of the oestrous cycle (the day of oestrus) in Expts. 1 and 2 ($n = 10$ females in each case)*
There are no significant differences between the replicates, implying that data in column 2 represent reliable baselines for data obtained during pregnancy and lactation (see Table 3). N.S., Not significant.

	Expt. 1	Expt. 2	
Harderian-gland weight (mg/100g)			
Mean	152	165	$t = 1.05$, N.S.
S.E.M.	±10	±9	
Range	112–207	120–195	
Porphyrin accretions/unit area			
Mean	195	169	$t = 0.94$, N.S.
S.E.M.	±17	±25	
Range	109–284	39–258	
Coproporphyrin (nmol/g)			
Mean	33.9	46.9	$U^1 = 30$, N.S.
S.E.M.	±8.4	±4.8	
Range	3.1–87.8	27.5–71.5	
Protoporphyrin (nmol/g)			
Mean	1210	889	$U^1 = 32$, N.S.
S.E.M.	±216	±154	
Range	269–2497	326–1922	
Total porphyrin (nmol/g)			
Mean	1244	936	$U^1 = 32$, N.S.
S.E.M.	±222	±159	
Range	262–2546	354–1977	

Finally, it is clear that, within each day's data, those individual females that have a small total number of accretions seem to have a higher number of large accretions than females with a large total number (Fig. 2*a*).

Porphyrin analysis (see Table 1). There is enormous individual variation in porphyrin concentrations, lowest individual values on each day of the cycle differing from highest individual values by factors of up to 30. Because of this, Table 1 only presents data collected during the dark period of each day, since (*a*) these were the females whose vaginal cycles were verified by behavioural oestrus and (*b*), because this results in a 24h gap between data collection points rather than a 12h gap, it was thought that this would minimize the likelihood of variance due to cyclic changes being obscured by the (much greater) variance produced by individual variation.

The concentrations of total porphyrins and protoporphyrin (which is by far the major constituent) both show significance over the oestrous cycle (for total

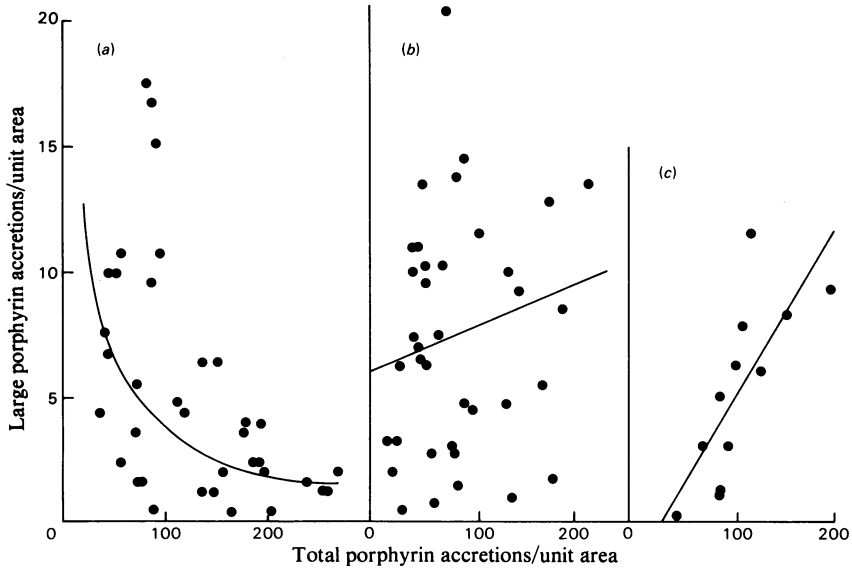

Fig. 2. *Relationship between the total number of porphyrin accretions per unit area and the number of large* (>0.12*mm*) *accretions within the Harderian gland of the female golden hamster during three phases of reproduction*
(*a*) During the oestrous cycle, there is an inverse relationship of the form $y = A/x^n$. The best line of fit was found to be: $\log y = -0.9 \log x + 3.3$. (*b*) During pregnancy there is a slight positive correlation between these two variables. Its linear regression takes the form: $y = 0.017x + 6.03$ ($r = 0.14$, not significant). (*c*) During lactation there is a marked positive correlation between these two variables. Its linear regression takes the form: $y = 0.069x - 2.3$ ($r = 0.76$, $P < 0.01$).

porphyrins, $H = 9.75$, $P < 0.05$; for protoporphyrin, $H = 9.73$, $P < 0.05$), and both exhibit similar changes, being highest on day 1 (oestrus) and lowest on day 2. Thus individual comparisons reveal that concentrations on day 2 are significantly lower than those on days 1, 3 and 4 ($U^1 = 82$, $P < 0.02$, and $U^1 = 85$, $P < 0.01$, respectively). No other inter-group comparisons reach significance.

Coproporphyrin concentrations, although considerably lower than protoporphyrin, show comparable cyclic variance ($H = 10.21$, $P < 0.05$). Again, concentrations are highest on day 1 (oestrus) and lowest on day 2, and individual comparisons reveal that they are significantly lower on day 2 than on day 1 or 4 ($U^1 = 87$, $P < 0.01$, and $U^1 = 79$, $P < 0.05$, respectively). No other comparisons are significant.

Changes in gland activity during pregnancy and early lactation (*Expt.* 2)

Data on oestrus females (*Table* 2). The day of oestrus is the period of highest gland activity during the oestrous cycle, as demonstrated in Expt. 1. It also acts in Expt. 2 as a baseline for comparing data obtained during pregnancy and lactation. Hence it is necessary to compare the data obtained on oestrus females in the two experiments in order to ensure comparability and reliability. Table 2 shows that none of the parameters of gland activity exhibited significant differences between the two replicates, and that the data can therefore be viewed as reliable.

Relative gland weight (*see Table* 3). The weight of the paired Harderian glands/100g body wt. did not show significant variance between oestrus, pregnant and lactating females; nor is any clear pattern evident in the minor fluctuations over these periods.

Porphyrin accretions (*see Table* 3). The number of intraluminal porphyrin accretions per unit area showed a significant variance over the period ($F = 3.49$, $P < 0.01$). Individual comparisons reveal that the number of accretions at oestrus was significantly higher than on any of the 4 days sampled during gestation (by Dunnett's test, for day 4, $t = 4.53$, for day 8, $t = 4.59$, for day 12, $t = 4.53$, and for day 16, $t = 4.11$; all $P < 0.01$), as well as significantly higher than on day 8 of lactation ($t = 3.43$, $P < 0.01$). There were no significant differences between any of the days during pregnancy and lactation.

Although there were fewer porphyrin accretions during pregnancy and lactation than during the oestrous cycle, a much higher percentage of them fell within the large (>0.12mm) range (see Table 3).

Table 3. *Weights* (±S.E.M.), *median* (+*range*) *concentrations of copro-, proto- and total porphyrins, and mean* (±S.E.M.) *numbers of accretions of porphyrin/unit area of the Harderian glands of female golden hamsters on the day of oestrus, on days* 4, 8, 12 *and* 16 *of gestation, and on days* 4 *and* 8 *of lactation*

Also shown are data on the size of porphyrin accretions, characterized as large (>0.12mm) or small (<0.12mm), present within the glands of females sampled during gestation and lactation. Results differing significantly from these on the day of oestrus are indicated thus: * $P<0.05$; ** $P<0.01$. N.S., Not significant.

		Day of gestation				Day of lactation		
	Oestrus	4	8	12	16	4	8	Analysis of variance
Weight of paired Harderian glands/100g	165 ±9	166 ±7	149 ±6	157 ±5	151 ±7	165 ±7	167 ±14	$F=0.88$, N.S.
Porphyrin accretions/unit area	169 ±25	83** ±13	82** ±13	83** ±14	91** ±25	127 ±16	97** ±16	$F=3.49$, $P<0.01$
Protoporphyrin (nmol/g)	889 ±154	4263** ±537	2267** ±454	1458** ±572	3015 ±929	3101** ±647	2137 ±513	$H=21.91$, $P<0.001$
Coproporphyrin (nmol/g)	47 ±5	74 ±11	51 ±9	79 ±9	59 ±9	65 ±9	55 ±10	$H=12.44$, N.S.
Total porphyrin (nmol/g)	936 ±159	4336** ±533	2318** ±451	4236** ±574	3074 ±937	3184** ±633	2192 ±522	$H=22.26$, $P<0.01$
Average frequency of small accretions (<0.12mm)		75.34	76.95	72.42	84.55	117.87	89.08	$F=0.83$, N.S.
Average frequency of large accretions (>0.12mm)		7.69	5.45	10.25	6.67	6.46	3.96	$F=2.04$, N.S.
Percentage of large accretions with largest dimension								
0.12–0.20mm		83	84	72	84	92	98	
0.20–0.28mm		14	13	23	13	7	2	
>0.28mm		3	3	5	3	1	0	

Moreover, although during the cycle those individuals with fewer total accretions tended to possess a greater number of large ones, during pregnancy and lactation the reverse is true. Thus for each day there was a positive correlation coefficient between total and large accretions, and this positive correlation reached significance towards the end of pregnancy and during lactation (Fig. 2*c*). This demonstrates clearly that there are differences in the pattern of accretion formation between the pregnant and non-pregnant female, and this may in turn reflect differences in porphyrin excretion by the gland.

Porphyrin analysis (*see Table* 3). There were significant analyses of variance for total porphyrin ($H=22.26$, $P<0.001$) and for protoporphyrin concentrations ($H=21.91$, $P<0.001$) over the 7 days sampled. Thus on all days during pregnancy and lactation concentrations of proto- and total porphyrin were higher than on the day of oestrus, and in most cases this reached statistical significance. Within the days of pregnancy and lactation, porphyrin concentrations on days 8 of pregnancy and lactation were lower than on other days, and concentrations of proto- and total porphyrins on both of these days proved significantly lower than on days 4 and 12 of pregnancy, when average concentrations were at their height. No other comparisons proved significant.

Coproporphyrin concentrations did not show significant variance over the 7 days sampled.

Discussion

These experiments demonstrate that the porphyrinogenic activity of the Harderian gland in the female golden hamster alters with the reproductive phase. Thus in Expt. 1 all the parameters of porphyrin production (together with gland weight) fluctuated over the oestrous cycle, being at their maximum on day 1 (oestrus) and minimal on day 2 or 3. Although coproporphyrin concentrations are considerably lower than those of protoporphyrin, their essentially simultaneous fluctuations suggest cyclic control of the haem-biosynthetic pathway as a whole, probably because of cyclic changes in δ-aminolaevulinate synthase (the rate-limiting enzyme for the pathway), which precede changes in porphyrin concentrations by 12–24h (Moore *et al.*, 1977). Similarly, Expt. 2 demonstrates that there is a marked increase in porphyrin concentrations during pregnancy and lactation compared with concentrations at oestrus.

Sex differences in porphyrin concentrations have been reported, usually with the female exhibiting higher concentrations than the male [e.g. the golden hamster (Woolley & Worley, 1954; Hoffman, 1971);

some strains of mice (Strong, 1942; Margolis, 1971); the rat (Joó & Kahán, 1975)]. However, no clear evidence of cyclic or gestational changes has previously been reported for the Harderian gland in laboratory rodents. Held & Przerwa (1976) have demonstrated cyclic changes in δ-aminolaevulinate synthase in the rat liver (and similar changes probably occur in the hamster liver; Moore *et al.*, 1977), but the genetic studies of Margolis (1971) suggest that different factors may control this rate-limiting enzyme in the liver as compared with the Harderian gland. Clinical studies have generally shown that porphyrin production in normal women rises during pregnancy (Lyberatos *et al.*, 1972) and that conditions of porphyrin overproduction, such as acute intermittent porphyria, are exacerbated during pregnancy (Goldberg, 1959; Brodie *et al.*, 1977) and also pre-menstrually (Perlroth *et al.*, 1965).

These present preliminary studies make no attempt to investigate the physiological basis of these cyclic and gestational changes in porphyrin content. Moreover, it is difficult to predict such a basis on the present evidence. Thus porphyrin concentrations in the Harderian gland have variously been stated to be decreased by androgens (Hoffman, 1971; Payne *et al.*, 1975, 1977*b*), to be controlled directly by gonadotropins (Hoffman, 1971; Clabough & Norvell, 1973), to be unaffected by ovariectomy or the administration of 17α-oestradiol (Hoffman, 1971), to be increased by thyroxine administration (Hoffman, 1971) and to be affected by changes in melanin-stimulating hormone and melatonin (Joó & Kahán, 1975).

It is not clear how much of the porphyrin within the Harderian gland is represented by the solid intraluminal accretions. Over the oestrous cycle, the actual number appears to be a good index of porphyrin content, as confirmed by fluorimetric assay. However, it must be remembered that the cycle is characterized by a large number of small accretions that fluctuate and a small number of larger accretions that appear to remain relatively stable. During pregnancy and lactation, this numerical measure is a poor index of actual porphyrin concentration within the gland, since these reproductive phases are characterized by a much smaller number of relatively large accretions. Clearly, size of accretion would be a more useful index under such circumstances. Since, during the oestrous cycle, it is the small accretions that fluctuate, it must be presumed that these are more readily secreted into the conjunctival sac than the large accretions and, moreover, that overall secretion of porphyrins by the gland may be substantially lowered during pregnancy and lactation, when large accretions prevail.

Thiessen *et al.* (1976) have suggested that the Harderian gland in the gerbil (*Meriones unguiculatus*) is a source of pheromones which reach the exterior by passing into the conjunctival sac and (secondarily) by leaving the nostrils having passed down the nasolachrymal ducts. Similar conclusions with regard to the pheromonal properties of the gland have been reached for the golden hamster on the basis of the behavioural sequelae of painting individuals with Harderian-gland homogenates (Payne, 1977). Thiessen & Rice (1976) have further suggested that porphyrins themselves may be the active pheromones; if this proves to be the case, then the changes in porphyrin concentration found here suggest an ideal basis for the transmission of information about the reproductive status of the female hamster, although it must be borne in mind firstly that the gland also produces a lipid secretion (Kanwar, 1960; Kühnel, 1971; Hoffman, 1971) and, secondly that (in the rat at least) it selectively accumulates androsta-4,16-dien-3-one, the precursor of the known pheromonal steroid 5α,16-androsten-3α-ol (Brooksbank *et al.*, 1973). Furthermore, the considerable individual variation in porphyrin concentration at all times, together with the individual differences in the method of laying down porphyrin accretions (large numbers of small mobile ones, or small numbers of large non-mobile ones) could provide a system for individual recognition by means of Harderian-produced olfactory cues.

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