in the untreated medium for 24 h were active (figure). With prolonged incubation time the activity of the CA declined. After 72 h about one half of the CA were inactive and after 120 h all the glands were devoid of activity.

Almost all CA incubated for 24 h in the medium containing precocene at a concentration of $10^{-4.35}$ M were inactive. The same incubation time with a precocene concentration of $10^{-5.35}$ M reduced the activity only slightly. However, the incubation for 48 h at the latter concentration inactivated 69% and the still longer incubation for 72 h inactivated all the glands. These 2 values differ with high significance from the control data in Fisher's test[14]. The lower concentration of 10^{-6} M did not affect the activity after 120 h of incubation.

In the second series of experiments CA were incubated in a medium recently described by Shields and Sang[11]. In the control medium 90% of the glands remained active even after 10 days of incubation (table). In the medium containing precocene at the concentration $10^{-4.35}$ M only 10% of the glands were active after 5 days. Similar results were obtained after 10 days incubation with a concentration 10 times lower. On the other hand, in the medium containing β-asarone at the high concentration of $10^{-4.32}$ M all the glands remained active after 5 days incubation.

Discussion. CA taken from fertile females of *O. fasciatus* and cultivated in vitro for a considerable period of time remain morphogenetically active. This activity declines faster in Landureau's medium S_{20F}[10] than in Shield and Sang's medium[11]. Addition of precocene II to either medium causes a rapid decline in activity of the cultivated glands. These glands do not regain their activity when transplanted into untreated last instar larvae. β-asarone[12] is chemically related to precocene yet it lacks any morphogenetic activity when applied in sublethal dosages to *O. fasciatus* in vivo (P.J. Müller, unpublished results). This compound was also found to be inactive in our in vitro system at a concentration 10 times higher than the active dose of precocene. The results indicate firstly that precocene acts directly on the CA and inhibits its morphogenetic activity and secondly that the inhibitory effect is specific to precocenes.

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Effect of testosterone on the hypothalamic-pituitary-gonadal axis of pinealectomized and pineal-intact male rats[1]

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Summary. Previous studies indicate that steroid hormones alter pineal biochemistry, and it has been suggested that at least part of the negative feedback effect of steroid hormones on pituitary gonadotropin release may be mediated by the pineal gland. In this study, pinealectomy did not alter the inhibitory effect of testosterone on neuroendocrine-gonadal activity in the male rat, suggesting that the pineal gland does not mediate the response of the rat hypothalamic-pituitary axis to testosterone.

The administration of either gonadal steroid hormones or the pineal product, melatonin, to rats inhibits neuroendocrine-gonadal activity[2,3]. A number of reports indicate that steroid hormones can modulate pineal melatonin synthesis[4,5] and this has led to the suggestion that the pineal gland may mediate, in part, the negative feedback effect of gonadal steroids on hypothalamic-pituitary activity[6]. Evidence for an interaction between melatonin and steroid hormones in male rats is found in the observation that castration leads to a decrease in pineal hydroxyindole-O-methyltransferase activity (HIOMT), the enzyme which converts N-acetyl serotonin to melatonin, while the administration of testosterone propionate stimulates pineal HIOMT activity[7]. Similar effects are found following ovariectomy and estrogen treatment in female rats[8,9]. If the inhibitory action of steroid hormones on pituitary gonadotropin release is indeed modulated by the pineal gland, then pinealectomy should alter the response of the neuroendocrine-gonadal axis to steroid treatment. In this study, we sought to test the hypothesis that the pineal gland mediates the action of steroid hormones, by determining if pinealectomy alters the response of the hypothalamic-pituitary-gonadal axis to the negative feedback effect of testosterone in intact and castrated male rats.

Mature male albino rats weighing 180–200 g were purchased from ARS Sprague-Dawley, Madison, Wisconsin. The animals were maintained throughout the studies in a room provided with 14 h of light per day (LD 14/10), and they were housed 4–5 per cage with food (Purina rat chow) and water provided ad libitum. After an acclimation period of 2–3 weeks, the animals were either sham-pinealectomized (sham-Px) or pinealectomized (Px) according to the method of Hoffman and Reiter[10]. In 1 study, rats were then implanted s.c. with either empty or testosterone-filled silastic capsules that were 10 or 20 mm long (8/group). In a 2nd study, sham-Px and Px animals were castrated and 9–11 days post-castration they were implanted with either empty or testosterone capsules that were 10 or 20 mm long (8/group). 60 days following capsule implantation, the animals were sacrificed by decapitation, blood was collected, and the testes were removed and weighed.

Serum luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were assayed following methods previously described[11], and values are expressed in terms of NIH-LH-S16 and NIAMDD-Rat-FSH-RP-1 per ml of serum. Crystalline testosterone was purchased from Sigma Chemical Company and the silastic capsules (Dow Corning Co., Cat. Nr. 602-305) were prepared as previously described[12]. The amount of testosterone released from subdermal silastic capsules is relatively constant and proportional to capsule length[13]. Data were analyzed by Student's t-test.

Pinealectomy in testes-intact rats that were implanted with either empty or testosterone filled capsules did not result in any significant change in testis weight or in serum levels of LH and FSH (figure 1). The 10-mm-long testosterone capsule induced a significant ($p < 0.05$) decrease in testis weight and serum FSH levels in both Px and sham-Px animals when compared to animals with empty implants, and the 20-mm testosterone induced a further significant ($p < 0.01$) decrease in these 2 parameters. Serum LH in the sham-Px and Px animals treated with empty capsules were already at the lower limits of detectability of our assay, and no further decreases following steroid treatment could be observed.

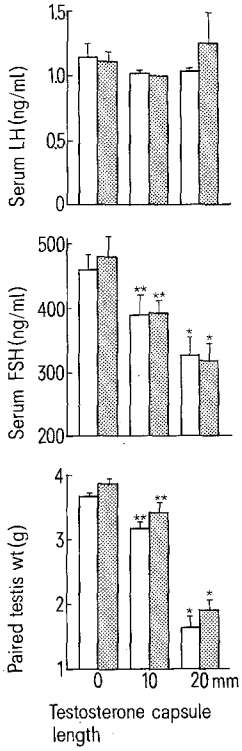

Fig. 1. Mean (±SE) paired testis weight, and serum levels of immunoreactive LH and FSH in sham-Px (open bars) or Px (striped bars) rats that were implanted with either empty or testosterone-filled silastic capsules that were 10 or 20 mm in length for 60 days. **$p < 0.05$, *$p < 0.01$.

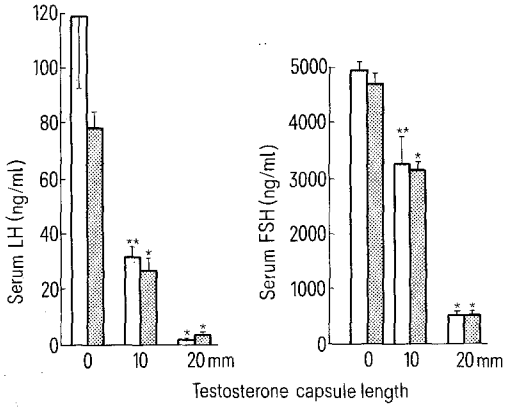

Fig. 2. Mean (±SE) serum levels of immunoreactive LH and FSH in castrated rats that were either sham-Px (open bars) or Px (striped bars). The animals were implanted with either empty or testosterone-filled silastic capsules that were 10 or 20 mm in length for 60 days prior to sacrifice. ** $p < 0.01$; * $p < 0.001$ vs empty capsules.

Castration of sham-Px rats resulted in an elevation of serum levels of FSH and LH (untreated animals in figure 1 and figure 2). Testosterone treatment via 10-mm-long capsules for 60 days caused a significant ($p < 0.01$) decrease in serum levels of LH and FSH while treatment with a 20-mm implant induced a further decrease ($p < 0.001$) in serum gonadotropin levels. Pinealectomy did not alter serum gonadotropin levels in castrated rats implanted with either empty or testosterone-filled capsules.

The results of the present study indicate that the pineal gland is not involved in mediating the negative feedback effects of testosterone on neuroendocrine-gonadal activity in either intact or castrated rats. If at least part of the inhibitory effect of testosterone on pituitary gonadotropin release was mediated via the pineal gland, testicular atrophy, and the reduction in serum gonadotropin levels which result from testosterone treatment were expected to be more pronounced in sham-Px than in Px animals. Instead, both a sub-maximum (10 mm) and a maximum (20 mm) inhibitory dose of testosterone had the same effect in both sham-Px and Px animals. Thus, the physiological significance of previously reported testosterone-induced changes in the biochemistry of the rat pineal gland remains to be determined.

A recent study on the golden hamster reports that pinealectomy does not significantly alter the inhibition of pituitary gonadotropin release by steroid hormones in animals maintained on LD 14/10, whereas, the increased responsiveness of the gonadotropin control center to steroid feedback observed in animals exposed to short days (LD 6/18) was suppresed by pinealectomy[14]. Thus, photic-induced changes in steroid-feedback sensitivity appear to be mediated via the pineal gland in the hamster. Although the photoperiod has very little, if any effect on testicular function in the rat[15], it should be noted that the present studies were carried out only in animals maintained on LD 14/10. Whether or not the pineal gland mediates the effect of testosterone on the neuroendocrine-gonadal axis of rats exposed to other photoperiodic conditions, remains to be determined.

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