80 Van Breemen, C., and Casteels, R., The use of Ca-EGTA in measurements of ^{45}Ca efflux from smooth muscle. Pflügers Arch. *348* (1974) 239–245.
81 Van Breemen, C., Farinas, B. R., Gerba, P., and McNaughton, E. D., Excitation-contraction coupling in rabbit aorta. Studied by the Lanthanum method for measuring cellular calcium influx. Circulation Res. *30* (1972) 44–54.
82 Wibo, M., Duong, A. T., and Godfraind, T., Subcellular localization of semicarbazide-sensitive amine-oxidase in rat aorta. Eur. J. Biochem. *112* (1980) 87–94.
83 Wibo, M., Morel, N., and Godfraind, T., Differentiation of Ca transport associated with plasma membrane and endoplasmic reticulum in intestine smooth muscle. Arch. int. Pharmacodyn. *250* (1981) 333–334.
84 Wuytack, F., DeSchutter, G., and Casteels, R., Partial purification of $(Ca^{2+} + Mg^{2+})$-dependent ATPase from smooth muscle and reconstitution of an ATP-dependent Ca^{2+}-transport system. Biochem. J. *198* (1981) 265–271.
85 Wuytack, F., Raeymaekers, L., DeSchutter, G., and Casteels, R., Demonstration of the phosphorylated intermediates of the Ca^{2+}-transport ATPase in a microsomal fraction and in a $(Ca^{2+} + Mg^{2+})$-ATPase purified from smooth muscle by means of calmodulin affinity chromatography. Biochem. biophys. Acta *693* (1982) 45–52.

0014-4754/85/07-0905-09$1.50 + 0.20/0

Full Papers

Titers of ecdysone, 20-hydroxyecdysone and juvenile hormone III throughout the life cycle of a hemimetabolous insect, the ovoviviparous cockroach *Nauphoeta cinerea*

B. Lanzrein, V. Gentinetta, H. Abegglen, F. C. Baker, C. A. Miller and D. A. Schooley*

University of Bern, Division of Animal Physiology, Erlachstrasse 9a, CH-3012 Bern (Switzerland), and Zoecon Corporation, Dept. of Biochemistry, 975 California Avenue, Palo Alto (California 94304, USA), 29 October 1984

Summary. Titers of ecdysone, 20-hydroxyecdysone and juvenile hormone III were measured in whole body extracts or hemolymph of embryos, first, penultimate and last stadium nymphs, and adult females of *Naupoheta cinerea*. We used a gas-chromatography/mass spectrometry method for quantifying juvenile hormone and a radio-immunoassay for ecdysteroid determination. Juvenile hormone III is particularly abundant in the embryonic stage (up to 960 ng/g), at a low level in first and penultimate stadium nymphs (2–10 ng/ml) and almost absent in the last nymphal stadium; in the adult female the juvenile hormone titer rises to 180 ng/ml in hemolymph during rapid oocyte growth. The titers of ecdysone and 20-hydroxyecdysone undergo similar fluctuations in the embryonic and nymphal stages, being highest at the time of cuticle formation in the embryo and a few days before the nymphal and adult molts (around 100–200 ng/ml for exdysone and 2–4 µg/ml for 20-hydroxyecdysone).
Key words. Ecdysteroids; juvenile hormone III; developmental changes; cockroach; *Nauphoeta cinerea*.

Introduction

According to the classical model for the regulation of insect development and metamorphosis (fig. 1) molting is induced by ecdysteroids but the nature of the molt depends on the concentration of juvenile hormone (JH) in the hemolymph[30]. In holometabolous insects JH is assumed to be high before larval molts, low at the larval-pupal transformation and absent before metamorphosis. The classical model derives mainly from ligation, transplant and parabiosis experiments[30], whereas titer measurements of either JH or ecdysteroids have been made only in a few cases at selected stages[5, 6, 13, 17, 18, 28]. The embryo is not included in the classical model (fig. 1) because ecdysteroids[11, 19, 20, 22] and JH[3] have only recently been identified and measured during embryonic development and in only a few species. There is still uncertainty surrounding the function of these hormones in embryos[26]. Nevertheless, ecdysteroids seem to play a role in cuticle formation in the embryo similar to their function during nymphal development[10, 22, 26]. The same hormones are found also in the adult female of most insects where JH acts as a gonadotropic hormone by stimulating both oocyte growth and yolk protein synthesis in the fat body[10]. At this stage ecdysteroids are located mainly in the ovary[14] but in some insects they also circulate in the hemolymph[12, 15, 20, 32].

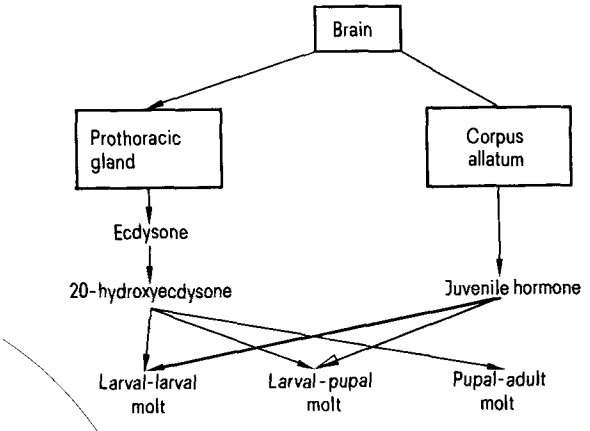

Figure 1. The classical model of the regulation of insect development and metamorphosis. The half-shaded arrow signifies a lower titer.

In view of the paucity of information available concerning variations in the titer of JH and ecdysteroids during development we considered it desirable to examine the validity of the classical model (fig. 1) by simultaneously measuring the quantity of ecdysone, 20-hydroxycdysone and JH during early, late and premetamorphic nymphal stages; for purposes of comparison we also included the embryonic and adult stages. Our experimental insect, *Nauphoeta cinerea* appeared suitable for this type of investigation because much is known about the biology and endocrinology of this species. The ecdysteroids in embryos[19,20], ovary[32] and hemolymph of adult females[25] have already been identified and quantified and the JH has been quantified in embryos[19,20] and adult females[24] using a bioassay. Furthermore we have identified JH III as the only JH in all stages during a study undertaken to reassess the identity of JH(s) in nymphal and adult *Nauphoeta cinerea*[2].

Material and methods

Nauphoeta cinerea were reared at 26 °C and 60% relative humidity on dog flakes and water in a 12-h light/12-h dark photoperiod. Under these conditions embryonic development lasts 35–40 days, the first nymphal stadium 9–10 days, the penultimate nymphal stadium 19–20 days, the last nymphal stadium 24–27 days, and oocyte maturation 12–13 days.

Work-up of samples for JH titer determination

Generally around 1 ml of hemolymph or 1–10 g of whole body samples were processed according to the method described by Bergot et al.[4], with minor modifications outlined by Baker et al.[2]. In this method the initial extraction steps are followed by fast and efficient purification procedures chosen to separate JH from several major lipid classes: glycerides, sterols and their esters, and free fatty acids. The 10,11-epoxide moiety of JH is converted to an 11-methoxy-d_{-3}-10-hydroxy derivative (methoxyhydrin), which is purified, completing the sample work-up. The derivatives are then analyzed by GC/MS with selected ion monitoring for detection. A tritium-labeled analog of JH is added during the extraction as an internal standard, which allows the recovery to be determined for each sample.

Work-up of samples for ecdysteroid determination

For each analysis approximately 20 µl of hemolymph was collected from first stadium nymphs (20–30 insects) and approximately 100 µl from penultimate and last stadium nymphs (6–10 insects); the hemolymph was then extracted and purified by TLC as described by Zhu et al.[32]. For ecdysteroid determinations a radioimmunoassay (RIA) was used[7,16]. The results are expressed as ng ecdysone or 20-hydroxyecdysone equivalents. Ecdysone and 20-hydroxyecdysone used as standards in each assay were purchased from Simes (Milan), antibodies were a generous gift from Dr J. D. O'Connor (Los Angeles) and 23,24-[^{3}H]-ecdysone from Dr J. Koolman (Marburg).

Results

JH titers measured in the various developmental stages are shown in the table. JH III is particularly abundant in embryos at stages between days 25 and 34; in the first and penultimate nymphal stadia comparatively low titers were observed and there seems to be only very little variation throughout any one stadium. The majority of samples from last stadium nymphs were devoid of JH. A high JH III titer is observed in adult females at the stage of rapid oocyte growth.

In order to investigate the existence of mutual interactions between JH III and ecdysteroids in nymphal development we measured the titers of ecdysone and 20-hydroxyecdysone in the first, penultimate and last nymphal stadia (fig. 2). The data obtained show that the concentration of these two compounds was closely synchronized in all three stadia investigated, 20-hydroxyecdysone always being predominant and showing a peak a few days before the molts. However, early in each stadium a slight

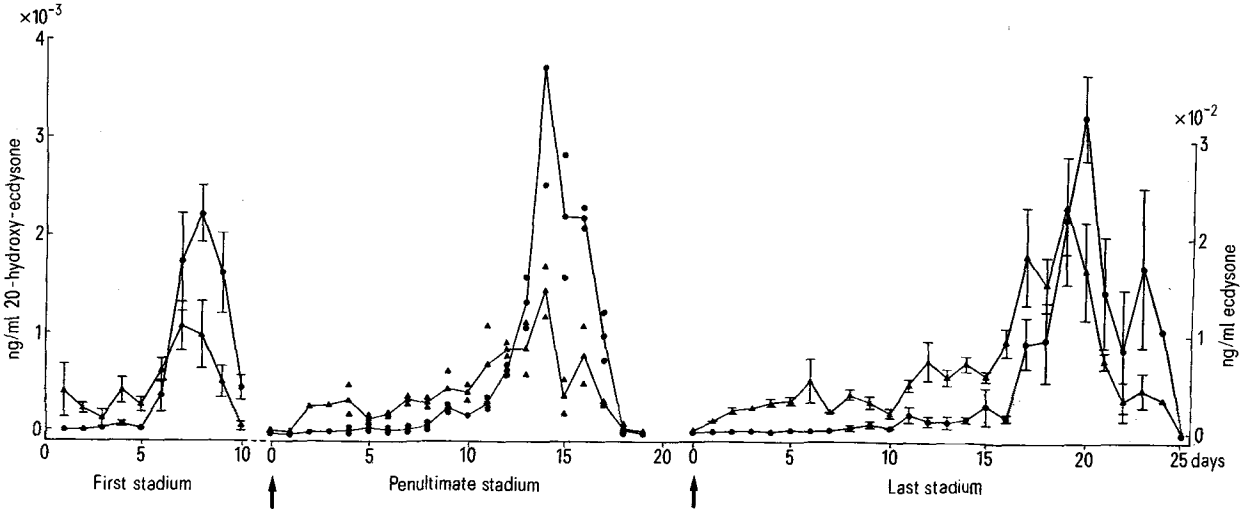

Figure 2. Titers of ecdysone (▲—▲) and 20-hydroxyecdysone (●—●) in first, penultimate, and last stadium nymphs. Data are given in ecdysone and 20-hydroxyecdysone equivalents and are means ± SEM for first and last stadium nymphs (based on 3–6 determinations per point) and single values for penultimate stadium nymphs. Note the difference in scale for ecdysone and 20-hydroxyecdysone.

but significant increase in the titer of ecdysone is observed, which is not paralleled by an increase in 20-hydroxyecdysone.

Discussion

Only JH III was found in another study of *Nauphoeta cinerea*[2] and in this one (table), in contrast to a prior investigation in which JH I and II were also reported[23]. The occurrence of only JH III is in agreement with studies conducted on other non-lepidopterous species using similar or related physicochemical analytical methods (See Schooley et al.[31] for review). Thus JH III assumes both morphogenetic and gonadotropic functions in this insect. The levels of JH III in *Nauphoeta cinerea* embryos between days 25 and 34 (table) are some of the highest titers of JH III ever observed. They are even more remarkable in the light of the fact that adults contain a JH level which is an order of magnitude lower than that in embryos, while in the nymphal stages investigated the levels of JH III were modest or undetectable. Furthermore, equally high levels of methyl farnesoate (the precursor of JH III in non-lepidopterous species) have been detected in *Nauphoeta cinerea* embryos[26]. Methyl farnesoate possesses a high degree of JH activity with respect to both morphogenetic and gonadotropic functions in this insect (Fehr and Lanzrein, unpublished results). At their peak the combined concentration of JH III plus methyl farnesoate in *Nauphoeta cinerea* embryos is about 10^{-5} M. Very few JH determinations in embryos have been performed, particularly using physicochemical methods. Levels of JH III in *Oncopeltus fasciatus* eggs are very low or nil (Bergot et al.[3] and Baker et al., unpublished results) although a report based on a bioassay procedure suggested huge levels of JH active material in eggs of this species[9]. *Locusta migratoria* eggs contain moderate levels of JH III (Pener et al., in preparation). Eggs of the lepidopteran species *Manduca sexta, Heliothis virescens* and *Hyalophora cecropia* lack JH III but all contain at least a low level of the higher homologs (JH II, I and 0)[3]. *Manduca sexta* is an interesting case, as the embryos contain high levels of JH 0 and JH I, together with a unique JH isomeric with JH O[3]. The embryonic stadia of *Manduca sexta* contain the highest JH titer throughout the life cycle, but the difference between embryonic and post-embryonic stages is not as great as in *Nauphoeta cinerea*. JH III levels in nymphs of the latter (table) are comparable to those obtained from other orthopteroidean species, e.g. *Locusta migratoria*[5,18], and *Teleogryllus commodus*[27]. The moderately high levels of JH III in the vitellogenic female (table) are also comparable to data obtained from certain other adult female insects of this superorder (*Locusta migratoria*[5,29], *Teleogryllus commodus*[27] and *Taeniopoda eques*[27]) and are in agreement with earlier data obtained using the *Galleria* wax test[24]. On the other hand, JH III titers in adult females of the cockroach, *Diploptera punctata* (Tobe et al., submitted for publication) are about an order of magnitude higher than in *Nauphoeta cinerea*. In other species encompassing several insect orders[31] similar or low levels of JH III have been found as compared to *Nauphoeta cinerea*; the significance of the relative magnitudes of JH titer in various species is at present unclear. From the similarity of the ecdysteroid titer profiles in the three nymphal stadia investigated (fig. 2), it seems that the presence of JH does not influence the fluctuations of ecdysone and 20-hydroxyecdysone nor their relative abundance. In *Locusta migratoria* similar titers of ecdysteroids have been observed[1,13], although no early peak has been found in penultimate stadium nymphs of this species[1].

The titer changes of JH III and the molting hormone 20-hydroxyecdysone during an entire life cycle of *Nauphoeta cinerea* are compared in figure 3, which also includes data from earlier publications (see legend to figure). The titer of 20-hydroxyecdysone in the embryo

Titers of JH III during development in *N. cinerea*

Embryo				First stadium nymphs				Penultimate stadium nymphs			Last stadium nymphs			Adult female		
Day	ng/g	x̄	(ng/ml)	Day	ng/g	x̄	(ng/ml)	Day	ng/ml	x̄	Day	ng/ml	x̄	Day	ng/ml	x̄
16	0			1	0.83			3–4	3.5		3	2.3		8	170	
	0	0			0.93	0.88	(4.2)		4.4	4.0		0			140	
17	2.2			3	0.58			5–6	1.9			0	0.76		180	163
	3.7				1.2				5.5	3.7	6	0				
	0.14				0.71			7–8	3.2			0	0			
	0				0.75	0.81	(3.8)		4.8		9	0.8				
	0			5	1.4				5.7	4.6		0	0.4			
	0	1.0	*		1.8			9–10	3.3		14	0				
18	0.1				0.73				4.8			0.4	0.2			
	0	0.05	*		0.99	1.2	(5.8)		4.5		19	0				
21	10.0	10	(42)	7	0.85				5.7	4.6		0	0			
25	840				1.1			11–12	2.2							
	750	795	(3400)		0.70				4.7							
32	430				1.5	1.0	(4.8)		8.4	5.1						
	960	695	(3300)	8	0.23			14–15	7.0							
34	340				0.41	0.32	(1.5)		9.3	8.2						
	510	425	(2000)	9	0			16–17	9.5	9.5						
35	1.2	1.2	(5.90)		4.0	2.0	(9.5)									

Values are given in ng/g for embryos and first stadium nymphs, and in ng/ml hemolymph for penultimate stadium nymphs and adult females. For a better comparison the values for the first group have also been converted into ng/ml (values given in parentheses) on the assumption that all JH circulates in the hemolymph and that the latter represents 21 % of the insect's fresh weight, as determined for penultimate stadium nymphs. For JH titers determined in embryo hemolymph using *Galleria* wax test see Imboden et al.[19]. * Circulatory system not yet developed.

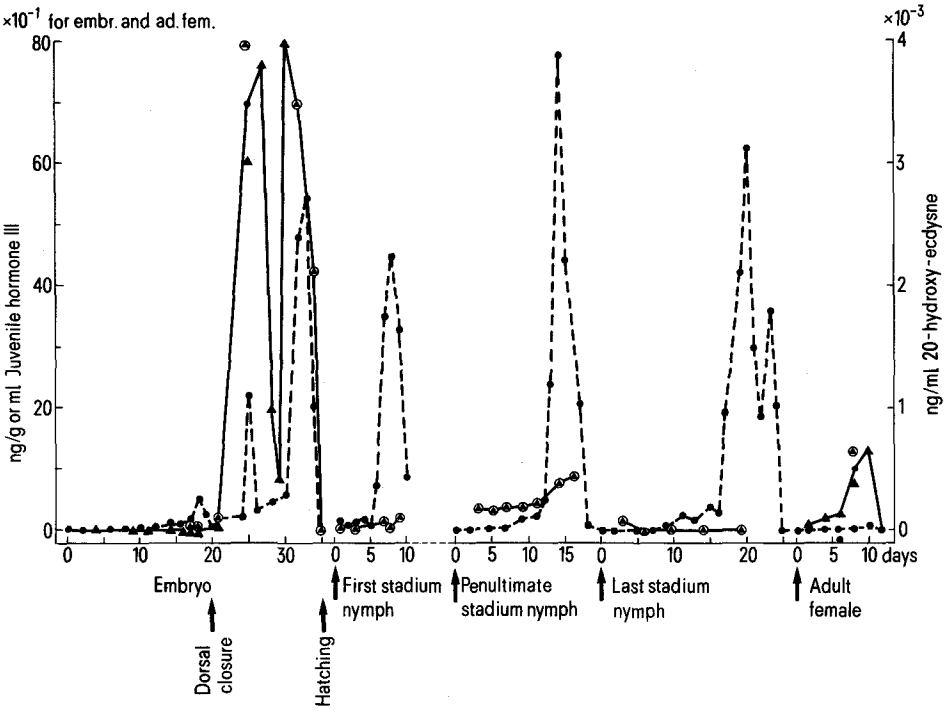

Figure 3. Titers of JH III (▲—▲) and 20-hydroxyecdysone (●---●) in embryos, first, penultimate and last stadium nymphs and adult females. (▲) are values obtained by the GC/MS method and the others by *Galleria* wax test (bioassay data for embryos redrawn from Imboden et al.[19] and for adult females from Lanzrein et al.[24]). JH III titers are given in ng/g for embryos and first stadium nymphs and in ng/ml for penultimate and last stadium nymphs and adult females. Data for 20-hydroxyecdysone in embryos are from Imboden et al.[19] and for adult females from Lanzrein et al.[25]. Note the different scales for JH.

reaches values similar to those observed in the nymphal stages. In the embryo the first small peak observed shortly before dorsal closure coincides with the formation of the first embryonic cuticle and the high peak is seen at the time when the first nymphal (= second embryonic) cuticle is formed. Since JH III is present in large quantities only at the time of formation of the first nymphal cuticle we have suggested that JH III plays a role in controlling this process[26]. Nevertheless it seems highly probable that JH exerts other functions in the embryo[8]; we do not yet know why the embryo produces so much JH. Comparatively little JH is present during nymphal development, when new nymphal cuticles are also formed. In the adult female the JH III titer rises, whereas only very little 20-hydroxyecdysone is found in comparison with the embryonic and larval stages. The slight but significant increase in 20-hydroxyecdysone titer at the time of chorion formation[25] is barely discernible in figure 3 (day 10) but nevertheless possibly has a biological significance[25,32].

In conclusion, our data show for the first time the fluctuation in the titers of the major developmental hormones throughout the life cycle of a hemimetabolous insect. They confirm the classical model of the regulation of development and metamorphosis as depicted in figure 1 insofar as JH is more or less absent before metamorphosis and 20-hydroxyecdysone is present before each molt. Regarding absolute quantities, we have observed similar values of ecdysone and 20-hydroxyecdysone in embryos and nymphal stadia, whereas the titers of JH are approximately 100 times higher in the embryos than in the nymphal stadia. It is difficult to interpret these data and to judge the significance of the absolute quantity of a hormone since its biological effect depends not only on its quantity but also on the responsiveness of the target tissues. Nothing, however, is known of the quality and quantity of JH receptors in embryonic and nymphal target tissues, and even the number of target tissues is by no means clear. The integument is certainly a target tissue for JH in nymphs[30] and probably also in the embryo[26], but other tissues such as the gut should probably also be considered[8,21]. As regards the adult female, the data presented here show the presence of high quantities of JH III at a stage of rapid oocyte growth, which is in agreement with its role in inducing yolk protein synthesis and oocyte growth[10]. There exists some information regarding the presence of JH-binding proteins (= receptors?) in fat body and ovary in a related cockroach species[10] but the precise biological significance of the relative titer of JH during development will have to be established in future investigations.

*Acknowledgments. We thank Mrs A. Tschan for rearing the cockroaches, Mr M. Kaltenrieder for drawing the graphs, Mr G. C. Jamieson and Mrs C. Reuter for GC/MS analyses. We are also grateful to the Swiss National Science Foundation (grant no. 3.291-0.82 to B. Lanzrein) and the United States National Science Foundation (grant no. PCM 82-08665 to D. A. Schooley) for their financial support.

1 Baehr, J.C., Porcheron, P., Papillon, M., and Dray, F., Haemolymph levels of juvenile hormone, ecdysteroids and protein during the last two larval instars of *Locusta migratoria*. J. Insect Physiol. *25* (1979) 415–421.

2 Baker, F.C., Lanzrein, B., Miller, C.A., Tsai, L.W., Jamieson, G.C., and Schooley, D.A., Detection of only juvenile hormone III in several lifestages of *Nauphoeta cinerea* and *Thermobia domestica*. Life Sci. *35* (1984) 1553–1560.

3 Bergot, B.J., Baker, F.C., Cerf, D.C., Jamieson, G., and Schooley, D.A., Qualitative and quantitative aspects of juvenile hormone titers in developing embryos of several insect species: discovery of a new juvenile hormone-like substance extracted from eggs of *Manduca sexta*, in: Juvenile Hormone Biochemistry; Developments in Endocrinology, vol. 15, pp. 33–45. Eds G.E. Pratt and G.T. Brooks. Elsevier/North-Holland Biomedical Press, New York 1981.

4 Bergot, B.J., Ratcliff, M., and Schooley, D.A., Method for quantitative determination of the four known juvenile hormones in insect tissue using gas chromatography-mass spectroscopy. J. Chromat. *204* (1981) 231–244.

5 Bergot, B.J., Schooley, D.A., and De Kort, C.A.D., Identification of juvenile hormone III as the principal juvenile hormone in *Locusta migratoria*. Experientia *37* (1981) 909–910.

6 Bollenbacher, W., Smith, S.L., Goodman, W., and Gilbert, L.I., Ecdysteroid titer during larval-pupal-adult development of the tobacco hornworm, *Manduca sexta*. Gen. comp. Endocr. *44* (1981) 302–306.

7 Borst, D.W., and O'Connor, J.D., Trace analysis of ecdysones by gas-liquid chromatography, radioimmunoassay and bioassay. Steroids *24* (1974) 637–656.

8 Cavallin, M., and Fournier, B., Characteristics of development and variations in ecdysteroid levels in *Clitumnus* embryos deprived of their cephalic endocrine glands. J. Insect Physiol. *27* (1981) 527–534.

9 Dorn, A., Struktur und Funktion des embryonalen Corpus allatum von *Oncopeltus fasciatus Dallas*. Verh. dt. zool. Ges. *1974* (1975) 85–89.

10 Engelmann, F., Vitellogenesis controlled by juvenile hormone, in: Endocrinology of Insects, pp. 259–270. Eds R.G.H. Downer and H. Laufer. Alan R. Liss Inc., New York 1983.

11 Gande, A.R., Morgan, E.D., Wilson, I.D., Ecdysteroid levels throughout the life cycle of the desert locust, *Schistocerca gregaria*. J. Insect Physiol. *25* (1979) 669–675.

12 Hanaoka, K., and Hagedorn, H.H., Brain hormone control of ecdysone secretion by the ovary in a mosquito, in: Progress in Ecdysone Research, pp. 467–480. Ed. J.A. Hoffmann. Elsevier/North-Holland Biomedical Press, Amsterdam 1980.

13 Hirn, M., Hetru, C., Lagueux, M., and Hoffmann, J.A., Prothoracic gland activity and blood titres of ecdysone and ecdysterone during the last larval instar of *Locusta migratoria L.* J. Insect Physiol. *25* (1979) 255–261.

14 Hoffmann, J.A., Lagueux, M., Hetru, C., Charlet, M., and Goltzené, F., Ecdysone in reproductively competent female adults and in embryos of insects, in: Progress in Ecdysone Research, pp. 431–465. Ed. J.A. Hoffmann. Elsevier/North-Holland Biomedical Press, Amsterdam 1980.

15 Hoffmann, K.H., Behrens, W., and Ressin, W., Effects of a daily temperature cycle on ecdysteroid and cyclic nucleotide titres in adult female crickets, *Gryllus bimaculatus*. Phys. Ent. *6* (1981) 375–385.

16 Horn, D.H.S., Wilkie, J.S., Sage, B., and O'Connor, J.D., A high affinity antiserum specific for the ecdysone nucleus. J. Insect Physiol. *22* (1976) 901–905.

17 Hsiao, T.H., and Hsiao, C., Simultaneous determination of molting and juvenile hormone titers of the greater wax moth. J. Insect Physiol. *23* (1977) 89–93.

18 Huibregtse-Minderhout, L., van den Hondel-Franken, M.A.M., van der Kerk van Hoof, A.C., Biessels, H.W.A., Salemink, C.A., van der Horst, D.J., and Beenakkers, M.T., Quantitative determination of the juvenile hormones in the hemolymph of *Locusta migratoria* during normal development and after implantation of *corpora allata*. J. Insect Physiol. *26* (1980) 627–631.

19 Imboden, H., Lanzrein, B., Delbecque, J.P., and Lüscher, M., Ecdysteroids and juvenile hormone during embryogenesis in the ovoviviparous cockroach, *Nauphoeta cinerea*. Gen. comp. Endocr. *36* (1978) 628–635.

20 Imboden, H., and Lanzrein, B., Investigations on ecdysteroids and juvenile hormones and on morphological aspects during early embryogenesis in the ovoviviparous cockroach, *Nauphoeta cinerea*. J. Insect Physiol. *28* (1982) 37–46.

21 Judy, K.J., and Gilbert, L.I., Effects of juvenile hormone and molting hormone on rectal-pad development in *Hyalophora cecropia* (L.). J. Morph. *131* (1970) 301–314.

22 Lagueux, M., Hetru, C., Goltzené, F., Kappler, C., and Hoffmann, J.A., Ecdysone titer and metabolism in relation to cuticulogenesis in embryos of *Locusta migratoria*. J. Insect Physiol. *25* (1979) 709–723.

23 Lanzrein, B., Hashimoto, M., Parmakovich, V., Nakanishi, K., Wilhelm, R., and Lüscher, M., Identification and quantification of juvenile hormones from different developmental stages of the cockroach *Nauphoeta cinerea*. Life Sci. *16* (1975) 1271–1284.

24 Lanzrein, B., Gentinetta, V., Fehr, R., and Lüscher, M., Correlation between haemolymph juvenile hormone titre, corpus allatum volume and corpus allatum in vivo and in vitro activity during oocyte maturation in a cockroach *(Nauphoeta cinerea)*. Gen. comp. Endocr. *36* (1978) 339–345.

25 Lanzrein, B., Wilhelm, R., and Gentinetta, V., On relations between *corpus allatum* activity and oocyte maturation in the cockroach *Nauphoeta cinerea*, in: International Conference on Regulation of Insect Development and Behaviour, part II, pp. 523–534. Wroclaw Technical University Press, Wroclaw, Poland 1981.

26 Lanzrein, B., Imboden, H., Bürgin, C., Brüning, E., and Gfeller, H., On titers, origin, and functions of juvenile hormone III, methyl farnesoate and ecdysteroids in embryonic development of the ovoviviparous cockroach, *Nauphoeta cinerea*, in: Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, pp. 454–465. Eds J. Hoffmann and M. Porchet. Springer-Verlag, Heidelberg 1984.

27 Loher, W., Ruzo, L., Baker, F.C., Miller, C.A., and Schooley, D.A., Identification of the juvenile hormone from the cricket, *Teleogryllus commodus*, and juvenile hormone titre changes. J. Insect Physiol. *29* (1983) 585–589.

28 Peter, M.G., Dahm, K.H., and Röller, H., The juvenile hormones of larvae and adults of *Manduca sexta*. Z. Naturforsch. *31c* (1976) 129–131.

29 Rembold, H., Modulation of juvenile hormone III titer during the gonotrophic cycle of *Locusta migratoria*, measured by gas-chromatography selected ion monitoring mass spectrometry, in: Juvenile Hormone Biochemistry, pp. 11–20. Eds G.E. Pratt and G.T. Brooks. Elsevier/North-Holland Biomedical Press, Amsterdam 1981.

30 Richards, G., Insect hormones in development, Biol. Rev. *56* (1981) 507–549.

31 Schooley, D.A., Baker, F.C., Tsai, L.W., Miller, C.A., and Jamieson, G.C., Juvenile hormones 0, I, and II exist only in *Lepidoptera*, in: Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones, pp. 373–383. Eds J. Hoffmann and M. Porchet. Springer-Verlag, Heidelberg 1984.

32 Zhu, X.X., Gfeller, H., and Lanzrein, B., Ecdysteroids during oogenesis in the ovoviviparous cockroach, *Nauphoeta cinerea*. J. Insect Physiol. *29* (1983) 225–235.

0014-4754/85/07-0913-05$1.50 + 0.20/0