# Porphyrin Metabolism in the Harderian Glands of Syrian Hamsters: *In Vivo* Regulation by Testicular Hormones, Lighting Conditions, Pineal Gland, and Pituitary Hormones (43389)

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*Abstract.* **The porphyrin concentration in the harderian glands of male hamsters subjected to several endocrine manipulations was studied. Prolonged bilateral gonadectomy resulted in a marked increase in harderian porphyrin concentration. This change was not prevented by either pinealectomy or by constant white light exposure. Castrated hamsters exposed to constant red light showed higher porphyrin concentrations than castrated hamsters kept under white light. Among several hormones studied, serum luteinizing hormone and thyroid-stimulating hormone levels were unexpectedly higher in the constant red light exposed group than in the other groups. In order to test whether luteinizing hormone was involved in the postcastrational rise in harderian porphyrins, we administered a potent luteinizing hormone-releasing hormone (LHRH) agonist. The chronic administration of the LHRH agonist resulted in a decrease in serum luteinizing hormone (because it desensitized the LHRH receptors on the gonadotropes) and, consequently, in serum testosterone levels. However, no rise in harderian porphyrin was observed. It is concluded that the absence of testicular hormones might not be the triggering factor involved in harderian porphyrogenesis.** [P.S.E.B.M. 1992, Vol 200]

The harderian glands are large accessory lacrimal glands that are especially prominent in rodents. Although the precise functional role of these tubuloalveolar glands is unknown, they have been increasingly used as a model in which to study porphyrin biosynthesis (1, 2). The harderian glands contain the highest porphyrin concentrations of all tissues studied. The porphyrin compounds, mostly protoporphyrin IV (the immediate precursor of heme), are produced in the secretory cells of the harderian glands (1–4). The enzymes involved in porphyrin synthesis in humans have been found in the harderian glands of mice, rats, and hamsters (5, 6). Among the rodents, the Syrian hamster harderian glands have been repeatedly studied because of their remarkable sexual dimorphism in porphyrin content. Female hamsters contain very high concentrations of porphyrins in their harderian glands, whereas the glands in male hamsters have low porphyrin levels (3). This sexual difference in the quantity of porphyrins seems to depend, to a large extent, on the endocrine status of the gonads. It has been proposed recently, however, that pituitary and thyroid hormones might be involved in harderian porphyrogenesis as well (7–9). Furthermore, ambient lighting conditions and the pineal gland might also affect the porphyrin content of these orbital glands (10–13).

In spite of the accumulated information, there are contradictory results that mask the precise roles of the various agents implicated in harderian porphyrin biosynthesis. As a result, in the present report, we have attempted to correlate porphyrin concentrations in the

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harderian glands with serum hormone levels in Syrian hamsters exposed to several experimental treatments.

## Materials and Methods

**Animals.** The animals used in this study were male Syrian hamsters (*Mesocricetus auratus*) purchased from Sasco (Omaha, NE). Ninety-one animals ranging from 75 to 100 g in weight were housed under a light:dark cycle of 14:10 hr (lights on at 0600 hr) before the beginning of the experimental procedures. Animals were maintained four per cage, with food and water provided *ad libitum*.

**Experimental Design.** *Experiment 1.* Since constant white light has been shown to increase harderian gland porphyrin content in rats (14) and constant red light has been alleged to prevent the porphyrin rise after castration in male hamsters (10), we designed an experiment to study the possible involvement of the pineal gland in these responses. Sixty-four hamsters were divided into eight equal-sized groups. Half of the animals were housed under a 14:10-hr photoperiod during the experiment and the remainder under a 24:00-hr cycle. The 32 hamsters under 14:10-hr white light photoperiodic conditions were grouped as unoperated controls, castrated animals, pinealectomized animals, and both castrated and pinealectomized animals (eight hamsters per group). Among the animals under continuous white light, there were control, castrated, and castrated plus pinealectomized hamsters. A final group of castrated animals (eight each) was maintained under continuous red light.

The animals were housed in light-tight cabinets. Each cabinet was illuminated independently and well ventilated by fans as described by Brainard *et al.* (15). Two months after the beginning of the experiments, the hamsters were sacrificed between 1100 and 1300 hr. The harderian glands were rapidly removed and frozen on solid $CO_2$. Serum was also collected.

*Experiment 2.* A second study utilizing 27 animals was designed to determine whether the porphyrin rise seen in castrated hamsters was due to low testosterone levels or to the corresponding high luteinizing hormone (LH) levels. Nine animals served as controls, nine animals were castrated, and, to avoid the usual pattern following castration of low testosterone and high LH levels, nine castrated hamsters were injected with a luteinizing hormone-releasing hormone (LHRH) agonist (D-Trpb-LHRH; courtesy of Dr. Andrew V. Schally). The LHRH agonist was given at a dose of 10 μg/day (50–100 μg/kg body wt) for 14 days, between 1600 and 1800 hr. This drug, when used chronically, acts like an LHRH antagonist because it desensitizes the LHRH receptors at the level of the pituitary gonadotropes, thereby diminishing serum LH levels and, as a consequence, testosterone levels.

**Assays.** Total porphyrin concentration was measured by fluorescence spectroscopy according to the method of Buzzell *et al.* (8). Tissues were weighed and homogenized (Polytron) in 2 ml of an organic phase consisting of ethyl acetate and acetic acid (8:2). Following centrifugation, 100-μl portions were extracted in 1.5 *N* HCl. Fluorescence was read in a Perkin-Elmer MPF-44A fluorescence spectrophotometer at an excitation wavelength of 405 nm and an emission wavelength of 604 nm. Samples were compared with standards made up of coproporphyrin III tetramethyl ester. The intra-assay variability using this technique was 2.2–4.4% and interassay variability was 3.9%. Porphyrin concentrations are expressed as μg porphyrin/mg tissue.

Serum testosterone was measured using a commercial kit from Diagnostic Products (Los Angeles, CA). Serum levels of LH and thyroid-stimulating hormone (TSH) levels were estimated in duplicate using a double antibody radioimmunoassay method. LH and TSH iodination hormones, standards, and antibodies were obtained from the National Institute of Arthritis, Digestive Diseases, and Kidney (Bethesda, MD).

**Statistical analysis.** Data are expressed as mean ± SE. Significance was tested by analysis of variance followed by a Student Newman-Keuls test. Significant differences were accepted with $P < 0.05$.

## Results

**Experiment I.** Harderian gland porphyrin concentrations of the various groups are shown in Figure 1A. The glands from control hamsters kept in either 14:10-hr or under continuous white light contained barely detectable porphyrin levels in their harderian glands. However, castration of hamsters kept under either 14:10-hr or 24:00-hr conditions dramatically increased the porphyrin content of the harderian glands ($P < 0.005$). Pinealectomy did not alter the porphyrin content under any of the conditions studied. Interestingly, the castrated hamsters exposed to constant red light contained higher porphyrin concentrations than those exposed to constant white light ($P < 0.05$).

Serum testosterone and LH levels are shown in Figures 1B and 1C, respectively. Serum testosterone was essentially undetectable in all castrated groups. Pinealectomized hamsters exposed to 14:10-hr conditions showed a slight reduction in serum testosterone ($P < 0.05$). LH levels were significantly increased by castration ($P < 0.05$). In addition, the castrated animals exposed to constant red light had higher LH levels than those exposed to constant white light ($P < 0.05$). Pinealectomy was ineffective in altering the changes caused by androgen deprivation.

TSH levels were similar in most of the groups (Fig. 1D). However, the pinealectomized-castrated animals exposed to 24:00-hr white light exhibited a slight increase in serum TSH levels ($P < 0.05$). Also, the exposure of castrated hamsters to constant red light resulted in a significant increase in serum TSH as compared

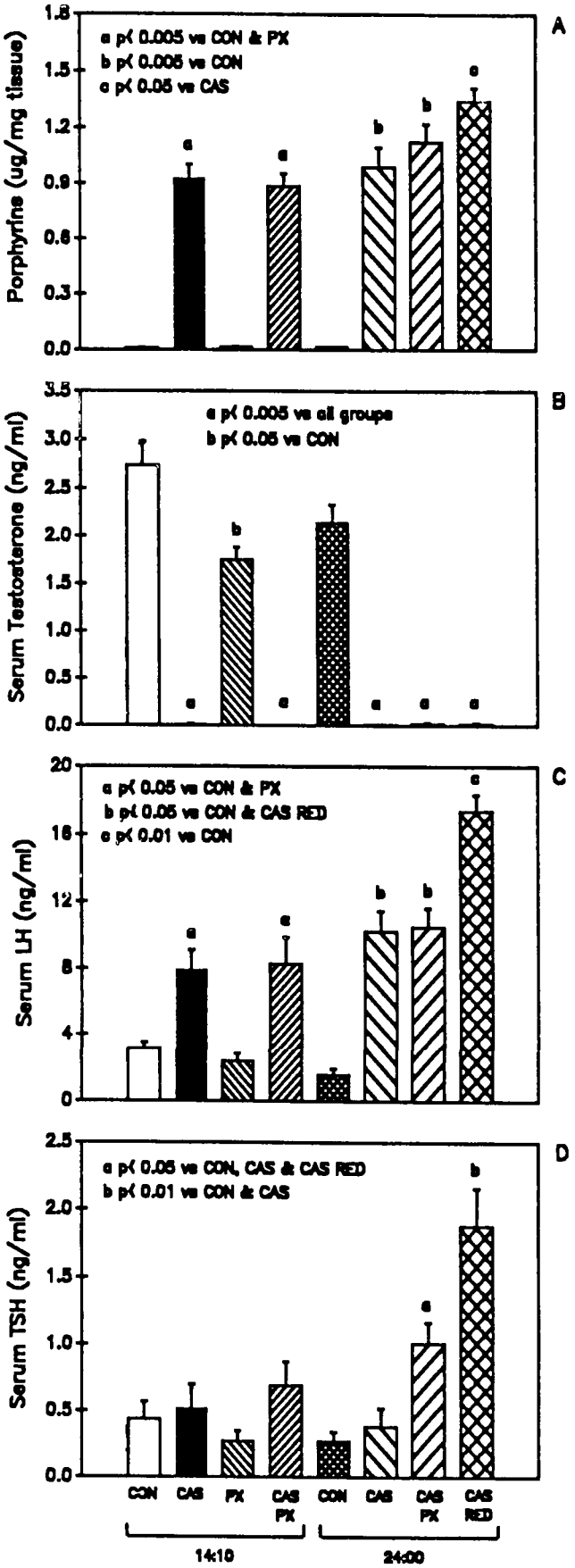


**Figure 1.** Effects of different experimental conditions on harderian gland porphyrin concentrations and serum levels of several hormones in male Syrian hamster. CON, control; CAS, castrated; PX, pinealectomized; CAS PX, castrated and pinealectomized; CAS RED, castrated and exposed to constant red light; 14:10, 14 hr of light and 10 hr of darkness; 24:00, constant light (white or red). (A) Porphyrin concentrations in male hamster harderian gland. The porphyrin concentrations are higher in castrated males under constant red light than in castrated males exposed to constant white light. (B) Serum testosterone levels. Testosterone levels were essentially absent in all castrated groups. (C) Serum LH levels. As in the case of porphyrin concentrations, the castrated group under constant red light exhibited higher LH levels than the castrated group exposed to constant white light. (D) Serum TSH levels. TSH levels were higher in castrated animals under constant red light than in castrated animals under constant white light.

with the castrated animals kept under constant white light ($P < 0.01$).

**Experiment II.** As expected, harderian gland porphyrin concentrations were higher in castrated animals when compared with control hamsters ($P < 0.01$). The animals injected with the LHRH agonist showed the same low porphyrin concentrations as those found in the harderian glands of the control animals (Fig. 2A).

Serum testosterone and LH levels are represented in Figure 2B and 2C, respectively. Testosterone levels were undetectable in the castrated group, whereas LH levels were significantly elevated ($P < 0.05$). As expected, the animals injected with the LHRH agonist had low LH levels ($P < 0.05$) and, consequently, low testosterone values ($P < 0.01$).

## Discussion

In the first study, we showed that testicular hormones, more than any other factor, are likely responsible for the inhibition of porphyrin synthesis in the harderian gland of the male Syrian hamster. Remarkably, however, continuous red light exposure increased porphyrogenesis in castrated hamsters above that seen in gonadectomized animals kept under 14:10-hr conditions. In an earlier report, Formgren and Wetterberg (4) claimed that exposure of castrated hamsters to red light prevented the castration-associated rise in harderian porphyrins. As shown by the present results, we did not confirm this observation and, in fact, obtained the opposite results. Hamsters exposed to constant red light can be considered "blind" in reference to the pineal indolamine metabolism (16); thus, the melatonin rhythm in these animals displays a "free running" pattern. A role for melatonin in harderian porphyrogenesis has been suggested (13) and may eventually help to explain the contradictory results obtained in different experiments in which blinding, constant darkness, short photoperiod, or red light exposure was used in order to manipulate the porphyrin content of the harderian glands.

Continuous white light exposure is reportedly a potent stimulator of porphyrogenesis in the liver (17) and the harderian glands of rats (13). However, using hamsters, we found continuous white light exposure incapable of changing the porphyrin concentration of the harderian glands.

Interactions between the harderian glands and the

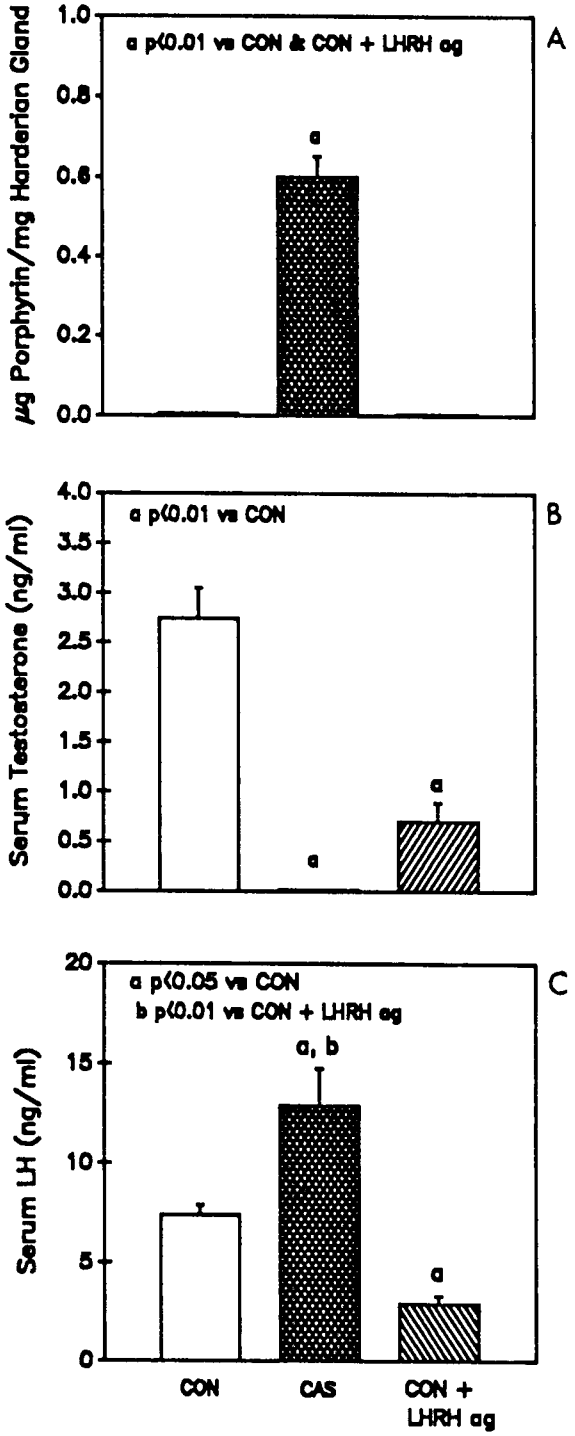


**Figure 2.** (A) Porphyrin concentrations in the harderian gland of intact male Syrian hamsters (CON), in castrated males (CAS), and after chronic injection of a potent LHRH agonist (which acts like an antagonist in terms of serum LH and consequently testosterone levels) to intact animals (CON + LHRH ag). (B) The porphyrin values of the agonist-treated animals did not increase, even though testosterone levels were very low. (C) Whereas castration increased serum LH levels, LHRH agonist treatment decreased them.

thyroid gland have been proposed on a number of occasions (3, 9, 13). Treatment with thyroxine reportedly increased the content but not the concentration of porphyrins in male harderian glands (3). In a more recent study, Buzzell *et al.* (18) did not observe changes in the porphyrin content of male hamster harderian glands after chronic treatment with the thyroid-inhibiting drug methimazole. In the present study, TSH levels were found to be high in the animals exposed to constant light coinciding with the higher concentrations of porphyrins. Hoffman and co-workers (9) have suggested that thyroid regulation of harderian porphyrinogenesis differs between the sexes.

Besides prolactin, LH could be a "stimulatory factor" for harderian gland porphyrogenic activity, a possibility reported by Hoffman (3). In the present study, we found a larger than normal increase in serum LH in castrated, constant red light-exposed hamsters. The reason for this unexpected increase remains unknown. High LH levels are induced by very low androgen levels present in gonadectomized animals; however, why red light exposure further increased these LH values is enigmatic. Since these animals had the high harderian gland porphyrin levels, LH may have an effect on harderian porphyrin levels.

We have recently shown that exposure of male hamsters to prolonged short photoperiod does not cause the dramatic rise in harderian porphyrin concentrations that is caused by castration (18), despite the fact that serum testosterone levels are markedly decreased in the short photoperiod-exposed hamsters (19). This may be due to the fact that short photoperiod exposure reduces both LH and testosterone levels (20), whereas castration reduces testosterone, but actually causes a rise in circulating LH values.

In the second experiment, we found that despite the low testosterone levels, the porphyrin concentrations in the LHRH-injected group were as low as in the intact animals. This could have been a result of the extremely high sensitivity of the harderian gland to testosterone, with very low levels of the steroid being capable of inhibiting porphyrin synthesis. However, there is another possible explanation that is independent of androgen levels. Thus, the virtual absence of LH due to treatment of the hamsters with the potent LHRH agonist (which down-regulated the LHRH receptors on the gonadotropes) caused the low porphyrin concentrations. The potential effects of LHRH itself on harderian porphyrin metabolism should be considered in subsequent studies.

Inasmuch as androgens seem to have a clear inhibitory role in porphyrogenesis, it is still not clear whether there is a factor that can stimulate the synthesis of porphyrins independently of the lack of androgens. Prolactin might be one of these "stimulating factors" since it has been shown that blinding, while reducing prolactin, also reduces the porphyrin content in harderian glands of female hamsters (8); also, bromocriptine, a dopamine agonist and consequently a potent prolactin inhibitor, is able to prevent postcastrational porphyrin rise in male Syrian hamsters (8). However,

in one study bromocriptine was ineffective in reducing the high porphyrin levels of female glands. Hence, the possible existence of dopamine receptors in the hamster harderian gland should be investigated.

We conclude that although testicular hormones are the most potent inhibitors of porphyrin biosynthesis in the harderian gland of Syrian hamster, the possibility that other hormones (e.g., LH, prolactin, etc.) may also be involved in porphyrogenesis in the harderian glands is still an open question.

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