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Juvenoid Biosynthesis by Corpora Allata of Adult Female and Male Loreyi Leafworm, *Mythimna loreyi* Duponchel (Lepidoptera: Noctuidae)

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Rong Kou and Meng-Ping Tu (1998) Juvenoid biosynthesis by corpora allata of adult female and male loreyi leafworm, *Mythimna loreyi* Duponchel (Lepidoptera: Noctuidae). *Zoological Studies* **37**(2): 119-125. In adult female *Mythimna loreyi*, the maximum rate at which the corpora allata (CA) incorporate L-[*methyl*- ^{3}H]-methionine in vitro was determined to be at 7 μM methionine concentration, and the greatest hourly incorporation (3.7 pmol/h/CA) occurred during 6-h incubations; both in vitro release of juvenile hormone (JH) II and JH III by female CA were age-dependent and showed similar trends, which peaked at day 4 (2.9 pmol JH III/4 h/CA) and day 9 (5.0 pmol JH III/4 h/CA) of adulthood. For adult male CA, the greatest hourly release of juvenile hormone acid (JHA) III occurred during the 1st 2-h incubations (27.7-37.8 pmol/h/CA); the in vitro release of JHA I, Iso-JHA II, and JHA III by male CA was also age dependent; the trends of JHA I and Iso-JHA II are similar, peaking at day 3 and day 10; for JHA III, the release rate peaked at day 4 and day 10. Male CA from different time intervals of a 24-h photoperiod showed different abilities to release JHA: from 5 h into photophase and 5 h into scotophase they showed the highest release rates of JHA I and JHA III, respectively; from 1 h and 5 h into scotophase, and 3 h into photophase they showed the higher release rates of Iso-JHA II; and from any time interval showed a constant low release rate of JHA II.

Key words: Juvenile hormone, Juvenile hormone acid, Radiochemical assay.

Juvenile hormones (JHs) are structurally related sesquiterpenoids secreted by the corpora allata (CA) of insects. Among the 6 identified JHs, all 4 ethyl-branched JHs (i.e., JH 0, Iso-JH 0, JH I, and JH II) are found in the Lepidoptera (Schooley and Baker 1985). In Lepidoptera, many JH studies have focused on the larval stages, with only a few on the adults life. In Lepidopteran adult females, such as *Manduca sexta*, the major release products of CA are JH II and JH III, when the in vitro release rate is measured for 9 days following adult emergence, no particular trend in the JH homologue ratio exists (Ishizaka et al. 1989). In *Helicoverpa zea*, the major release products of CA also are JH II and JH III, and there is an increase in the release of JH II and III with age, while the release of JH I remains low and uniform (Satyanarayana et al. 1991). In *Pseudaletia unipuncta*, the major release products of CA are JH I and JH II, and the release of these JHs increases with age under different rearing conditions (Cusson et al. 1990 1993). In Lepidopteran adult males, the major release products of CA are JH acid (JHA) I and II for *Hyalophora cecropia* (Peter et al.1981), and JHA I, JHA II, and homo-farnesoic acid (FA) for *P. unipuncta*. The release of these JHA homologues increases with age under different rearing conditions (Cusson et al. 1993).

In previous studies, we reported that *Mythimna* (*Leucania*) *loreyi* adult moths possess isolated cell type CA, and that the male hypertrophic CA is more than 20 times larger than that of the female (Kou et al. 1995). The major release products of CA in *M. loreyi* are identified as JH II and JH III for females, and JHA I, JHA II, Iso-JHA II, and JHA III for males (Ho et al. 1995). Here we further inves-

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tigate the quantities of JHs and JHAs released by the isolated cell type CA of *M. loreyi* females and males, under varying incubation conditions, age, and photoperiods as a basis for further research on how moths control production of JHs and JHAs.

MATERIALS AND METHODS

Animals

Larvae of *M. loreyi* were reared on a modified artificial diet (Shorey and Hale 1965) and the sexes separated at the pupal stage. Larvae and pupae were maintained under a 16L:8D regime at 26-28 °C. Emerged moths were fed with an 8% sucrose solution.

L-methionine concentration

For female CA, the optimum cold L-methionine concentration required to produce maximum incorporation rates was determined by incubating 1 pair of CA in 50 μl medium TC 199 lacking methionine and suplemented with calcium chloride (5 mM), Ficoll 400 (20 mg/ml, Sigma Chem. Co.), various amounts of cold L-methionine and always 1 μCi L-[*methyl*- ^{3}H]-methionine (NEN, final specific radioactivity of 83.1 Ci/mmole) to obtain total methionine concentrations of 0. 24, 7, 20, 40, 60, 80, 100, 200, 300, and 400 μM. Incubations were conducted for 4 h at 28 °C with shaking. At the end of incubation, the medium was extracted 3 times with 150 μl isooctane, and this female extract was dried under N_2; 400 μl cocktail (Ready Safe$_{TM}$, Beckman) was added and analyzed for radioactivity by liquid scintillation spectrometry (LSS). A total of 5 replicates were taken for this experiment.

Incubation duration

To select the optimum incubation duration of female CA, incubations were conducted for 0.5, 1, 2, 3, 4, 5, 6, 8, 10, 12, and 24 h. Each pair of CA from 4-d-old females was incubated in 50 μl medium TC199 containing 7 μM L-methionine and 1 μCi L-[*methyl*- ^{3}H]-methionine. For male CA, incubations were conducted for 1, 2, 3, 4, 6, and 12 h. Each pair of CA from 4-d-old males was incubated in 100 μl medium TC199. This experiment used 5 and 12 replicates for females and males, respectively.

Thin-layer chromatography (TLC)

TLC of female extract and synthetic standards (JH I, JH II, and JH III) were performed on 0.25-mm plastic-backed silica F254 plates (Merck, Germany) with ether-hexane (1:3, v/v). Synthetic standards of JH I and JH III were obtained from Sigma Chem. Co., and JH II was obtained from the Institute of Organic Chemistry and Biochemistry, Academy of Sciences of the Czech Republic.

Influence of age on the biosynthetic activity of female and male CA

Female CA were dissected at predetermined ages and each pair of glands was incubated in 50 μl medium TC199 containing 7 μM cold L-methionine and 1 μCi L-[*methyl*- ^{3}H]-methionine. Because the results of the above incubation showed no significant difference between 4 h and 6 h duration, incubations in this experiment were conducted for 4 h at 28 °C with shaking. Eighteen pairs of glands were included for each age group. For male CA, the procedure was similar except that each pair of glands was incubated in 100 μl medium TC199 for 24 h. The 24-h incubation was needed because only JHA III could be detected with our GC method if glands were incubated for only a few hours.

At the end of incubation, glands were removed and the released products extracted from the medium with isooctane (female) or chloroform (male) (Pratt and Tobe 1974, Feyereisen and Tobe 1981). Female extracts were reduced under N_2 to 5 μl/female; because the major release products of female CA were identified as JH II and JH III (Ho et al. 1995), the reduced female extract and synthetic JH II and JH III were spotted on alternate TLC lanes. Following development, the synthetic JH bands were visualized with UV and marked, and the appropriate zones of female extract were analyzed with LSS. Each male extract was dried under N_2 and treated with 10 μl ethereal diazomethane (Fales et al. 1973) to convert the acids to methyl esters, then 2 μl of the male ether solution was injected into a 35 m × 0.25 mm ID fused silica capillary column coated with a 0.25-μm film of DB-1 phase; the column was installed in a Shimadzu 14-A gas chromatograph (GC) equipped with a flame ionization detector. Chromatographic conditions were as follows: nitrogen carrier 0.5 kg/cm^2, and column temperature was isothermal at 250 °C. The amount of each JHA was calculated by multiplying the amount of each JHA methyl ester detected by GC and quantified with the external standard method of quantitative analysis (Ho et al. 1995) by 0.95 (The ratio of molecular weight of

JHA/JH is approximately 0.95). This experiment was replicated 12 times for both females and males.

Influence of photoperiod on the biosynthetic activity of male CA

From the beginning of the 3rd day scotophase, 10 pairs of male CA were dissected at 2-h intervals through the scotophase and photophase of that day. Each pair of CA was incubated in 100 µl medium 199 for 24 h at 28 °C with shaking. Detection of released JHA in the medium was described above. This experiment was replicated 12 times.

Statistical analysis

All results were analyzed with the Student-Newman-Keul's test (Steel and Torrie 1980)

RESULTS

L-methionine concentration

The influence of L-methionine concentration (cold methionine + 1 µCi L-[*methyl*- ^{3}H]-methionine) on in vitro incorporation by 4-d-old adult female CA is shown in Fig. 1. Maximum incorporation (8.2 pmol/4 h/CA) occurred at 7 µM total methionine with 1 µCi L-[*methyl*- ^{3}H]-methionine. L-methionine concentrations above 40 µM resulted in lower amounts of L-[*methyl*- ^{3}H]-methionine incorporation (below 0.28 pmol/4 h/CA). When L-methionine concentrations were above 100 µM, the incorporation almost stopped (below 0.09 pmol/4 h/CA).

Incubation duration

The greatest hourly L-[*methyl*- ^{3}H]-methionine in vitro incorporation of 4-d-old female CA occurred during 6-h incubations (3.7 pmol/h/CA); as the incubation time increased, the incorporation rate steadily declined and remained so up to 24 h (Fig. 2). For males, only JHA III could be detected from the hourly incubations, the greatest hourly release of JHA III from 4-d-old male CA occurred during 1-h incubation (37.8 pmol/h/CA), then the in vitro release rate of JHA III declined steadily up to 12 h (Fig. 3).

Influence of age on the biosynthetic activity of female and male CA

For female CA, the trends of JH II and JH III in vitro release as a function of age were very similar from day 1 to day 10 (Fig. 4). The release rate of JH III ranged from 0.7 pmol/ h/CA on day 9 to 0.08 pmol/h/CA on day 1; while JH II ranged from 0.27 pmol/h/CA on day 9 to 0.02 pmol/h/CA on day 1. The release rate on day 9 was significantly higher than that at the other ages ($p < 0.05$).

The trends of JHA release by different-aged male CA are shown in Fig. 5. For JHA I (Fig. 5A), the release rate increased significantly ($p < 0.05$) from 1.8 x 10^2 pmol/24 h/CA on day 1 to 5.8 $\times$ 10^2 pmol/24 h/CA on day 3, then decreased to about 1.5 $\times$ 10^2 pmol/24 h/CA on day 4 ~ 9; a 2nd peak of release rate was shown on day 10 (2.7 $\times$ 10^2 pmol/24 h/CA); for JHA II (Fig. 5B), the release rate was constantly low (below 1.0 $\times$ 10^2 pmol/24 h/

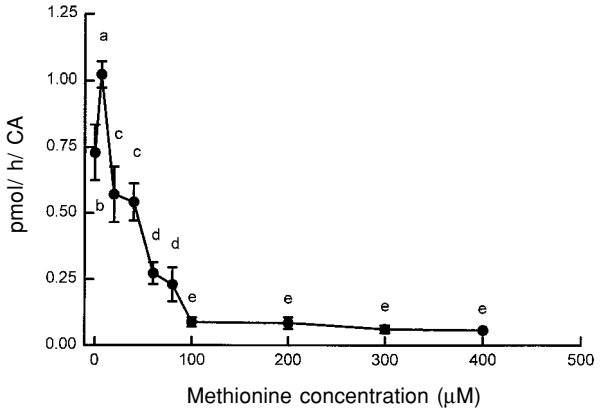

Fig. 1. Effect of methionine concentration (cold methionine + 1 µCi L-[*methyl*- ^{3}H]-methionine) on [*methyl*- ^{3}H] incorporation by the 4-d-old *Mythimna loreyi* adult female CA in vitro. Error bars: SEM.

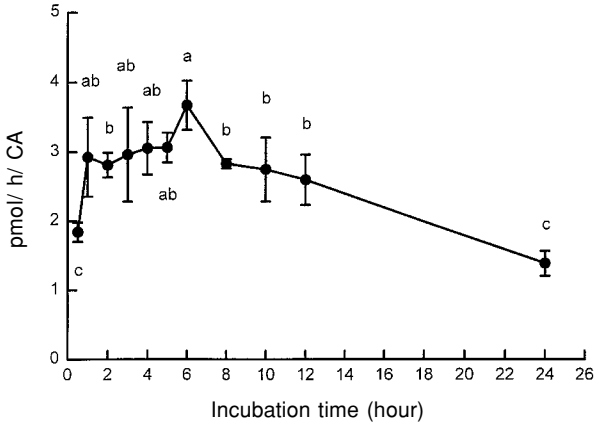

Fig. 2. Effect of incubation duration on [*methyl*- ^{3}H] incorporation by 4-d-old *Mythimna loreyi* adult female CA in vitro. Error bars: SEM.

CA) from day 1 to day 10; for Iso-JHA II (Fig. 5C), the release rate increased from 0.4 x 10^2 pmol/24 h/CA on day 1 to 2.7 $\times$ 10^2 pmol/24 h/CA on day 3, then decreased to about 0.8 $\times$ 10^2 pmol/24 h/CA on day 4 ~ 9, a 2nd peak of release rate was shown on day 10 (2.7 $\times$ 10^2 pmol/24 h/CA); for JHA III (Fig. 5D), the release rate increased significantly ($p < 0.05$) from 0.2 $\times$ 10^2 pmol/24 h/CA on day 1 to 4.9 $\times$ 10^2 pmol/24 h/CA on day 4, then decreased sharply to 0.7 $\times$ 10^2 pmol/24 h/CA on day 5, a 2nd peak also appeared on day 10 (3.5 $\times$ 10^2 pmol/24 h/CA).

Influence of photoperiod on the biosynthetic activity of male CA

Male CA from different time intervals of a 24-h photoperiod on the 3rd day showed different abilities to release JHAs (Fig. 6). For JHA I (Fig. 6A), significantly higher release rates were shown by CA at 5 h into photophase (4.8 $\times$ 10^2 pmol/24 h/ CA) ($p < 0.05$). For JHA II (Fig. 6B), a constantly

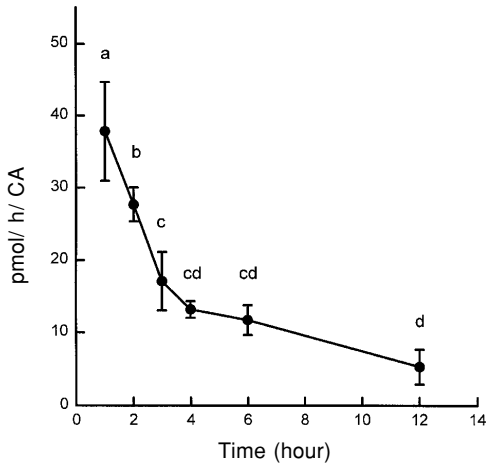

Fig. 3. Effect of incubation duration on JHA III release by 4-d-old *Mythimna loreyi* adult male CA in vitro. Error bars: SEM.

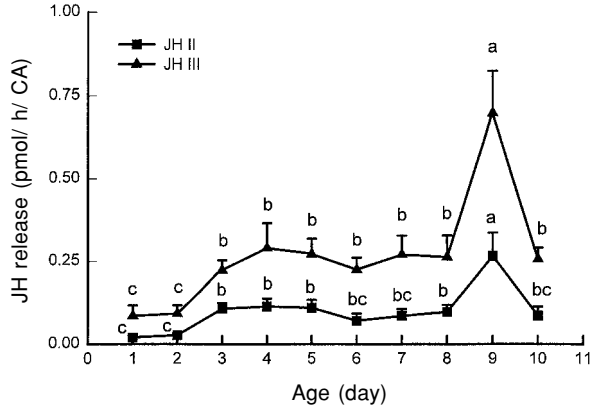

Fig. 4. Rate of juvenile hormone (JH) release in vitro (pmol/4 h/pair CA) by CA of 4-d-old *Mythimna loreyi* females as a function of age. Error bars: SEM.

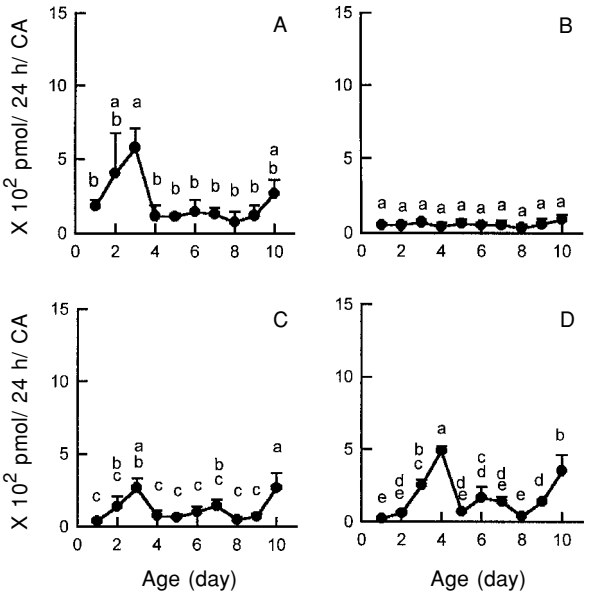

Fig. 5. Rate of juvenile hormone acid (JHA) release in vitro (pmol/24 h/pair CA) by CA of 4-d-old *Mythimna loreyi* males as a function of age. (A) JHA I, (B) JHA II, (C) Iso-JHA II, (D) JHA III. Error bars: SEM.

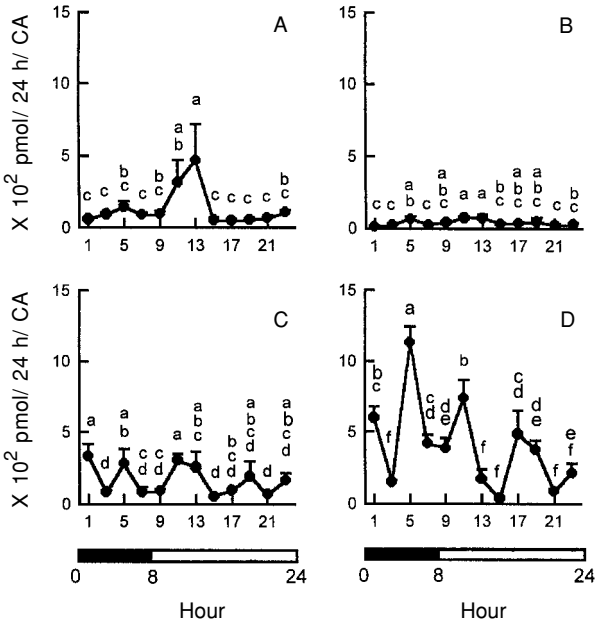

Fig. 6. Ability of CA from 3-d-old *Mythimna loreyi* males to release JHA in vitro (pmol/24 h/pair CA) at different time intervals of a 24-h photoperiod. (A) JHA I, (B) JHA II, (C) Iso-JHA II, (D) JHA III. Error bars: SEM.

low release rate was shown by CA from any time interval of the 24 h photoperiod. For Iso-JHA II (Fig. 6C), higher release rates were shown by CA at 1 h and 5 h into the scotophase ($3.3\text{-}2.8 \times 10^2$ pmol/24 h/CA), and 3 h into photophase (3.0×10^2 pmol/24 h/CA). For JHA III (Fig. 6D), a significantly higher release rate was shown by CA at 5 h into scotophase (12.5×10^2 pmol/24 h/CA) ($p < 0.05$).

DISCUSSION

The data presented here indicate that certain in vitro conditions can influence JH homologues released from female CA, and that the quantities of JH and JHA homologues released in vitro by the isolated cell type CA of female and male *M. loreyi* vary with age and time of the day in a 24-h photoperiod. CA of adult female *M. loreyi* are capable of high synthetic activity within a range of L-methionine (0. 24 ~ 40 μM, always accompanied with 1 μCi L-[*methyl*- ^{3}H]-methionine), and with a maximum incorporation response at 7 μM. This is the 1st such measurement in adult Lepidoptera. In *Lymantria dispar* larva, the maximum incorporation occurred at 25 μM total methionine (Jones and Yin 1989). In *Schistocerca gregaria* adult females, the optimum incorporation of hot-methionine occurred at 300 ~ 400 μM of total methionine (Tobe and Pratt 1974). Whether the concentration of L-methionine in adult *M. loreyi* haemolymph lies within the range that supports in vitro synthetic activity still needs further study.

The greatest hourly release of JH and JHA homologues of *M. loreyi* female and male CA occurred during the 6- (3.7 pmol/h/CA) and 3-h incubations (37.8 pmol JHA III/h/CA), respectively. In *L. dispar* larva, the greatest hourly incorporation (0.14 pmol/h/pair CA) occurred during 6-h incubations (Jones and Yin 1989); in adult female *S. gregaria*, labelled JH was detectable after a 10-min incubation and the rate of incorporation was constant for up to 4 h (Tobe and Pratt 1974). Whether the hourly in vitro release of JH and JHA homologues from CA of *M. loreyi* reflects the in vitro endocrinological activity of the gland will be further investigated.

In female *M. loreyi*, age-related trends of JH II and JH III in vitro release were quite similar, with JH III as the dominant form; the release rate was significantly higher for both JHs on day 9 (Fig. 4). In male *M. loreyi*, age-related trends for JHA I and Iso-JHA II were similar; this phenomenon may imply their biosynthetic similarity. But age-related trends for JHA II and JHA III were quite different from those of the other 2 JHAs, and lower release rates of JHA II were very uniform from day 1 to day 10 (Fig. 5B). In adults of noctuid moths, control of vitellogenesis by JH has been found in all species examined, such as in the corn earworm *H. zea* and the true armyworm *P. unipuncta* (Wyatt and Davey 1996). In *H. zea*, JH is the only hormone required in egg maturation, and no reproducible variations in the ratios of JH homologues were related to the age of the female (Satyanarayana et al. 1991); in *P. unipuncta*, the production of JH and JHA in females and males matches the rising proportion of females calling and males responding to the sex pheromone at 25 °C, so JH or JHA was proposed as a regulator of the maturation of a pheromonal communication system (Cusson et al. 1993). Also in this species, male sexual receptivity showed a correlation with the rate of JH synthesis which suggests dependence on JH action (McNeil et al. 1994). In *M. loreyi*, both female JHs and male JHAs peaked at 3 to 4-d old and 9 to 10-d old after emergence, respectively; generally, adults reached their strongest mating ability at 3 to 4 days after emergence, and become old and weak on day 10. But in *H. zea*, the general tendency of CA from old females to produce more JH is puzzling, because it is unrelated to ovarian development (Satyanarayana et al. 1991). We should further investigate whether this age-related JH and JHA in vitro release reflects the real physiological conditions in *M. loreyi*, such as ovarian maturation and pheromonal behavior mentioned in the above species.

The CA of male *M. loreyi* at certain time intervals of a 24-h photoperiod showed significantly higher in vitro biosynthetic activity of certain JHAs (Fig. 6); this phenomenon may imply that each JHA compound has its own physiological function, but this should be further elucidated.

In Lepidoptera, the mechanisms which regulate the production of different proportions of various JHs have received limited attention. The availability of precursors such as acetate, propionate, mevalonate, and homomevalonate has been shown to influence the proportion of JHs produced (Brindle et al. 1988). Recently, the synthesis of ethyl-branched JHs (EBJH) has been reported to be determined by the existence of transaminase, and it is hypothesized that variation in the activity or concentration of the branched-chain amino acid transaminase will change the composition of JHs (Brindle et al. 1992); if so, the biosynthetic pathway of male's JHAs may also be related to transaminase, and this will be further studied.

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羅氏夜蛾成蟲咽喉側腺之青春激素類物質合成作用

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羅氏夜蛾雌性成蟲咽喉側腺於體外培養，當 methionine 之濃度為 7 μM 時，腺體顯示出對 L-[*methyl*- ^{3}H]-methionine 之最大攝入率，再以培養之時間測量，則其最大攝入率為培養後六小時內，而腺體於體外培養時所分泌之青春激素 II 及青春激素 III 亦顯示出年齡依存之現象，此二激素具有相似之分泌趨勢，在第 4 及第 9 日齡各具有一分泌高峰期。

雄性成蟲之咽喉側腺於體外培養時，其青春激素酸之最大分泌率出現於培養後 3 小時內，同時各青春激素酸（青春激素酸 I，異－青春激素酸 II 及青春激素酸 III）之分泌亦為年齡依存，青春激素酸 I 及異－青春激素酸 II 顯示出相似之分泌趨勢，於第 3 及第 10 日齡之分泌量最大，而青春激素酸 III 則於第 4 及第 10 日齡之分泌量最大；在 24 小時光週期各時段之腺體亦顯示出不同之分泌能力。

關鍵詞：青春激素，青春激素酸，放射化學檢定。

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