may fulfil the functional requirements imposed by the geometry of tubes to differing extents and in different ways. The reactions of a variety of excised, pressurized blood vessels need to be examined under many different experimental conditions, including pathophysiological conditions. Acute renal hypertension did not obviously disturb the negative feedback mechanism (fig. 1B), but this does not mean that the mechanism may not be upset in other pathophysiological situations.

The main advantage of pressurized blood vessels over rings and strips of these vessels is their potential ability to react to stretch and, under the appropriate conditions, to do so reproducibly. A pressurized, blood vessel preparation is more physiological in that the diameter is measured rather than calculated and the stresses on the wall (circumferential and axial) more closely approximate those in vivo[6]. Pressurized vessels may also be used to study other phenomena like phasic activity and spontaneous constriction (tone) which are rarely seen with strips or rings of blood vessels[18]. The pressurized ear artery of the rabbit may constrict spontaneously (figs 2 and 3) and the constriction can become unstable (fig. 3). Figure 3 also indicates that maximal activation needs to be used judiciously as it impairs active reactions to distension.

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Full Papers

Juvenile hormone titre and regulation in the cockroach *Diploptera punctata*

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Summary. Titres of juvenile hormone (JH) have been determined in both hemolymph and whole body extracts of female *Diploptera punctata* during the first gonotrophic cycle using a method employing gas chromatography/mass spectrometry for qualitative and quantitative analysis. JH III is the sole JH found in both adult and last instar *D. punctata*. Maximum values of ~ 1500 ng/ml (~ 6 μM) were observed at the middle of the gonotrophic cycle, when basal oocyte growth rate was greatest. Changes in rates of JH release in vitro by corpora allata paralleled closely the changes in JH titre, suggesting that biosynthesis is a major regulator of titre. JH levels per animal were calculated from observed JH titres, and at certain time points in the gonotrophic cycle JH levels obtained from analysis of whole bodies were significantly greater than those predicted from hemolymph titres. These results suggest the existence of a nonhemolymph JH pool in *D. punctata*.

Decay in JH titre after allatectomy of 5 day females has also been studied. Following a rapid initial decline, the rate of decay slowed appreciably 4 h post-operation. Thus, use of a first-order rate constant to estimate half-life of JH significantly underestimated the longevity of the hormone. The apparent persistence of JH following allatectomy may be due to the existence of a nonhemolymph JH pool.
Key words. Cockroach; *Diploptera punctata;* juvenile hormone titre; juvenile hormone half-life; juvenile hormone release rate; gonotrophic cycle; allatectomy; nonhemolymph juvenile hormone pool.

Introduction

Juvenile hormone (JH) plays an important role in the regulation of both metamorphosis and reproduction in most insect species[7, 40]. Declining JH titres are believed to be responsible in part for metamorphosis in both hemi- and holometabolous insects whereas in adult females, high JH titres are believed to be associated with vitellogenic oocyte growth[7, 39, 40]. These conclusions have been based largely on experiments involving bioassay of extracts of hemolymph, whole bodies or corpora allata (CA)[13, 39, 41], the organs of JH biosynthesis. Data on JH biosynthesis and release by the CA in vitro, as determined by radiochemical assay[29, 34], have also confirmed that high rates of JH synthesis can be associated with periods of rapid oocyte growth[34–36, 38] in the donor whereas reduced rates of in vitro biosynthesis have been observed during the last two nymphal stadia[12, 32].

The development of a sensitive, reproducible method for JH titre determination using gas chromatography/mass spectrometry (GC/MS)[1] has allowed accurate JH titre determination in vivo which avoids the uncertainties of bioassay procedures. We here report on the identity and titre of JH in the hemolymph of the cockroach *Diploptera punctata* throughout one gonotrophic cycle and at two time points during the last larval stadium. These measurements reveal an excellent correlation between in vivo JH titre and rates of JH release from CA in vitro, and between periods of rapid oocyte growth and high JH titre. We also studied JH levels in whole body extracts during the first gonotrophic cycle; our results suggest the existence of a 'pool' of JH outside the hemolymph. Finally, we determined JH titre following allatectomy and observed a rate of disappearance considerably slower than that predicted by first-order kinetics. These results suggest a persistence of JH effects far longer than previously expected.

Materials and methods

Cockroaches were reared as described previously[30, 36]. Mating occurs on day 0, immediately after imaginal emergence. Oviposition occurs on day 8; parturition on day 63. Newly emerged females were isolated from the colony 0–4 h after ecdysis. Successful mating was confirmed by the presence of a spermatophore.

JH levels were measured in hemolymph (collected as described previously[25, 26]) and in whole body samples according to the method of Bergot et al.[1]. However, a single sample of day 4–5 female hemolymph containing 0.87 ng/ml of internal standard was worked-up by a modified procedure: the sample was purified as usual, but prior to formation of the methoxyhydrin derivative, the JH III was removed by liquid chromatography (LC) on a 25 × 0.46 cm silica column (Spheri-5) using 5% ether in pentane (solvent half-saturated with H_2O). Additional internal standard (470 ng/ml of hemolymph) was added to the JH III fraction. Then it and the JH III-free zones (the latter containing the original internal standard and any JH 0, JH I, and/or JH II) were separately processed through the remaining steps of the methodology for quantification. Length of basal oocytes were measured in all females used for JH titre determinations; care was taken to avoid loss of hemolymph from animals analyzed for whole body JH content.

Allatectomies and sham-operations were performed as described previously[31], using care to avoid loss of hemolymph during surgery. For JH half-life experiments, surgery was performed on mated females 5 days (± 2 h) post-emergence; groups of animals were bled at intervals up to 8 h later in one experiment and up to 16 h later in a second experiment. Oocyte lengths were measured after hemolymph collection. Rates of in vitro JH biosynthesis and release were determined by a radiochemical assay[34, 36]. JH release is defined as the quantity of JH found in the medium at the end of incubation whereas JH biosynthesis is the quantity of JH released during incubation plus the quantity of JH contained within the CA at the end of incubation[36]. JH release is linearly related to both JH biosynthesis and JH content of the CA[36].

JH content of selected tissues was determined by the whole-body method of Bergot et al.[1] following homogenization of dissected tissues. Tissues were rinsed in cockroach Ringer, blotted and weighed prior to homogenization.

Results

a) Hemolymph JH titre. JH titres determined by physicochemical methods have been reported for adults of several species, but these studies have not spanned a gonotrophic cycle nor been compared directly with data on JH biosynthesis by CA in vitro. One purpose of our study was to determine if there were any correlation between *D. punctata* JH titre in hemolymph and JH biosynthetic rates in vitro. The results are shown in figure 1. JH III (C_{16}JH) was the only JH detected in the hemolymph of *D. punctata* at all stages. Titre was relatively low in the last nymphal stadium but began to increase at adult emergence and reached a maximum of 1600 ng/ml 5 days later. Titre then declined dramatically in the following 24 h period to less than 20% of the day 5 values. JH titre continued to decline and by day 54 was 43 ng/ml. At this age, females possessed nearly mature embryos (parturition usually occurred on day 63). The lowest JH titre we observed was in female nymphs between days 14 and 17 of the last stadium (fig. 1) (35 ng/ml). The JH III titre in adult males 5 days after emergence was ~ 50 ng/ml (data not shown). Relative rates of in vitro JH release essentially mirrored the hemolymph JH III titre profile throughout comparable life stages investigated (fig. 1).

Thus, in vitro rates of JH release appear to be an accurate indicator of the in vivo JH titres in *D. punctata*.

Two other parameters are also shown in figure 1: basal oocyte length (inset) and JH esterase activity (solid line). In all females, vitellogenesis began between days 2 and 3 after adult emergence; by days 7–8, chorion formation was complete. Oviposition occurred on day 8. The period of maximum oocyte growth corresponded to the periods of highest JH titre and maximum in vitro rates of JH III biosynthesis by the CA. JH esterase activity (from Rotin et al.[25] and Szibbo et al.[32]) is high at the end of the last larval stadium and declines over the imaginal moult to a minimum on day 3. By day 7 after adult emergence, JH esterase activity is again high. In general, the pattern of JH esterase activity is the opposite of the profiles of JH titre and biosynthesis.

b) Occurrence of solely JH III. While our data show JH III to be the major JH in *D. punctata*, the occurrence of low levels of other JHs ($\leqslant 0.05$ ng/ml) could not be ruled out in samples containing ~ 1000 ng of JH III/ml of hemolymph. The method employed for quantification of JH has limitations of dynamic range in the detection and peak integration systems; for accurate results the ratio of endogenous hormone: internal standard should not exceed a factor of 10^2 (or 10^{-2}). Thus, we specially processed a sample of female day 4–5 hemolymph to allow analysis of JH III separately from traces of other JHs (see 'methods'). Analyses revealed a JH III titre of 880 ng/ml, but no JH 0, JH I, or JH II at a limit of detection of 0.02 ng/ml. If any of the higher JH homologues occurred in *D. punctata*, they were present at levels $\sim 5 \times 10^4$ lower than that of JH III.

c) Whole body JH titre. To determine the proportion of JH in the hemolymph relative to the tissues, the quantity of JH in whole body extracts was determined at selected times during the first gonotrophic cycle (table 1). JH titres reached a maximum on days 4 and 5 and declined rapidly thereafter in agreement with haemolymph titres and in vitro JH biosynthetic rates. The lowest value of 46 ng/g was obtained from day 7 animals.

We then calculated JH levels for whole bodies on a per animal basis (table 1). Using data from figure 1, we also calculated values for JH levels in the hemolymph on 2 days for which replicate data were available and using hemolymph volume data obtained by standard ^{14}C-inulin dilution techniques[15] (56.5 ± 1.6 µl – day 2; 57.4 ± 2.0 µl – day 5). On day 2, the amount of JH in the hemolymph (on a per animal basis) was 25.6 ± 7.9 ng (n = 3) (mean ± SD) and on day 5, the amount of JH in the hemolymph was 91.3 ± 13.1 ng (n = 7). The quantity of JH in hemolymph thus appears to be less than that in whole body extracts (table 1) (79.8% on day 2 and 68.1% on day 5) and using Student's t-test for comparison of means, on day 2, $p < 0.15$ and on day 5, $0.025 \geqslant p \geqslant 0.01$. These data suggested that there may be a pool of JH outside of the hemolymph.

In an attempt to establish the existence of an extrahemolymph JH pool, we determined JH titre in several tissues of female *D. punctata* at selected times during the gonotrophic cycle. The tissues surveyed included ovary, fat body, midgut, hindgut, and accessory gland/brood sac. Fat body, ovary and accessory gland/brood sac contained appreciable quantities of JH when expressed on a unit weight basis, particularly on days 4 and 5; however, in no case did these values exceed those for whole body extracts (table 2). Moreover, levels of JH in these organs

Table 1. JH III levels in adult female *D. punctata* whole body extracts

Age (days)	JH III[a] (ng/g)	Whole body JH III content[d] (ng)	number of animals	Basal oocyte length[b] (mm)
2	127 ± 37[c]	32.1 ± 8.0[c]	15, 11, 12	0.80 ± 0.01
4	740	196	14	1.45 ± 0.05
5	504 ± 161[c]	134 ± 41.5[c]	13, 12, 12, 12	1.48 ± 0.01
6	103	25.5	14	1.71 ± 0.03
7	46	12.8	15	1.71 ∓ 0.03

[a] Titre values are based in each case on a single determination except on day 2, where n = 3 and day 5, where n = 4. [b] Mean ± SEM. [c] Mean ± SD. [d] (Including hemolymph) on a per animal basis.

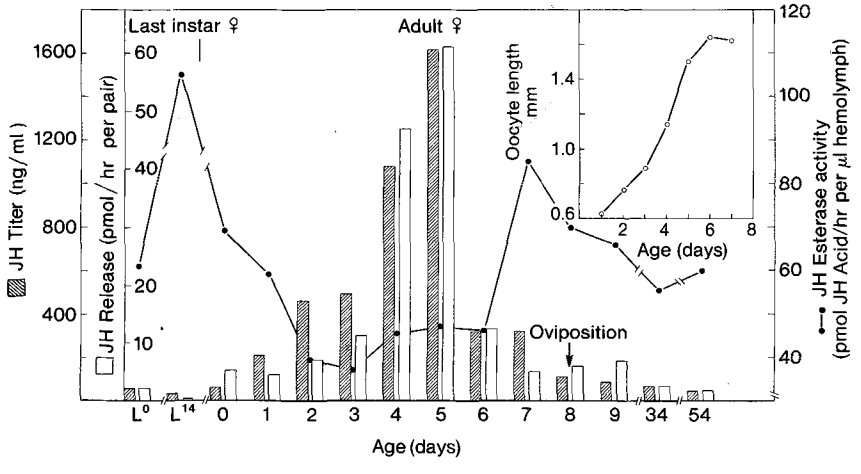

Figure 1. Hemolymph JH titre and rates of JH release in last instar and adult females of *D. punctata*. Also shown are basal oocyte lengths (inset) for those animals from which hemolymph was collected for JH titre determinations; each point represents the mean – SEM are less than the diameters of the points. JH release by CA in vitro was determined on a separate group of animals by a radiochemical assay[23,24]. Each bar for JH release represents the mean of 5–10 individual determinations and are taken in part from[36]. Also shown are values for JH esterase activity taken from[25]. Each bar for JH titre represents an individual determination on a pooled hemolymph sample of 205–1045 µl, obtained from 16 to 200 animals.

Table 2. JH III content of tissues of female *D. punctata* in ng/g

Age (days)	Ovary	Fat body	Gut	Accessory gland/brood sac	No. of animals	Basal oocyte length[a] (mm)
2	42 (0.03)[b]	16 (0.35)	9 (0.26)	ND[c]	36–59	0.73 ± 0.01
4	258 (0.75)	131 (3.6)	6 (0.30)	ND	30–60	1.36 ± 0.02
5	119 (0.29)	80 (1.4)	ND	204 (0.84)	30	1.45 ± 0.01
7	6 (0.03)	4 (0.10)	0.3 (0.01)	ND	34–67	Oviposited

[a] Mean ± SEM. [b] Figures in parentheses show calculated quantity of JH in tissue in ng per animal. [c] ND = not determined.

were rather low when expressed on a per animal basis. After correcting for ~ 50% recovery of fat body during dissection, we calculated that the total JH in fat body on day 4 was ~ 7.5% of that present in the whole body. Because the JH content of tissues in day 7 animals was very low, most of the JH in the tissues must have been catabolized rapidly.

d) Half-life of JH. The high levels of JH III in *D. punctata* (fig. 1) afforded a good model for the determination of physiological $t_{1/2}$. Because of the sensitivity of the GC/MS method for JH titre determination, quantities of JH as low as 0.1–0.5 ng/ml can be detected when basal levels of 1000 ng/ml are monitored. Day 5 mated females were allatectomized or sham-operated (time 0) and hemolymph JH titre was determined 1, 2, 4, and 8 h (exp. 1) or 1, 2, 4, 8, and 16 h (exp. 2) after the operation (see fig. 2). The JH titre declined rapidly after allatectomy. No attempt was made to generate a curve of best fit for the data. From the JH titres at time 0, we have determined the initial $t_{1/2}$ (see fig. 2) for experiment 1 = 1.0 h and for experiment 2 = 1.4 h (mean $t_{1/2}$ = 1.2 h). However, the rate of catabolism decreased after 4–8 h and thus the decay in endogenous JH did not obey first order kinetics. The JH titres for sham-operated animals are also shown in figure 2 and at least during the first 0–4 h, there was no appreciable difference between individual values and the mean value (1420 ± 140, n = 8). However, one sham value at 8 h and the 16 h value were significantly different from the mean, probably reflecting the dramatic decline in JH titre normally observed between days 5 and 6 (see fig. 1).

Figure 2 also shows mean basal oocyte length for the various groups of animals used in the half-life study. With the exception of one group of sham-operated and allatectomized animals at 8 h, which possessed oocytes somewhat smaller than the mean, the oocytes of virtually all other animals were of similar length; so the animals were assumed to be of similar physiological age.

Discussion

a) JH titre. The JH titre values reported in this study are some of the highest reported for any species and, in fact, exceed by at least an order of magnitude values reported for other adult orthopteroidean species (e.g. *Locusta migratoria*[2]; *Teleogryllus commodus*[19]) obtained using similar physicochemical methods. These values are also considerably greater than those determined in other dictyopteran species as well as in other orders such as Coleoptera, Hymenoptera, and Diptera (see Schooley et al.[28] for review). Titre values in certain Lepidoptera are low[28], whereas in adult male *Hyalophora cecropia*[3], they are very high (> 2000 ng/g of whole bodies).

The JH titre of ~ 6 μM in hemolymph of day five adult females approaches the limit of solubility of JH III in aqueous solution in the absence of proteins (see Giese et

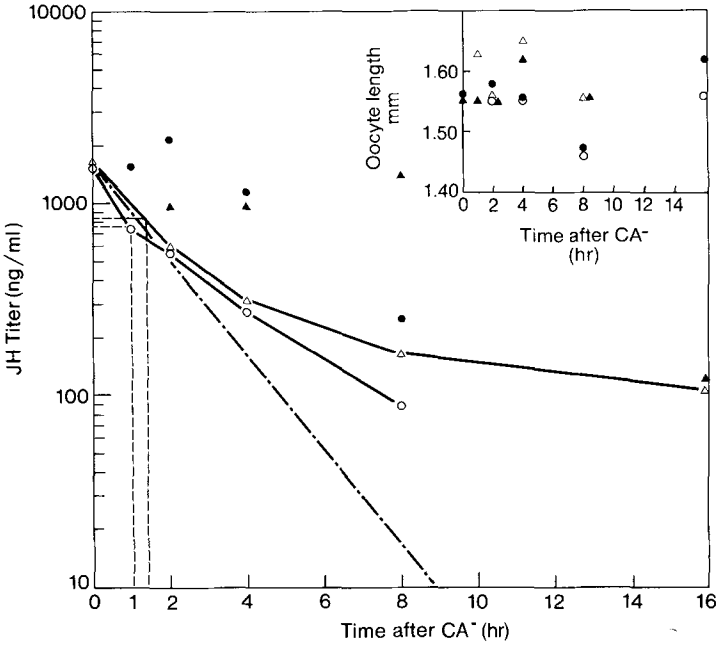

Figure 2. Decay in hemolymph JH III titre following allatectomy of day 5 female *D. punctata* at t = 0 h. At t = 0 h, JH titre for the two experimental series was 1600 ng/ml. One experiment was terminated at 8 h (○) and the other at 16 h (△). Also shown are values for sham-allatectomized animals from those studies terminated at 8 h (●) and 16 H (▲). Each point represents an individual determination on a pooled hemolymph sample of 209–377 μl from 16 to 25 animals. Also shown (inset) are mean basal oocyte lengths for all animals from which hemolymph was obtained. Initial half-life values based solely on the time required for the JH titre to decline to half of that at t = 0 h are shown for the two experimental series by the intersection of the dotted lines with the X-axis; apparent initial half-life for the two experiments was 1.0 and 1.4 h. Using the mean of these two half-life values, 1.2 h, the hypothetical JH titre after allatectomy has been predicted and are shown by the dot-dash line.

al.[9] and Reibstein et al.[24]). Although JH solubility is enhanced by proteins[9] this is still a remarkably high physiological concentration.

We were also able to show that at the time of maximum JH III titre (day 4–5, adult females) higher JH homologues were undetectable in hemolymph or present at levels 5×10^4 lower than those of JH III. JH I and JH II are more active than JH III in many bioassays; in the *Galleria* wax test and *Tenebrio* assays JH I is 100 times or 16,000 times more active, respectively, than JH III[3]. However, there can be little doubt that JH III is the only physiologically significant JH in *D. punctata*. The detection of only JH III in both nymphs and adults of *D. punctata* supports previous observations on the product from CA incubations in vitro[32,36]. Analysis of in vitro incubations of CA of *Periplaneta americana* using GC with a radioactivity monitor has also confirmed the absence of JH I and JH II at a detection limit of 10^{-5} that of JH III[11]. It is surprising that such large differences exist in JH titres between species. Although we cannot account for this range of titres, it is intriguing that variation in rates of JH release in vitro of up to 10^3 between different species have also been observed. It is also striking that the minimum JH titres we observed in *D. punctata* (in nymphs or in pregnant females) are similar to or greater than the maximum values observed in many other species. This perhaps implies that *D. punctata* may be relatively insensitive to JH and indeed previous results obtained following treatment with a JH analogue support this interpretation[37].

The striking correlation between in vivo JH titre and in vitro rates of JH release from animals of corresponding ages (fig. 1) suggests that JH synthesis and release can be used in *D. punctata* to predict relative in vivo JH titres although there is some question if this relationship holds for individual insects of another cockroach species (*Nauphoeta cinerea*)[18]. A similar correlation between JH biosynthesis in vitro and JH titre, as determined by *Galleria* bioassay, has been reported for *N. cinerea* as well[18]. In *D. punctata*, the changes in hormone synthesis/release are not due to changes in availability of substrates since in vitro measurements were performed in tissue culture medium under constant conditions. The precision of the relationship between JH release and hemolymph titre was tested by plotting JH titre as a function of JH release rate (data taken from fig. 1) for each day; an excellent correlation was observed: $r = 0.97$, $n = 14$. These observations lend support to the hypothesis that a major component of regulation of JH titre is the rate of release as suggested previously for adult insects[4,33] as well as for larval insects[8]. The profile of JH esterase activity (fig. 1)[25] bears an inverse relationship to in vivo JH titre and in vitro rates of JH biosynthesis. However, even the lowest rates of JH degradation by esterase are about three orders of magnitude greater than in vitro rates of JH biosynthesis. It is difficult to rationalize these very high levels of JH esterase activity although they may be somewhat artifactual in that rates of JH hydrolysis were measured with diluted hemolymph[25]. Nothing is known concerning binding proteins in *D. punctata* hemolymph which may protect JH from esterases, as do the JH binding proteins of lepidopterans[16,27]. Recent studies in *L. migratoria*[21] and *L. decemlineata*[6] call into question many prior studies on rate of metabolism of JH using exogenously applied labeled JH III or JH I. The unnatural 10*S* optical isomer of JH III is degraded more rapidly than (10*R*)-JH III in both species[6,21], because the hemolymph binding proteins have very high affinity for exclusively (10*R*)-JH III. Therefore, the unbound 10*S* isomer of exogenous [^{3}H]JH III is not protected from the non-enantioselective JH-specific esterase. Moreover, rate of hydrolysis of 10^{-7} M (10*R,S*)-[10-^{3}H]JH III is much faster in dilute (1:20) hemolymph of *L. decemlineata*[6], as compared to more concentrated (1:1) hemolymph. Such complex interactions may only become clear from studies of properties of separated preparations of the esterase and binding protein. We also do not know the relative contributions of low-affinity nonspecific esterases and high-affinity JH-specific esterases with respect to hydrolysis of JH in *D. punctata*. The apparent sequestration of JH into protected compartments (see below) which are likely in equilibrium with the hemolymph pool offers an additional mechanism for JH protection. Further studies may assist is resolving this paradox of massive catabolic activity greatly exceeding synthetic activity.

b) Whole body JH titre. Our results are consistent with the occurrence of a pool of JH outside of the hemolymph at selected ages in adult female *D. punctata*. This pool appears to be largest on day 5, when JH titre is maximal. The existence of such a pool has been hypothesized in *T. molitor* to explain the apparent persistence of JH effects following JH analogue treatment[10]. Our half-life experiments suggest that the non-hemolymph pool is in equilibrium with the hemolymph pool. Rapid uptake and reflux of JH by fat body in vitro has been claimed in *M. sexta*[20]; although the apparent uptake of the JH was attributed to specific 'binding' in this study, it is equally plausible that a sequestration of JH into protected pools within the fat body had also occurred. Interestingly, most of the radiolabeled product released from the *M. sexta* fat body was JH and not metabolites[20] and accordingly, the sequestered JH had been protected from catabolic enzymes.

An alternative explanation for the discrepancy between hemolymph and whole body JH titres may reside in the possible difference between hemolymph volume determined using radiolabeled inulin and actual volume of body fluids occupied by JH. Such a phenomenon has been proposed for ecdysteroid volume *vs* inulin volume in *Calliphora vicina*[15]. In the latter species, the body volume occupied by ecdysteroids appears to be much greater than the volume accessible to inulin and a similar situation may pertain to JH in *D. punctata*.

c) Half-life of JH. Although the $t_{1/2}$ of JH in the hemolymph has been determined in several species, most studies have measured the decay in exogenous radiolabeled JH for the calculation of $t_{1/2}$ (e.g. Kramer et al.[17]). While some studies have used JH bioassay to monitor the decline in endogenous JH following allatectomy or neck ligation to estimate $t_{1/2}$[8,13], the present study used endogenous JH titres determined by GC/MS for subsequent $t_{1/2}$ estimation. The initial $t_{1/2}$ of ~ 1.2 h for JH in 5 day adult female *D. punctata* is of a magnitude similar to that estimated for several other species including for example *L. migratoria* (1.5 h)[14] and fourth stadium *M. sexta* (1.5 h)[8]. A shorter half-life for JH has been observed in *L. de-*

cemlineata (25 min)[17]. The $t_{1/2}$ of 1.2 h for *D. punctata* is in fortuitous agreement with the value estimated from the disappearance of low doses of exogenous radiolabeled JH III (1.7 h)[26]. However, many of these half-life values were obtained using methodology which is now known to be deficient, especially as regards interactions with binding proteins. It should be noted further that $t_{1/2}$ values are valid only for the specific stages in the life cycle which were measured, because of large fluctuations in catabolic activity in insects as a function of age.

d) Use of the rate constant for degradation to estimate rate of biosynthesis. In view of the non-first-order decay in JH titre discussed above, the use of the rate constant for degradation to calculate a corresponding rate of biosynthesis is dubious at best. Levels of JH esterase and rates of JH release, and accordingly JH titre, change appreciably during the gonotrophic cycle and hence steady-state conditions, where rates of synthesis would equal rates of catabolism, may seldom be achieved. Other authors have used the rate constant obtained from different species and approaches[5,8] to calculate theoretical rates of JH biosynthesis necessary to maintain the JH titre. These authors[5,8] suggested that observed rates of JH biosynthesis in vitro were far less than those required to equal the rate of catabolism, probably because the CA were being maintained in vitro under suboptimal conditions. We find the same situation to pertain to our data from *D. punctata* half-life measurements, particularly at those times of high biosynthesis/release (fig. 1)[36]. However, because JH titre in *D. punctata* does not decline as steeply in vivo as anticipated based on $t_{1/2}$ values from in vitro degradation studies, it is probable that rates of JH biosynthesis necessary to maintain a steady titre are much lower than previously calculated using various kinetic parameters. Hence, in vitro rates of JH biosynthesis may reflect the in vivo situation more accurately than was originally presumed (see de Kort et al.[5]). Further $t_{1/2}$ studies will be necessary in order to confirm if non-first-order decay of endogenous JH occurs in other species.

e) Persistence of JH effects. The observed persistence of JH after allatectomy is consistent with the existence of a sequestered JH pool outside the hemolymph. The existence of a substantial JH pool within the male accessory glands of *H. cecropia* has been well documented[22,29]. However, this pool results from the methylation of JH acid, derived from the CA, within the accessory glands. This JH pool has been stated to exist exclusively in the accessory glands, although there appears to be no data on hemolymph JH or JH acid content. If sequestered JH pools in equilibrium with hemolymph pools also occur in other species, reinterpretation of data of JH metabolism and its effects may be necessary.

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Short Communications

Interhemispheric asynchrony of the sleep EEG in northern fur seals

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Summary. In northern fur seals the two brain hemispheres can generate the EEG slow waves during sleep not only simultaneously, as in all the terrestrial mammals investigated, but also independently as in dolphins.
Key words. Sleep; interhemispheric relations; pinnipeds.

Electrophysiological studies on sleep in the bottlenose dolphin, *Tursiops truncatus*[1,2], and in the porpoise, *Phocoena phocoena*[3], have revealed that the main stage of sleep in both dolphin species is unihemispheric slow wave sleep. This type of sleep was not found in the Caribbean manatee, *Trichechus manatus*[4], but this study was based only on two night-recording sessions on a single animal. No unihemispheric sleep has been recorded in the Caspian seal, *Phoca caspica*[5], or in the harp seal, *Pagophilus groenlandicus*. Two other studies on Phocidae[6,7] provide no information about interhemispheric interrelations during slow wave sleep.

The present investigation is an attempt to test the existence of unihemispheric slow wave sleep in a member of the Otariidae, the other pinniped family; the northern fur seal, *Callorhinus ursinus*. A detailed characterization of the sleep-wakefulness cycle for this species will be given later.

Methods. Sleep was studied in 12 fur seals of both sexes (6 males and 6 females) and different ages (from 2 months old to 22 years old). Electrodes were implanted under chloralose anesthesia (30 mg/kg i.m. for cubs and 50 mg/kg i.m. for subadults and adults). The skull dorsal surface was exposed. Epidural electrodes (steel screws, 0.8 mm in diameter) were situated over different cortical areas. Standard for all the animals were bipolar recordings of the electroencephalogram (EEG) from each hemisphere through the electrodes located symmetrically over the frontal and occipital cortical fields. Pairs of nichrome wire electrodes, 0.3 mm in diameter, were implanted in one of the orbits to record the electrooculogram, in the neck muscles to record their electromyogram and in the nasal muscles for the recording of the nostril respiratory motions and whisker twitches during paradoxical sleep. There was always a pair of the implanted electrodes available to record an electrocardiogram. The lead wires from all the electrodes were soldered to a 10-pin socket and embedded in acrylic cement. The skin cut was sewn up. All the seals were allowed to recover from the operation for several days. Thereafter polygraphic recording was started to last 3–6 days continuously. The electrodes were connected to the electroencephalograph input by special artefact-free cables. The experimental cage (3 × 3 m) was housed in a spacious open-air tank. Prior to the operation, the animals were allowed to adapt to the cage for up to two weeks.

The sleep of the fur seals was studied in two experimental situations: on the land and in the water. In the former case the water level was maintained at a minimum to ensure the seals' normal thermoregulation and a platform was set up above the water surface. In this situation the seals slept on the platform only. In the latter case, the platform was removed, and the water level was raised to reach 1.2 m so that the animals could sleep only in the water.

Results and discussion. The major EEG patterns in fur seals were