Effect of Lighting Conditions on the Porphyrin Content and Morphology of the Harderian Gland and on Uterine Morphology in the Golden Hamster

Recent work suggests that the Harderian gland and possibly its porphyrin content is involved in the photoregulating mechanism influencing the diurnal variation of indoleamine concentration in the pineal gland of immature rats[1]. The Harderian gland may also be involved in reproduction since removal of this gland causes enlargement of the uteri of adult female albino rats[2].

Due to a sexual dimorphism of porphyrin content in the Harderian gland of the golden hamster, this animal offers special opportunity to study the importance of the porphyrin content in relation to light and the regulation of the pineal and gonadal function[3,4].

In the present study we report the effect of long periods of continuous light and continuous darkness on the porphyrin content and histological appearance of the Harderian gland. The morphology of the uterus was also examined as an indicator of gonadal function in relation to the changes in the Harderian gland.

Material and methods. Forty-one sexually mature female golden hamsters (*Mesocricetus auratus*, $2n = 44$) were used. The hamsters were housed in individual plastic cages in isolated rooms without windows with a temperature approximating 22°C. The hamsters were given drinking water and laboratory pellets ad libitum. The experiment was conducted during the months of February through May.

Animals were kept in the 3 lighting conditions light: dark (L:D) 0:24, 12:12 or 24:0 for periods of 9, 36 or 72 days. At the appropriate times the animals were sacrificed, the Harderian gland removed, rinsed in cool, 1.15% KCl and weighed. The left gland was kept for assay of the porphyrin content and the right gland was placed in 2.5% glutaraldehyde in Soerensen's phosphate buffer embedded in paraffin, cut at 5 µm and stained with hematoxylin-eosin and Masson.

The left Harderian gland was homogenized and the porphyrin content of the gland was examined in a spectrophotofluorometer. The results were statistically analyzed using the Student *t*-test.

Results. The mean weight of the Harderian gland calculated per g of body weight was higher for animals kept in total darkness for 72 days than for animals kept in light (Table). Conversely the total porphyrin content of the gland was more than 4-fold higher in the group maintained in continuous light (144 µg versus 30 µg porphyrin per gland). The difference in porphyrin content for hamsters in light and darkness for 36 days was 2-fold (Table).

The Harderian glands of hamsters kept in continuous light for 72 days were richer in amorphous pigment bodies

Harderian gland weights (mg/100 g body wt.), total porphyrin content (µg/gland) and concentration in Harderian gland (µg/100 gland) in female golden hamsters maintained in different lighting conditions.

Treatment	*N*	Harderian gland		
		weights	porphyrin per gland	porphyrin per 100 mg gland
Light: Dark 12 h: 12 h	(7)	78 ± 4.3	50 ± 10	61 ± 10
Continuous light				
9 days	(5)	73 ± 3.9	51 ± 7.3	66 ± 8.6
36 days	(4)	83 ± 14	87 ± 17	109 ± 21
72 days	(8)	72 ± 2.9[a]	144 ± 20[a]	212 ± 25[a]
Continuous darkness				
9 days	(5)	74 ± 10	54 ± 15	86 ± 21
36 days	(5)	81 ± 6.8	44 ± 16	56 ± 19
72 days	(7)	85 ± 3.3[a]	30 ± 6.9[a]	46 ± 11[a]

All values are expressed as means ± standard errors. [a] Designates means that differ significantly ($p < 0.01$) between animals kept in continuous light or darkness.

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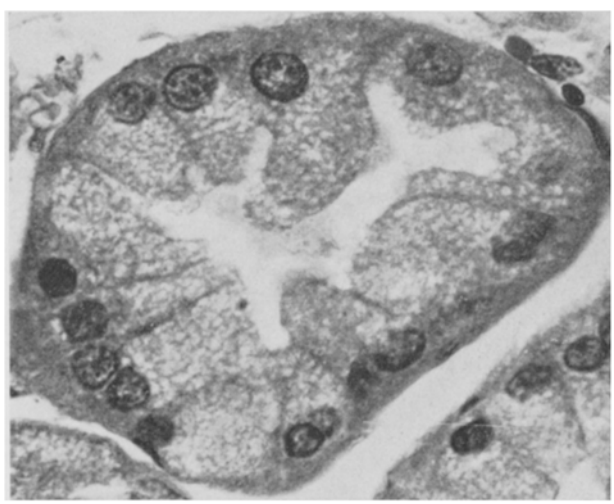

Fig. 1. Harderian gland of a female hamster kept in continuous light for 72 days. All alveolar cells are small-vacuolated. Massen staining. ×440.

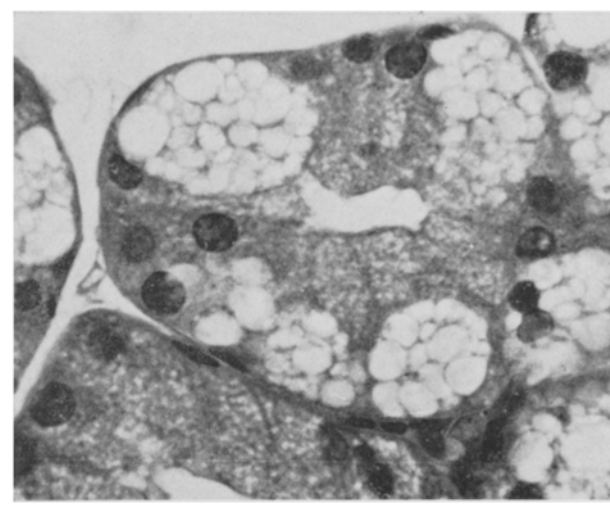

Fig. 2. Harderian gland of a female hamster kept for 72 days in continuous darkness. In addition to small-vacuolated alveolar cells, there are also cells with large vacuoles. Masson staining. 440×.

present in the alveolar luminae, than were the glands of animals kept in continuous darkness. The alveolar cells of glands from animals kept in continuous light were cylindrical, fairly uniform in appearance, stuffed with small vacuoles (Figure 1). This type of alveolar cell could also be seen in glands from animals kept in continuous darkness (Figure 2). In addition these animals showed another type of cell with very large vacuoles (Figure 2).

The uterus from hamsters kept in darkness for 72 days atrophied whereas continuous light for 72 days resulted in a much thicker uterus.

Discussion. After 72 days of continuous light the porphyrin concentration and content in the Harderian gland was increased more than 4-fold compared with animals kept in darkness, despite a higher mean gland weight for the latter. This could be a direct effect of light on the Harderian gland or an effect via the eye and the pineal gland[1], which in its turn could be direct or mediated through the hypothalamic-adrenal-gonadal axis[2]. The high content of porphyrin in glands from animals kept in continuous light was parallelled histologically by a higher amount of luminar pigment in these glands than in animals kept in darkness. The large-vacuolated alveolar cells seen in the latter animals could sometimes be seen in animals kept on the diurnal lighting condition 12 L: 12 D but never in animals kept in continuous light. There may thus be 2 basic types of alveolar cells of which only 1 seems to respond to changes in lighting conditions. 2 types of secretory cells are previously described in the rat[5] and the mouse[6].

The morphology of the hamster uterine tissue showed hypertrophy after continuous light and atrophy in animals kept in darkness. The same characteristic morphological responses of the uterus to different lighting regimes have previously been described by REITER et al.[7].

Zusammenfassung. Bei Goldhamstern, welche längere Zeit im Dunkelversuch (72 Tage) gehalten wurden, konnten in der Harder'schen Drüse zwei Zellarten nachgewiesen werden, bei konstantem Licht dagegen nur eine einzige Zellart. Der Porphyringehalt in diesen Drüsen ist bedeutend höher.

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Pea Phytohemagglutinin Selective Agglutination of Tumour Cells

The recently characterized phytohemagglutin of pea[1], the biological activity of which has not yet been established, was compared with concanavalin A (con A) for the ability to agglutinate tumour cells. Pea phytohemagglutinin was prepared as a mixture of 2 substances of the same molecular weight, of very similar amino acid composition, and identical hemagglutinating activity[1]. Con A lyophilized in NaCl was purchased from Calbiochem (grade A, lot 010229). For agglutination assay cells all cultivated in vitro were harvested with 0.02% EDTA solution in PBS and washed 3 times with PBS. Normal embryonic fibroblasts of human (HuEF), rat (LWF) and RIF-free chicken (BLEF) origin were examined. Tumour cells were represented by one clonal line of spontaneously in vitro transformed Lewis rat fibroblasts (LW13K2)[2], 3 Rous sarcoma virus in vitro transformants of LW13 cells (LW13-RsK4[3], RsK4-A4[4], RsK4-A4K1[4]) and 1 in vivo induced Rous Wistar rat sarcoma (CZW1)[4]. The agglutination assay was done in test tubes. To 0.2 ml of phytohemagglutinin solution 0.2 ml of cell suspension was added. The results were read after 15 min both macroscopically and microscopically. Trypsinization (Trypsin Spofa, Czechoslovakia, crystallized) of normal cells was carried out in suspension at trypsin concentration of 0.1% in PBS for 6 or 10 min at 37 °C. Trypsin action was stopped by soybean trypsin inhibitor. 30 m*M* methyl α-D-glucopyranoside was used to inhibit phytohemagglutinins in a concentration of 500 μg/ml. The mixture of phytohemagglutinin and hapten was incubated at least 15–30 min before the addition of cell suspension.

Agglutinability of normal and tumour cells by con A and pea phytohemagglutinin is shown in the Table. Normal rat and human cells show the usual patterns of interaction with phytohemagglutinins. They are agglutinated only when the highest concentration (1500 μg/ml) of both con A and pea phytohemagglutinin is used. After trypsin treatment the amount of phytohemagglutinins necessary for minimum significant agglutination of normal cells decreases – less for con A and more for pea phytohemagglutinin. Agglutinability of in vitro spontaneously transformed highly malignant rat tumour cells LW13K2 lies between normal cells and rat Rous sarcoma cells. Rat Rous sarcoma cells are agglutinated even at a concentration of 10 μg of con A per 1 ml or 5 μg of pea phytohemagglutinin per 1 ml. Clumping of cells caused by pea phytohemagglutinin is always easier to read than a similar effect of con A as pea phytohemagglutinin leaves less free single cells in suspension. Specificity of phytohemagglutinin tumour cell agglutination is in all positive cases proved by the inhibition of agglutination by sugar. In our experiments methyl α-D-glucopyranoside was chosen for its relatively high inhibitory activity for both pea phytohemagglutinin and con A[5]. High degree of inhibition of tumour cell agglutination is obtained only when the sugar is preincubated with phytohemagglutinin solutions for 15–30 min before the addition of cells. Addition of sugar to already agglutinated cells had no

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