Eur. J. Entomol. **90**: 37–44, 1993
ISSN 1210–5759

Immunoreactive progesterone concentration in some tissues of the cockroach *Nauphoeta cinerea* (Blattodea: Panchloridae) during development

PETER TAKÁČ [1], MILAN KOZÁNEK [1], ERIKA SOMOGYIOVÁ [2] and PAVEL VÝBOH [2]

[1] Institute of Experimental Phytopathology and Entomology, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovak Republik

[2] Institute of Animal Biochemistry and Genetics, Slovak Academy of Sciences, 900 28 Ivanka pri Dunaji, Slovak Republik

Physiology, progesterone, steroid hormones, ontogenetic development, radioimmunoassay, *Nauphoeta cinerea*

Abstract. The levels of progesterone radioimmunoassay-positive material in the gonads, intestine and haemolymph of the cockroach *Nauphoeta cinerea* during adult ontogenetic development were studied. The animals were divided into two groups: group 1 – control males and females reared for a period of 36 days after adult ecdysis at temperature 29 ± 1°C; group 2 – experimental males and females reared from day 8 to day 18 at 15°C and then at the same temperature as cockroaches of group 1. The immunoreactive progesterone concentration in gonads and intestine of females from group 1 reached the highest value on day 24 and 27. In females from group 2, the progesterone peak shifted to days 35–38. Females from group 1 gave birth to nymphs on day 36, females of group 2 on days 42–45. In males of group 2, a similar shift of the progesterone peak was observed.

INTRODUCTION

The question of the presence, distribution and functional role of vertebrate sex steroid hormones in insects has been studied during the last few years. Steroids have been demonstrated in a wide variety of living organisms (Lehoux & Sandor, 1970; Sandor & Mehdi, 1979; Sandor, 1980; Ando, 1982; De Loof et al., 1987). However, the recent development of more rigorous analytical techniques has led to the unequivocal identification of these steroids in several insect species. Testosterone, progesterone and other steroids have been identified in haemolymph of larvae of *Sarcophaga bullata* (Diptera: Sarcophagidae) (De Clerck et al., 1983; 1984) and *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae) (Diederik et al., 1984). Androgens and estrogens were identified in the body of adults of *S. bullata* and *Periplaneta americana* (Blattodea: Blattidae) (Mechoulam et al., 1984; Denlinger et al., 1987). Ohnishi (1985) demonstrated the presence of estradiol in ovaries of *Bombyx mori* (Lepidoptera: Bombycidae). Pregnenolone, testosterone and estradiol were identified in the locust *Locusta migratoria* (Orthoptera: Acrididae) (Novak et al., 1989). In the cockroach *Nauphoeta cinerea* nonecdysteroid steroids have already been demonstrated. Some were identificated by means of gas chromatography-mass spectrometry (GC-MS) (Novak et al., 1989), while the presence of estradiol, progesterone, testosterone and dihydrotestosterone was shown by radioimmuno-assay (Takáč et al., 1988).

These studies showed that vertebrate steroids could be demonstrated by GC-MS in whole body extracts, gonads and haemolymph and that the concentrations of vertebrate

steroid immunoactive substances measured by RIA were high enough to presume a hormonal role for insects.

Many recent reports have dealt with the occurrence of vertebrate-type steroids in insects (Bradbrook et al., 1990), with their biosynthesis and metabolism by insect tissues (Swevers et al., 1991), with reviews of vertebrate-type steroids in invertebrates and their possible functions (Lafont, 1991).

To investigate the latter possibility we report in the present paper changing progesterone concentrations in intestine, gonads and haemolymph of the cockroach *N. cinerea* during adult ontogenetic development. The aim of our work was to measure immunoreactive-progesterone concentration during the time of the first gonotrophic cycle of females *N. cinerea* and the corresponding time in males, including control group and group chilled to delay ontogenetic development.

MATERIAL AND METHODS

Animals

Cockroaches *Nauphoeta cinerea* were reared at 29 ± 1°C, 60 ± 5% r.h. and a 12 hours reversible light-dark cycle. Food (a semisynthetic food for young turkeys) and water were provided ad libitum. Under these conditions oocyte maturation takes 12 days and the embryos hatch from the brood sac at day 36.

Our experiments involved newly moulted adult females and males. Each experimental group comprised five females or five males.

The progesterone concentration in gonads, intestine and haemolymph of females and males cockroaches *Nauphoeta cinerea* during the investigated period of development was studied. The animals were divided into two groups: group 1 – control females and males reared during a period of 36 days after adult ecdysis at 29 ± 1°C, group 2 – females and males reared from day 8 (after adult ecdysis) to day 18 at a temperature of 15°C (cooled animals). Thereafter, animals of group 2 were reared at the same temperature as those of group 1.

Extraction and isolation of progesterone

Gonads and intestines were dissected in a Petri dish cooled with ice, weighed and homogenised in glass microhomogenisers containing 500 μl 0.1 $mol.l^{-1}$ phosphate buffer saline pH 7.0. IR-progesterone was extracted with 3 ml redistilled petroleum ether. The haemolymph was obtained by cutting off the cockroaches antennae, followed by centrifugation of the animals in conical glass tubes at 500 g for 10 min. Then, 50 μl of haemolymph with 500 μl of 0.1$mol.l^{-1}$ phosphate buffer, pH 7.0 was extracted with 3 ml of petroleum ether. After extraction, the samples were centrifuged at 1500 g for 10 min. The organic phase was transferred to plastic tubes and evaporated to dryness.

Radioimmunoassay (RIA)

The residues were dissolved in 100 μl of 0.1$mol.l^{-1}$ phosphate buffer saline pH 7.0 with 0.1% gelatin and 0.1% NaN_3 (assay buffer) and analysed by RIA for progesterone (adopted method, Kolena & Channing, 1985). The anti-progesterone serum (a gift of Dr Slebodzinski, Polish Acad. Sci., Poznań, Poland), was prepared by immunisation of a rabbit with Progesterone-11α Succinyl-BSA conjugate. The antiserum was diluted 1 : 100 000 (v/v) in assay buffer pH 7.0 and 100 μl was added to each assay tube. The cross-reactivity of 8 tested steroids with the antiserum did not exceed 0.1%. The cross-reactions are given below.

^{125}I progesterone (URVJT Košice, SR) was dissolved in assay buffer and 100 μl (15 000) CPM was added to the each assay tube. Tubes were mixed and incubated at 37°C for 30 min and then at 4°C for 2 hours. After incubation, 0.4 ml of 0.3% dextran-coated charcoal solution was added to each tube. The tubes were incubated at 4°C for 20 min and then centrifuged at 4°C for 10 min and the bound fraction was

counted with a Gamma Counter (Tesla, CR). The average activity was 12,5 pg/tube. The calibration curve ranged from 6.25 pg to 3200 pg/tube. The recovery after extraction was about 65%.

Antiserum	Steroid	% cross reaction
Anti-progesterone	Progesterone	100.00
	Pregnenolone	0.01
	Hydrocortisone	0.01
	Testosterone	0.08
	Estradiol-17α	0.01
	Estradiol-17β	0.0001
	Estradiol	0.0001
	Estrone	0.01

Results were analysed statistically by analysis of variance followed by Newman-Keul's multiple comparison test. Significance of differences between mean values was evaluated on the levels $p < 0.05$ and $p < 0.01$.

RESULTS

The highest IR-progesterone concentration of fertilised females of group 1 was found in intestine (Fig. 1) and in gonads (Fig. 2) with lower levels in haemolymph (Fig. 5). The titre of IR-progesterone in the investigated period showed changes. The progesterone concentration in the intestine of fertilised females in group 1 (Fig. 1), increased from day 21 and reached the highest value on day 27. By day 30 the progesterone concentration had

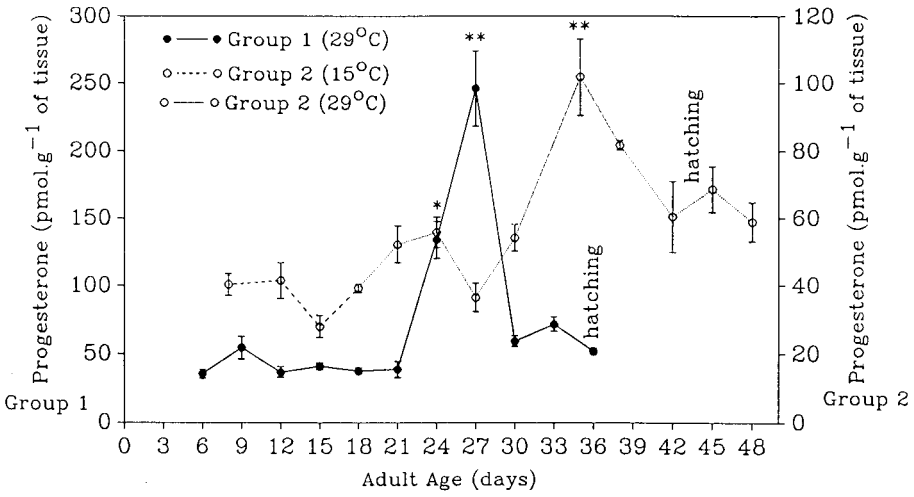

Fig. 1. Progesterone concentration in intestine of females *N. cinerea*.

Fig. 1–6. Group 1 – control animals reared for a period of 36 days after adult ecdysis at temperature 29 ± 1°C. Group 2 – animals reared from day 8 to 18 at 15°C and at 29°C thereafter. Each point represents the mean of 5 individually measured animals ± SEM and * $p < 0.05$, ** $p < 0.01$.

decreased rapidly. The IR-progesterone concentration in female intestines in group 2 (Fig. 1) increased from day 15 and reached maximum on day 35.

The changes in gonads of females from group 1 (Fig. 2) were of similar magnitude and time course as the change in the intestine. The IR-progesterone concentration increased

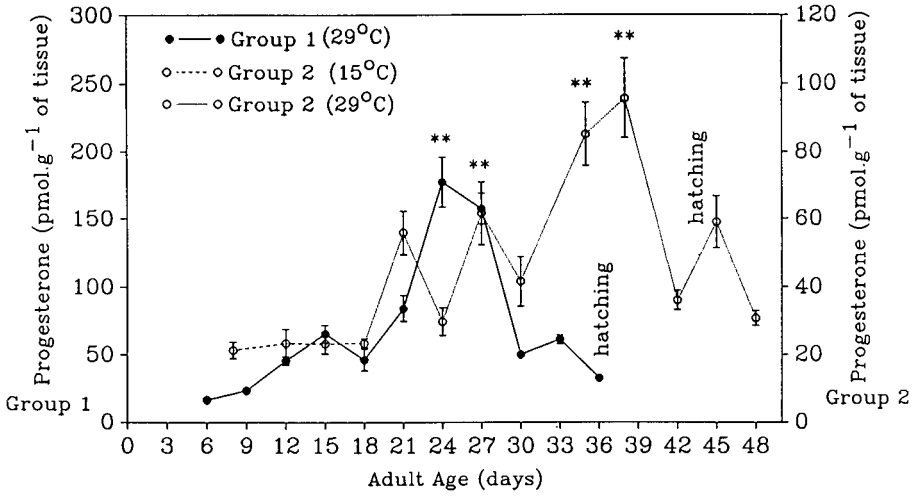

Fig. 2. Progesterone concentration in gonads of females *N. cinerea.*

from day 9 and the highest levels were observed on day 24 and 27. In gonads in group 2 (Fig. 2) the increase started on day 18 and continued up to day 38.

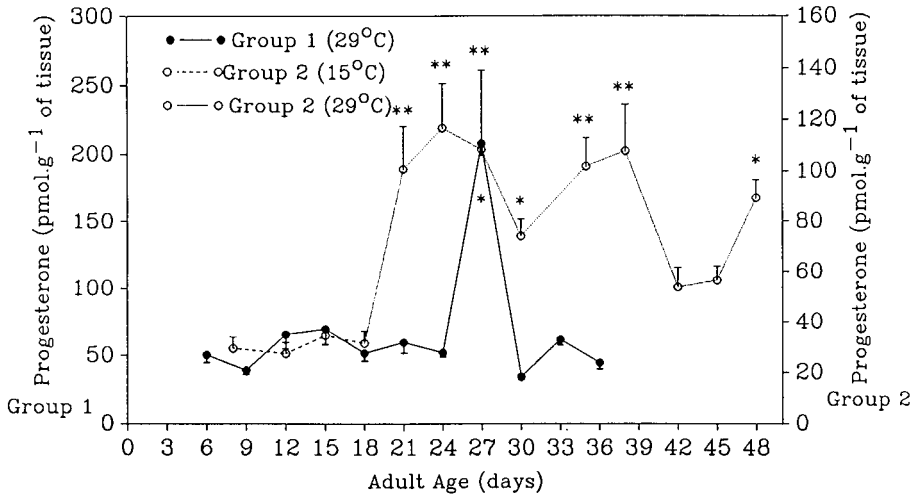

Fig. 3. Progesterone concentration in intestine of males *N. cinerea.*

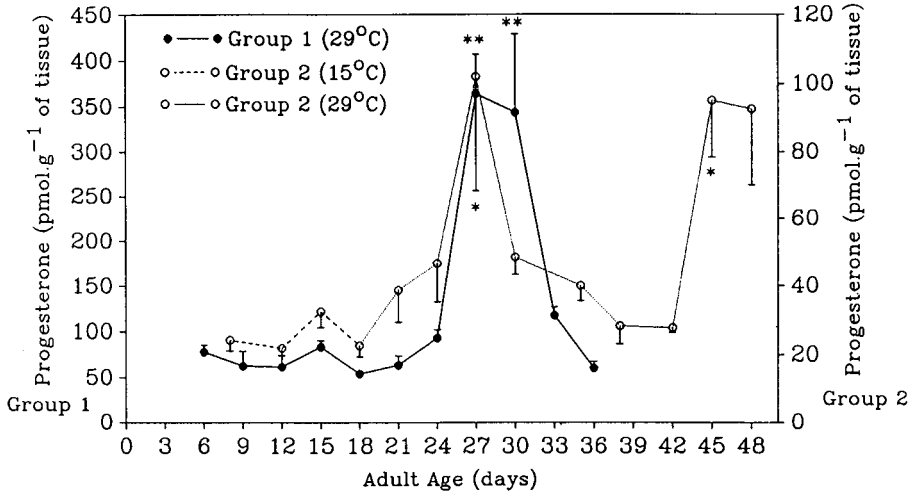

Fig. 4. Progesterone concentration in gonads of males *N. cinerea*.

In the males of group 1, the IR-progesterone concentration in intestine (Fig. 3) and in gonads was similar (Fig. 4) but in the haemolymph (Fig. 6) the progesterone level was lower. In the intestine of control males from group 1 the maximal content (200 pmol.g^{-1}) found on day 27 fell to 34 pmol.g^{-1}of tissue on day 30 (Fig. 3). The progesterone levels in intestines of cooled males (group 2) varied from 27 pmol.g^{-1} of tissues (day 12) to 117 pmol.g^{-1} of tissues (day 24). The highest concentrations were measured on days 24, 27, 35 and 38. In male gonads (Fig. 4) the IR-progesterone increase started in group 1 on day 18

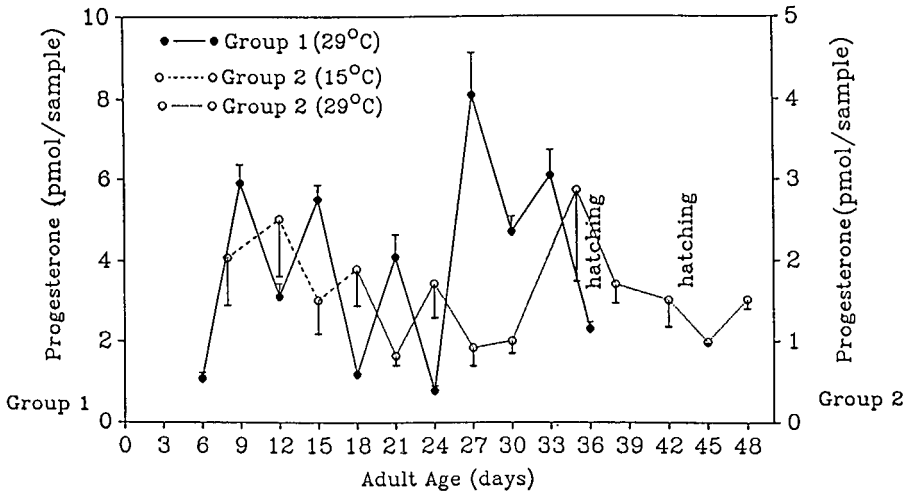

Fig. 5. Progesterone concentration in haemolymph of females *N. cinerea*.

and continued up to day 30. The highest concentrations being measured on day 27 and 30. In gonads of cooled males (group 2), the progesterone concentration varied from 22 pmol.g^{-1} of tissue (day 12) to 102 pmol.g^{-1}(day 27), the highest concentrations being found on days 27, 45 and 48. The IR-progesterone concentration in haemolymph of females (Fig. 5) and also of males (Fig. 6) was lower than in the intestine and gonads. The levels varied

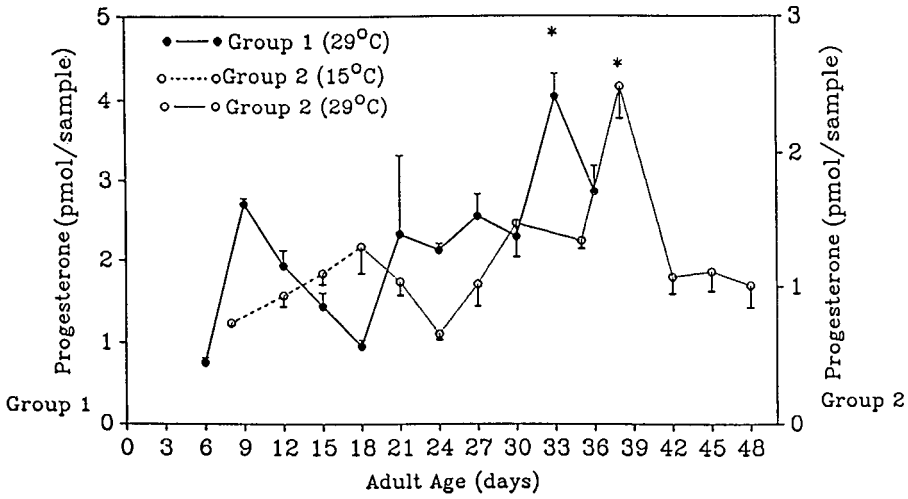

Fig. 6. Progesterone concentration in haemolymph of males *N. cinerea*.

from 2 pmol/sample to 8 pmol/sample. The highest concentration of this steroid in haemolymph of females in group 1, was on day 27 and in group 2 was on day 35 (Fig. 5). In the haemolymph of males in group 1, the highest concentration was measured on day 33 and in group 2 on day 38 (Fig. 6).

Fertilised females in group 1 bore nymphs around day 36 and in group 2 around days 42–45.

It is interesting that the IR-progesterone concentration in all investigated tissues of cooled males and females (group 2) was lower than in control animals (group 1).

DISCUSSION

Our results demonstrate the presence of IR-progesterone in gonads, intestine and haemolymph of females and males of the cockroach *Nauphoeta cinerea*. We found that the progesterone concentration in both females and males varied during adult life which in females included the development of the embryos in the brood sac. Similar results were obtained by Novak et al. (1987), who studied changes of progesterone concentration in haemolymph of *Locusta migratoria*. Progesterone levels varied in female haemolymph from 37 to 77 pg/ml and in males from 58 to 119 pg/ml. They found that the highest concentration of progesterone in haemolymph was in three-week-old males. The progesterone concentration reached the highest levels on day 24 or 27, after adult ecdysis. In the fertilised females it is the period of late embryogenesis, from dorsal closure until hatching.

Comparing our results with the titre of ecdysteroids and juvenile hormone (JH) in embryos of fertilised females of *N. cinerea* during embryogenesis (Imbodenet al., 1978) shows that ecdysteroid activity was detected in small amounts at the time of dorsal closure and in higher amounts around day 28 and 32. JH-active material could be detected only after the dorsal closure had occurred. Two peaks were observed, namely one on day 27 and one on day 33, at the time of the chorion rupture. Ecdysteroids as well as JH were accumulated in the haemolymph of the embryo. These results probably refer to a common mechanism for embryonic and ontogenetic development. However the site of synthesis of JH and ecdysone in the embryos is not known.

However, it is also interesting that the birth of nymphs in control females in group 1 was observed on day 36 and in the second experimental group (cooled females reared from day 8 to 18 at a temperature 15°C) both the shift in the time of the birth of nymphs and progesterone culmination were observed. These findings indicate that progesterone could play a role in the regulation of reproduction *N. cinerea.*

From our results we can suppose, that there is certain correlation between the progesterone concentration and the rate of gonotrophic cycle, but it's precise nature has yet to be confirmed. The real physiological function of progesterone in this process is still unknown.

The importance of the occurence of vertebrate-type steroids in insects is a contentious issue and much argument concentrate on the origin of these steroids. Evidence relating to an insect's ability to biosynthesise these steroids from cholesterol has already been discussed, but another possibility is that the steroids are sequestered from food sources. Bradbrook (1990) shown that insect food from both animal and plant sources apparently contains steroidal material, but that there appears to be no obvious relationship between the levels in the insect and in its food. The influence of food contents on the IR-progesterone was investigated in our previous experiments where, besides the diet for young turkeys, a semisynthetic food was used and also the effects of starvation (only with water supply) was investigated. No significant differences in IR-progesterone levels were found. The previous results indicate that the *N. cinerea* possess a mechanism to transform some non-ecdysteroidal steroids and this may be facilitated by gut symbionts.

Several suggestions about the possible functions of vertebrate-type steroids have been suggested, but their hormonal role has not yet been proven unequivocally. It is necessary to search for the presence of steroid receptors in various tissues. Better understanding of insect physiology may also bring greater information upon the interactions with other hormones, such as steroids, juvenile hormones and neuropeptides.

ACKNOWLEDGMENTS. Thanks are due to Professor B. Stay (Department of Biology, University of Iowa, Iowa City, USA) and Professor A. De Loof (Zoological Institute, Catholic University, Leuven, Belgium) for their critical reading of the manuscript and for many valuable comments.

REFERENCES

ANDO T. & BARBIER M. 1982: Steroid hormons of vertebrates and invertebrates. In: Nikitina S.M. (ed.): *Steroid Hormons of Invertebrates.* Izdatelstvo Leningradskogo Universiteta, Leningrad, pp. 24.

BRADBROOK D.A., CLEMENT C.Y., COOK B. & DINAN L. 1990: The occurence of vertebrate-type steroids in insects and a comparison with ecdysteroid levels. *Comp. Biochem. Physiol.* **95 B**: 365–374.

De Clerck D., Eechaute W., Leusen I., Diederik H., & De Loof A. 1983: Identification of testosterone and progesterone in haemolymph of larvae of the fleshfly Sarcophaga bullata. *Gen. Comp. Endocrin.* **52**: 368–378.

De Clerck D., Diederik H. & De Loof A. 1984: Identification by capillary gas chromatography-mass spectrometry of eleven non-ecdysteroid steroids in the haemolymph of larvae of Sarcophaga bullata. *Insect Biochem.* **1**: 199–208.

Denlinger D.L., Bruggemeier R.W., Mechoulam R., Katlic N., Yocum L.B. & Yocum G.D. 1987: Estrogens and androgens in insects. In Law J.H. (ed.): *Molecular Entomology*. Alan R. Liss, New York, pp. 189–199.

De Loof A., Huybrechts R. & Verhaert P. 1987: Vertebrate-peptide hormone like materials in arthropods: identification methods and functions. *Bull. Acad. Serbe Sci. Cl. Sci. Math. Nat., Sci. Nat.* N 29.

Diederik H., De Clerck D. & De Loof A. 1984: Identification of 14 non-ecdysteroid steroids in the haemolymph of larvae of the Colorado potato beetle Leptinotarsa decemlineata. *Gen. Comp. Endocrin.* **53**: 449.

Imboden H., Lanzrein B., Delbeque J.P. & Luscher M. 1978: Ecdysteroids and juvenile hormone during embryogenesis in the ovoviviparous cockroach Nauphoeta cinerea. *Gen. Comp. Endocrin.* **36**: 628–635.

Kolena J. & Channing C.P. 1985: Stimularory action of follicular fluid components on maturation of granulosa cells from small porcine follicles. *Hormone Res.* **21**: 185–198.

Lafont R. 1991: Mini-Review, Reverse endocrinology, or "hormones" seeking functions. *Insect Biochem.* **21**: 697–721.

Lehoux J.G. & Sandor T. 1970: The occurence of steroids and steroid metabolising enzyme systems in invertebrates. *Steroids* **16**: 141–171.

Mechoulam R., Bruggemeier R.W. & Denlinger D.L. 1984: Estrogens in insects. *Experientia* **40**: 942–944.

Novak F., De Clerck D., Paesen G., Swevers L. & De Loof A. 1987: Radioimmunological quantification of C21, C19 and C18 steroids in haemolymph of the insect Locusta migratoria. *Int. J. Invert. Reprod. Devel.* **11**: 255–264.

Novak F., Takáč P., Schoonen W., Paesen G., Lambert J., & De Loof A. 1989: Identification and concentration of non-ecdysteroid steroids in a few insect species. *Gen. Comp. Endocrin.* **74**: 260.

Ohnishi E., Ogiso M., Wakabayashi K., Fujimoto Y. & Ikekawa N. 1985: Identification of estradiol in the ovaries of the silkworm Bombyx mori. *Gen. Comp. Endocrin.* **60**: 35–38.

Sandor T. & Mehdi A.Z. 1979: Steroids and evolution. In Barrington E.J.W. (ed.): *Hormones and evolution. Vol. 1.* Academic Press, New York, pp. 1–72.

Sandor T. 1980: Steroids in invertebrates. In Clark W.H. & Adams T.S. (eds): *Advances in Invertebrate Reproduction*. Elsevier-North Holland Inc., New York/Amsterdam, pp. 81–96.

Swevers L., Lambert J.G.D. & De Loof A. 1991: Synthesis and metabolism of vertebrate-type steroids by tissues of insects: a critical evaluation. *Experientia* **47**: 687–698.

Takáč P., Výboh P., Kozánek M., Hučková A. & Slovák M. 1988: Estradiol, progesterone, testosterone and dihydrotestosterone concentrations in some tissues of cockroach Nauphoeta cinerea. *Proceedings of the International Conference on Endocrinological Frontiers in Physiological Insect Ecology. Vol. 2.* Technical University, Wroclaw, pp. 899–905.

Received May 5, 1992; accepted September 28, 1992