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Short Contribution

Effects of prepubertal manipulation with androgens on the development of sexual differences in the Harderian glands of Syrian hamsters

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ABSTRACT The onset of sexual differences in the metabolism of porphyrins and melatonin in the Harderian glands of Syrian hamsters was studied. Three weeks after birth, the porphyrin concentrations were already higher in glands of females than in those of males. Castration of 22-day-old male hamsters led to an increase in Harderian porphyrin concentrations, although the levels of intact females were not reached. The administration of testosterone to 22-day-old female hamsters resulted in a marked decrease in porphyrin concentrations. Study of the development of sexual differences in the enzymes involved in melatonin synthesis, N-acetyltransferase (NAT) and hydroxyindole-O-methyltransferase (HIOMT) indicated that not all the sexual differences observed in these glands begin at the same time. Thus, while differences in NAT activity were detected after the age of 3 weeks, male-female differences in HIOMT activity were only observed after 7 weeks. Castration of prepubertal male hamsters lowered NAT but not HIOMT activities. The administration of testosterone to prepubertal female hamsters led to male activity levels in both enzymes. Although circulating androgens seem to have a crucial role in maintaining sexual differences, other hormones including those from the pituitary and thyroid glands are probably also important for generating these sexual differences.

KEY WORDS: *porphyrin, melatonin, Harderian gland, hamsters*

The Syrian hamster Harderian gland has recently been used as a model of extrapineal melatonin production and porphyrin biosynthesis (Hoffman *et al.*, 1985; Menéndez-Peláez *et al.*, 1987; Buzzell *et al.*, 1989; Marrufo *et al.*, 1989). Although these are common orbital glands in mammals, Harderian glands are without an assigned physiological role. Suggested functions include interaction with the light-pineal-gonadal axis (Hoffman *et al.*, 1985) and secretion of hormones (Menéndez-Peláez, 1990). Harderian glands of Syrian hamsters display a marked sexual dimorphism and have been extensively studied. Female hamster Harderian glands, which are dark-gray in color and possess a single secretory cell type, contain large amounts of porphyrins and detectable concentrations of melatonin and other indoleamines. On the other hand, male glands, which are pale and possess two types of secretory cells, contain relatively little porphyrin and melatonin (Hoffman, 1971; Hoffman *et al.*, 1985). Castration of male hamsters induces a feminization of the gland, and androgen replacement restores the male phenotype (Hoffman, 1971; Payne *et al.*, 1977; Marrufo *et al.*, 1989). For some of the parameters studied, this androgenic control is similar to that in male accessory sex glands, where testosterone is intracellularly converted to 5α-dihydrotestosterone (Menéndez-Peláez *et al.*, 1990).

Both porphyrin and indole metabolism have been extensively studied in Harderian glands of adult Syrian hamsters (Hoffman *et al.*, 1989; Buzzell *et al.*, 1990; Menéndez-Peláez and Buzzell, 1991). There are also some studies on the postnatal development of the rat (Feria-Velasco *et al.*, 1984; Osuna *et al.*, 1990) and the hamster (Bucana and Nadakavukaren, 1973; López *et al.*, 1990) Harderian glands. However, there are no studies on the appearance of sexual differences in porphyrin and indole metabolism during postnatal development in the hamster Harderian gland.

In this study we have found that the concentration of porphyrins in the Harderian glands of male and female hamsters after 10 days

Abbreviations used in this paper: NAT, N-acetyltransferase; HIOMT, hydroxyindole-O-methylatransferase; LH, lutenizing hormone; FSH, follicle stimulating hormone; TSH, thyroid stimulating hormone.

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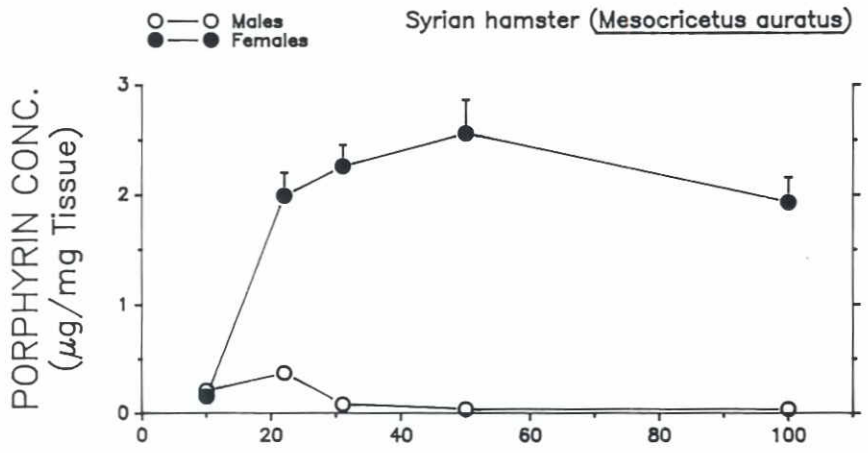


Fig. 1. Porphyrin concentration in the Harderian glands of male and female Syrian hamsters from 10 to 100 days of age.

of postnatal development is low and that in male hamsters, these low levels remained unaltered through all the stages studied. However, in the Harderian glands of female hamsters, an increase in porphyrin concentrations was observed 22 days after birth ($p<0.01$). These high levels appeared constant through the other stages studied, with a slight decrease in the 100-day-old group (Fig. 1).

Clearly, female glands contain more porphyrin than do male glands at 50 days of age ($p<0.01$). Porphyrin deposits can be visualized in the Harderian glands from female but not from male Syrian hamsters (Figs. 2, 3). Castration of prepubertal male hamsters induced an increase in the porphyrin concentration ($p<0.05$) 28 days later (Fig. 4, bottom panel). However, the porphyrin concentration of the intact females continued to be higher than that of castrated males ($p<0.05$). Administration of testosterone to prepubertal female hamsters resulted in a significant decrease in the porphyrin concentration of their Harderian glands ($p<0.01$) (Fig.4, bottom panel).

Sexual differences in NAT activity appeared at 22 days of age ($p<0.01$) (Fig. 5, top panel). At this age, female glands reached their peak in NAT activity. On the other hand, NAT activity in male Harderian glands did not plateau until the 30-day stage ($p<0.05$). After this point, NAT activity in male glands was three times higher than that of females. Castration of male prepubertal hamsters resulted in a significant decrease in NAT activity ($p<0.01$) (Fig. 4, top panel). The subcutaneous implantation of testosterone into prepubertal female hamsters produced a drop in NAT activity ($p<0.01$), reaching values comparable to those of intact males.

In contrast to porphyrin and NAT activity, the sexual differences in HIOMT activity began later, at the 50-day stage ($p<0.05$) (Fig. 5, middle panel). The activity of this methylating enzyme was low in the male Harderian gland at all stages. In the Harderian glands of female hamsters, HIOMT activity increased at the 50-day stage and maintained its high values in the 100-day group.

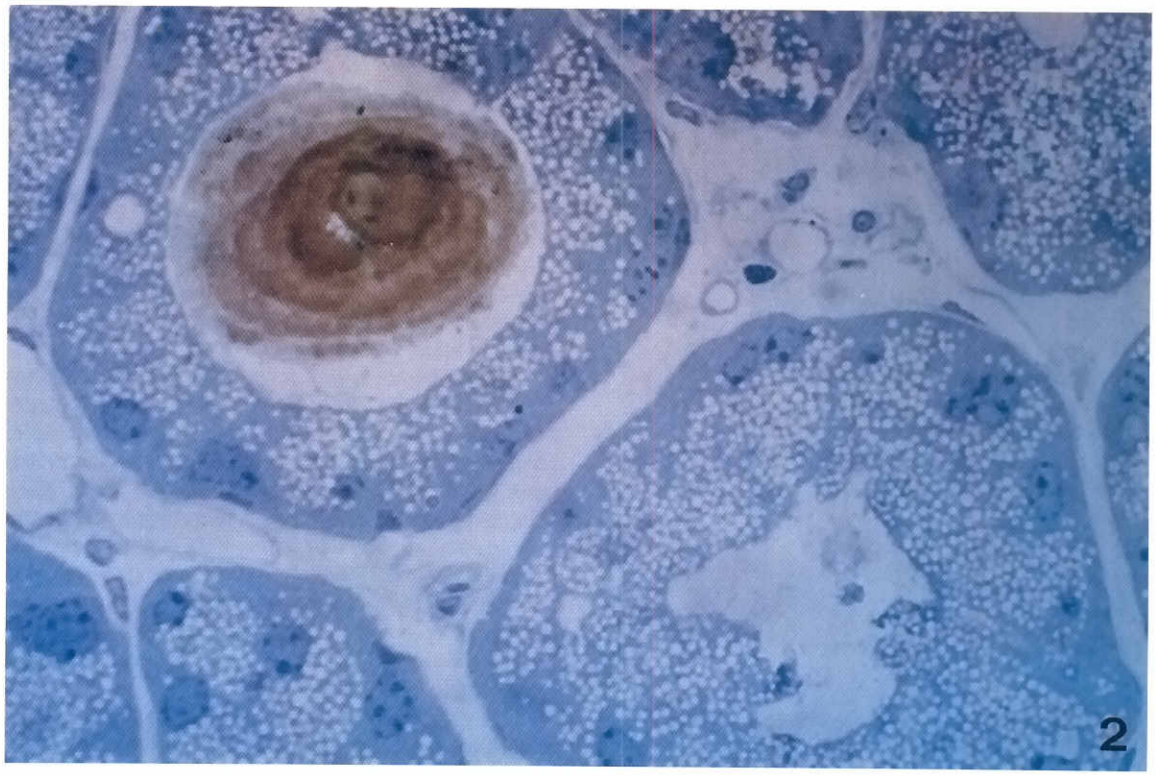
Castration of prepubertal male hamsters did not modify HIOMT activity. However, testosterone implants in young females produced a significant decrease in HIOMT activity ($p<0.01$) (middle panel). These values did not differ from those of intact and castrated males.

The melatonin concentration of both male and female glands was relatively high in the early stages of development (Fig. 5, bottom panel). In males, the melatonin concentration of Harderian glands decreased during postnatal development, reaching by 22 days the same low levels observed in adult animals. Female Harderian glands contained similar high melatonin concentrations during the entire study.

The postnatal development of porphyrin content in the Harderian glands of Syrian hamsters has received little attention. Previous studies have been performed in mice Harderian gland (Strong, 1942; Figge and Davidheiser, 1957) and in the hamster from 2 to 24 months of age (Spike *et al.*, 1988). However, this is the first report analyzing the porphyrin content of the hamster Harderian gland during the first 2 months of life. The marked sexual differences already evident by 22 days should be carefully discussed. It is generally accepted that female hamsters do not reach sexual capabilities before the first month after birth (Bex and Goldman, 1977). In male hamsters, androgens rise sharply after day 30, peaking on day 50 (Vomachka and Greenwald, 1979). However, it has been proposed that circulating androgens are exclusively responsible for the sexual differences observed in Harderian porphyrin content (Payne *et al.*, 1977). The results presented in our studies suggest that factors other than gonadal androgens might be involved in porphyrinogenesis, since the concentration of Harderian porphyrin increased between the 10- and 22-day stages in female, but not in male glands. Structural and ultrastructural changes have been found in the Harderian glands of male and female hamsters during this period of time (Bucana and Nadakavukaren, 1973; López *et al.*, 1990). There can be little doubt that testosterone inhibits Harderian porphyrinogenesis since the administration of testosterone to prepubertal females led to a marked decrease in the porphyrin content. We have previously shown that administration of 5α-dihydrotestosterone is also effective in decreasing porphyrin levels in female glands (Marrufo *et al.*, 1989). Treating castrated male hamsters with other androgens prevents the rise in Harderian porphyrin content (Payne *et al.*, 1977). Moreover, androgen receptors are present in the Harderian glands of hamsters (Vilchis *et al.*, 1987; Stankov *et al.*, 1989).

The data suggest that the lack of testosterone in females is not a porphyrinogenic factor; rather that testosterone is a potent inhibitor of porphyrinogenesis. However, other factors might be involved in the onset of the sexual differences observed, since serum testosterone levels are very low in male hamsters until 30 days of age (Vomachka and Greenwald, 1979), by which time the porphyrin differences are well established.

Hoffman (1971) and Clabough and Norvell (1973) suggested some time ago that gonadotropins might be involved in Harderian porphyrinogenesis. In female but not in male hamsters, serum LH



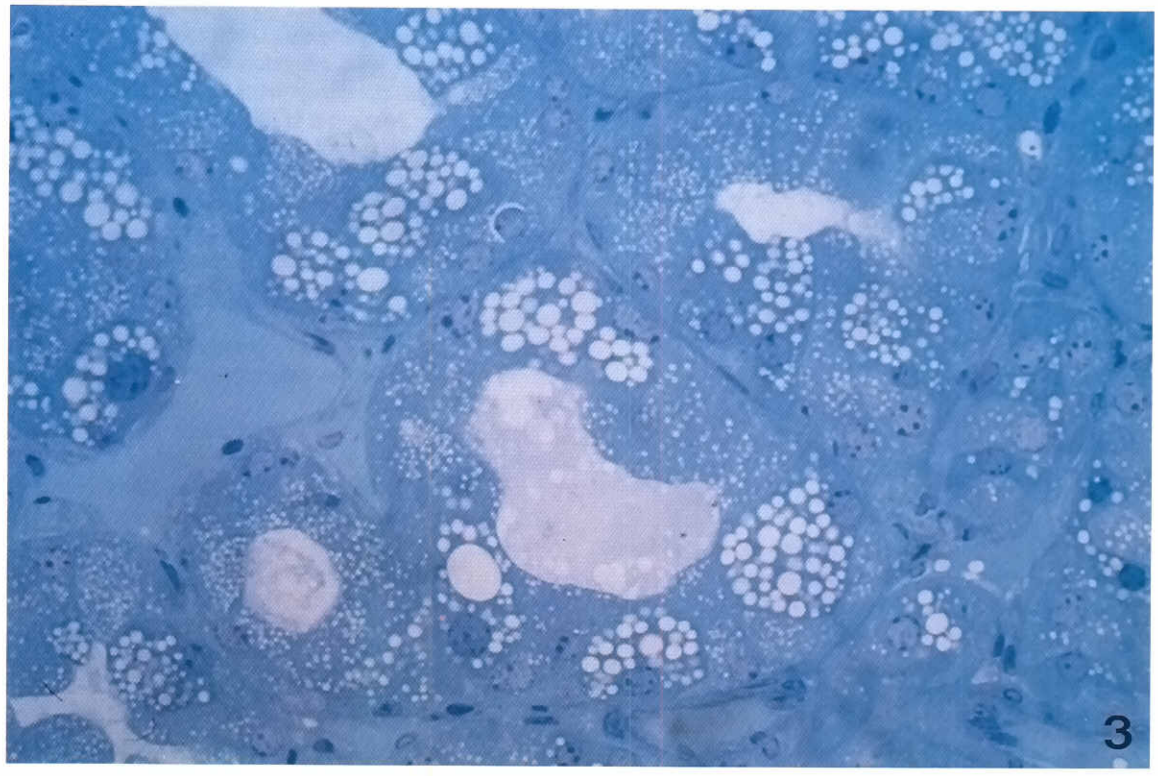


Fig. 2. Semithin section from a female Syrian hamster Harderian gland showing a dark brown intraluminal deposit of porphyrin. *A single secretory cell type is observed.*

Fig. 3. Semithin section from a male Syrian hamster Harderian gland. *No porphyrin deposits are observed. Two secretory cell types are clearly distinguishable.*

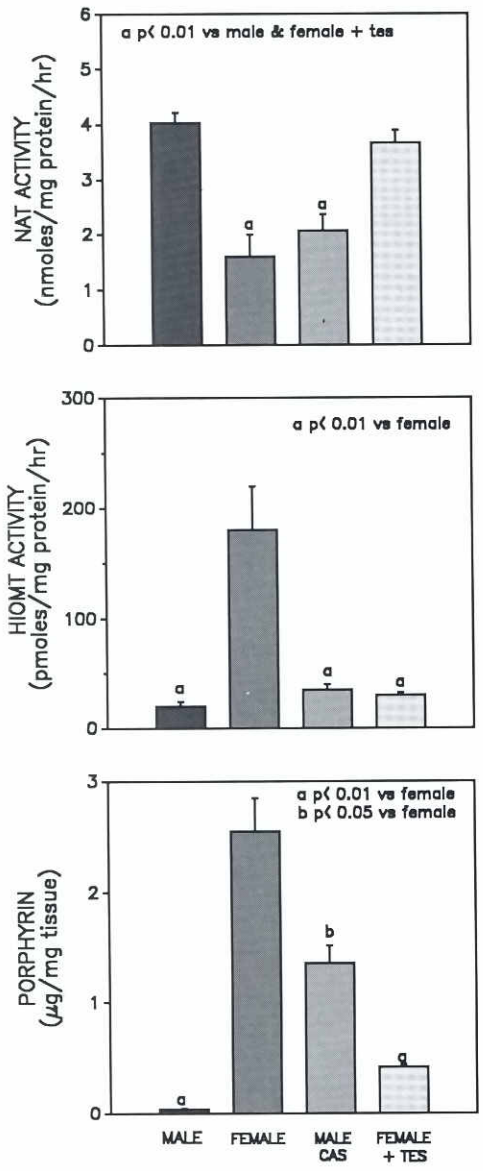


Fig 4. Effects of prepubertal male castration (CAS) or exogenous administration of testosterone to prepubertal females (TES) on NAT activity (top panel), HIOMT activity (middle panel), and porphyrin concentration (bottom panel).

levels peak at days 19-21 and FSH levels peak between days 20 and 25 (Bex and Goldman, 1977; Vomachka and Greenwald, 1979), which are near the times when we found female Harderian porphyrin levels rising dramatically above those of males (Fig. 1). These results are consistent with gonadotropins controlling the onset of porphyrinogenesis. The sharp increase in testosterone levels after the first month in males would then inhibit porphyrinogenesis in male (but not female) Harderian glands.

Prolactin has also been proposed as a porphyrinogenic hormone since reducing serum prolactin levels with blinding or with dopamine against bromocriptine, prevented the castration-induced rise in porphyrin concentration in male hamsters (McMasters and Hoffman, 1984; Buzzell *et al.*, 1989). However, serum prolactin levels are identical in male and female hamsters until at least day 30 (Vomachka and Greenwald, 1979), so the sexual differences observed at 22 days of age would not be explained by differences in circulating prolactin. The possibility that other hormones control the onset of porphyrinogenesis is open. Thus, it is known that the injection of newborn rats with thyroxine leads to an early appearance of Harderian porphyrins, although the attainment of maximal porphyrin concentration is delayed (Wetterberg *et al.*, 1970).

Indole metabolism in the Harderian glands also seems to be under androgenic control (Hoffman *et al.*, 1985). NAT activity is higher in male than in female Harderian glands (Menéndez-Peláez *et al.*, 1988). Androgen deprivation in males or androgen administration to female hamsters modifies these phenotypes (Menéndez-Peláez *et al.*, 1988, 1989). In our study, sexual differences could be observed at 22 days of age when female glands reached the NAT peak while the Harderian NAT activity in male glands continued increasing until 30 days of age. As was the case of porphyrinogenesis, the attainment of sexual differences in NAT is unlikely to be due to circulating androgen because male testosterone levels begin to rise after NAT activity is maximal. In addition, although serum testosterone level reaches a peak on day 50 (Vomachka and Greenwald, 1979), no differences in NAT activity were observed between 30 and 50 days of age.

Testosterone and other androgens have important roles in the maintenance of high NAT activity in male hamsters, but the activity observed in females, though lower than that of males, is respectable, suggesting that other factors are involved in the regulation of this acetylating enzyme. Supression of prolactin levels with bromocriptine has no effect on Harderian NAT activity in either male and female Syrian hamsters (Buzzell *et al.*, 1989). Castration and short days both lead to reduced Harderian NAT activity in male hamsters, but these experimental conditions lead to increased and decreased gonadotropin levels respectively (Turek *et al.*, 1975), suggesting that gonadotropins do not directly influence the activity of NAT.

It has been suggested that TSH or thyroid hormones influence Harderian gland NAT activity in Syrian hamsters. Treatment of intact female or intact and castrated male Syrian hamsters with methimazole, resulting in a decrease in thyroid hormones and an increase in TSH, produced a rise in NAT activity (Buzzell *et al.*, 1990). Unfortunately, there is insufficient data concerning serum TSH or thyroid hormone levels in female and male hamsters during postnatal development.

The sexual differences observed in HIOMT activity became evident later than the other parameters studied. The hormonal control of the Harderian methylating enzyme seems to be very complex. In a previous study, we showed that castration of prepubertal (25 days old), but not adult, hamsters produced an increase in HIOMT activity 50 days later (Menéndez-Peláez *et al.*, 1988). In the present study we were unable to repeat these results with animals castrated on the 22nd day. We also found that testosterone administered to prepubertal females led to reduced HIOMT activity. However, in a previous study, the administration of androgens to adult female hamsters did not modify HIOMT activity (Marrufo *et al.*, 1989). Thus, the roles of androgens and other hormones in the control of Harderian HIOMT activity is still unclear.

Melatonin has been detected in the Harderian glands of several rodents (Menéndez-Peláez, 1990; Menéndez-Peláez and Buzzell, 1991). Although this indole may be taken up into the Harderian

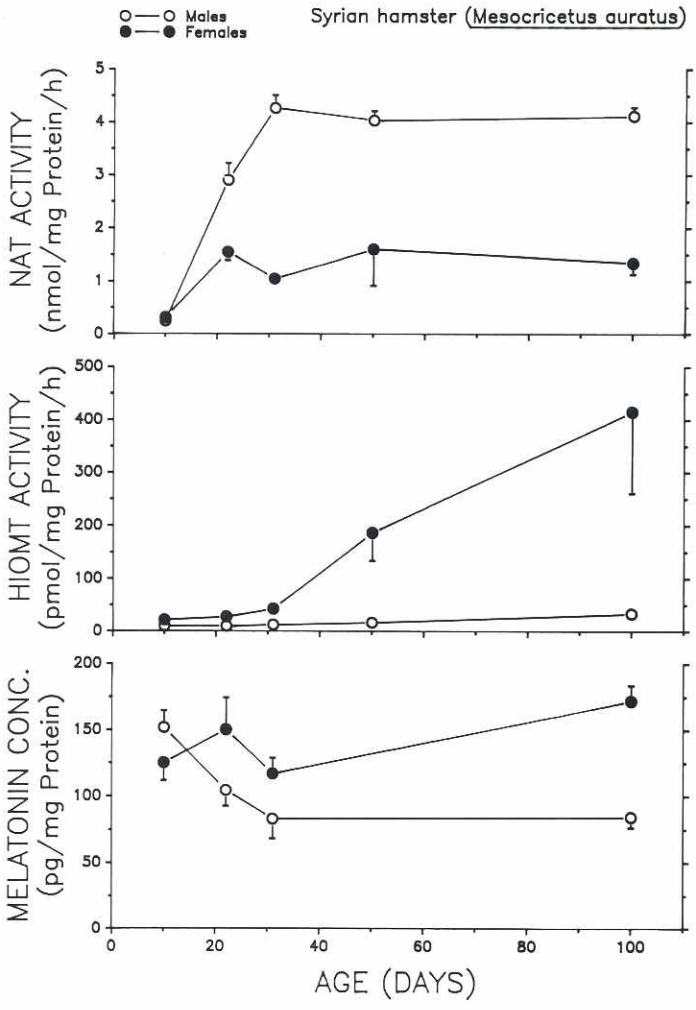


Fig. 5. NAT activity (top panel), HIOMT activity (middle panel), and melatonin concentration (bottom panel) in the Harderian glands of male and female hamster during postnatal development.

gland from the circulation, (Hoffman *et al.*, 1985), we have shown that melatonin can be synthesized within the Harderian glands of female hamsters (Menéndez-Peláez *et al.*, 1987). The results obtained in the present study suggest that the high melatonin values found in the Harderian glands of both sexes during the first 3 weeks of extrauterine life might be a consequence of uptake from circulation, since both NAT and HIOMT activities were very low at this time. Contrary to the situation in the pineal gland, Harderian melatonin concentrations seem to be correlated with HIOMT instead of NAT activities (Menéndez-Peláez *et al.*, 1987, 1988). In the present study, the increase in melatonin observed in the Harderian glands of female hamsters from 30 to 100 days of age runs parallel to the increase in HIOMT activity.

In conclusion, the sexual differences observed both in the porphyrin and indole metabolism in the Harderian glands of Syrian hamsters develop independently of the circulating levels of androgens. The hormones or factors generating these differences might be produced by the pituitary, but their precise nature is still unknown.

Experimental Procedures

Sixty-three male and 66 female Syrian hamsters *(Mesocricetus auratus)* were used in this study. The animals, which were born and maintained in the Colgate University colony, were kept grouped by sex (4 per cage) under a light:dark cycle of 14:10 h (lights on at 0900 h) and a temperature of 22 ± 2°C. Food and water were provided *ad libitum*.

The animals were sacrificed by decapitation 10, 22, 31, 50 and 100 days after birth (10-12 hamsters per group). Eight 22-day-old male hamsters were bilaterally gonadectomized under ether anaesthesia and killed at the same time as the 50-day intact group. Eight 22-day-old females were subcutaneously implanted with testosterone (1 mg in 24 mg of beeswax) twice (at the 22- and 47-day stages) and sacrificed at the same time as the 50-day-old group. Harderian glands were rapidly removed and frozen on solid CO_2. Some of the samples were fixed and processed for conventional light microscopy.

Porphyrin concentrations were determined according to routine methods (Buzzell *et al.*, 1989). Both N-acetyltransferase activity (NAT) and hydroxyindole-O-methyltransferase activity (HIOMT) were measured following the method described by Champney *et al.* (1984) and Menéndez-Peláez *et al.* (1989). Melatonin concentrations of the glands were determined by radioimmunoassay (Rollag and Niswender, 1976). The protein content of the samples was estimated by the method of Lowry *et al.* (1951).

Data were analyzed by a one-way analysis of variance (ANOVA) followed by a Newman-Keuls test.

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