Ann. Biol. anim. Bioch. Biophys., 1979, **19** (6), 1827-1836.

A radioimmunological assay for naturally occurring insect juvenile hormones using iodinated tracers : Its use in the analysis of biological samples

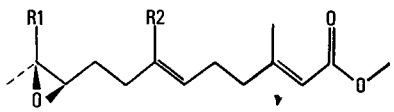
par J. C. BAEHR, P. PRADELLES*, F. DRAY*

Université Pierre et Marie Curie, Laboratoire de Cytophysiologie des Arthropodes, ERA 620, CNRS, 105, Boulevard Raspail, 75006 Paris.

* *FRA n° 8 INSERM, Unité de Radioimmunologie Analytique, Institut Pasteur, 28, Rue du Dr. Roux, 75724 Paris Cedex 15.*

Summary. Three sensitive and specific radioimmunological assays (RIA) using iodinated tracers have been developed for the quantitative measurement of naturally occurring juvenile hormone (JH). The antigens were prepared by binding the JH's to human serum albumin. Iodinated tracers were prepared by binding histamine to the JH molecule, and then subsequently binding one molecule of ^{125}I per molecule of histamine. The RIA's were carried out in 0.02 M phosphate buffer containing 0.1 p. 100 of BSA. The assay detection limit was 20 pg for all the three juvenile hormones. RIA sensitivity (at B/BO = 50 p. 100) of JH_1, JH_2 and JH_3 was 0.75, 1 and 2.6 ng/ml, respectively. Antiserum to JH_1 cross-reacted 7.3 p. 100 with JH_2 and 0.8 p. 100 with JH_3 ; antiserum to JH_2 cross-reacted 26 p. 100 with JH_1 and 12 p. 100 with JH_3, and antiserum to JH_3 cross-reacted 0.3 p. 100 with JH_1 and 0.9 p. 100 with JH_2. Various methods of preparing biological samples for the RIA of JH's have been described.

Radioimmunological assays (RIA) are useful tools in investigative research, and are commonly used in the field of vertebrate physiology. In insect physiology, radioimmunoassays for ecdysteroids are used routinely in many laboratories. However, the first RIA described for JH's (fig. 1) (Lauer *et al.*, 1974), which used a tritiated tracer, was not sufficiently sensitive to measure the low levels of hormone present. We have developed a RIA for JH_1 (Baehr *et al.*, 1976), and more recently for JH_2 and JH_3, using high specific activity ^{125}I-labelled tracers. The present work describes the preparation of the immunogens and the iodinated tracers, the methods used for the assays,



JH1 : R1 = R2 = C_2H_5
JH2 : R1 = C_2H_5 R2 = CH_3
JH3 : R1 = R2 = CH_3

FIG. 1. — *Structures of three natural juvenile hormones.*

and their sensitivity and specificity. The application of these assays to the measurement of juvenile hormones in biological material is discussed.

Methods and results.

JH Radioimmunoassay.

When the RIA for JH_1 was being developed, each step in the preparation of the immunogen and the iodinated tracer could be controlled by using a tritiated JH_1 tracer (JH_1, NEN, 13 Ci/mM) (Baehr *et al.*, 1976). Radioactively-labelled JH_2 and JH_3 were not available ; immunogens and iodinated tracers for the assay of these two hormones were prepared by following the methodology devised for JH_1. The JH_1 was the generous gift of Roussel Uclaf (Romainville, France) ; the JH_2 and JH_3 were obtained from Calbiochem (San Diego, California, USA).

Preparation of the immunogen. — 20 mg of juvenile hormones were dissolved in 0.25 ml of methanol and mixed with 15 μCi of tritiated hormone (for the preparation of the JH_1 immunogen only) ; 0.4 ml of methanol/NaOH (2N) (v/v) was added to the JH methanolic solution and then complexed to N'-hydroxysuccinimide (NHS) in the presence of N-N'-dicyclohexyl-carbodiimide. In an alkaline medium, an amino radical of the lysine of human serum albumin was substituted for NHS. On the basis of isotopic dilution twenty six molecules of acid JH were fixed per molecule of HSA.

Immunization schedule. — For each immunogen, five white rabbits were immunized intradermally with 600 μg of the conjugate emulsified in complete Freund's adjuvant. Booster injections were given intradermally with same amount of conjugate at 5-week intervals. The animals were first bled 10 days after booster injection and then weekly. The best blood samples were obtained 2 or 3 weeks after the second booster injection. For each immunogen, two rabbits gave antisera with a high binding level.

Preparation of the iodinated tracer. — In an alkaline medium, one molecule of histamine was substituted for the NHS in the JH-NHS complex. The JH-histamine (JH-His) was labelled with ^{125}I using the chloramine T method. To 1.5 nmol of JH-His in 5 μl of PBS 0.5 M, pH 7.4, were added 320 μCi of ^{125}I and 4 μl of chloramine T (3 mg/ml in 0.05 M PBS, pH 7.4). After 1 min, the reaction was stopped by 4 μl of sodium metabisulfite (15 mg/ml in 0.05 M PBS, pH 7.4).

The reaction products were purified by TLC (chloroform/methanol/water 80 : 20 : 2 on silica gel plate F254 Merck) ; a labelled compound ($R_F = 0.64$) was separated from unlabelled JH-His ($R_F = 0.40$) and free iodine ($R_F = 0.03$). The yield of iodination was estimated to be 30 p. 100 (on the basis of the isotopic dilution of unlabelled JH-His). Since the excess of JH-His in proportion to the amount of iodine did not allow diiodo-derivative synthesis, the specific activity of the JH-His-^{125}I was close to the theoretical value of 2 000 Ci/mmol.

Radioimmunoassay. — JH is strongly adsorbed onto a variety of substrates (Giese, Spindler and Emmerich, 1977). This adsorption was reduced in our experiments by carrying out the assay in an aqueous medium using a 0.02 M phosphate buffer containing 0.1 p. 100 of BSA (fraction V). In these conditions, there was about 0.2 p. 100 adsorp-

tion onto siliconized glassware (1 p. 100 siliclad), about 1.5 p. 100 onto polystyrene, and about 1.4 p. 100 onto Eppendorf cones. In the absence of BSA, adsorption onto plastic materials was about 40 p. 100.

100 µl of the sample to be assayed, 100 µl of the tracer and 100 µl of diluted antiserum were pipetted successively into polystyrene tubes. The reaction was allowed to equilibrate at 20 °C for 2 hrs or at 4 °C for 12 hrs ; it was then stopped by the addition of 1 ml dextran-coaded charcoal (Norit A, 2.5 g, Dextran T70, 0.25 mg in 100 ml buffer) to each tube. After incubation at 0 °C for 20 min, the tubes were centrifuged at 2 200 g for 20 min at 4 °C. The radioactivity in the precipitate was counted in a gamma counter.

The antisera having the best sensitivity also showed the best specificity and elevated antibody concentration. Only these antisera were used routinely. They were diluted 1/300 000 to give a binding of 30 to 40 p. 100 in the absence of any added JH (Bo). The sensitivity of the JH_1, JH_2 and JH_3 assays (B/Bo = 50 p. 100) were 0.75, 1 and 2.6 ng/ml, respectively (fig. 2).

The standard deviations for the three assays were 0.10 ng/ml (10 pg/tube).

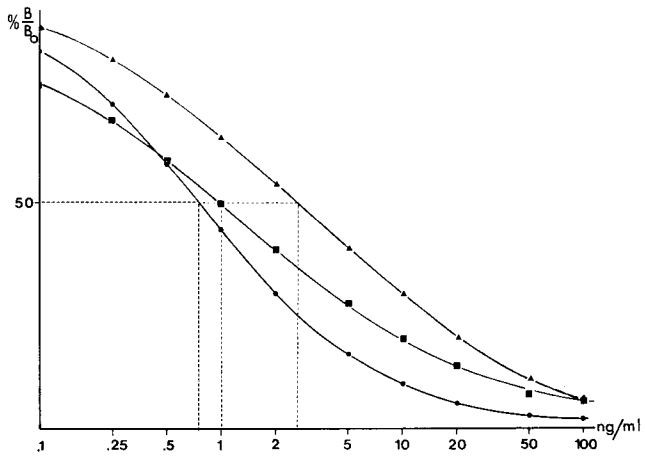


FIG. 2. — *Inhibition of labelled JH_1 binding to anti JH_1 in the presence of increasing amounts of unlabelled JH_1 (●).*

Inhibition of labelled JH_2 binding to anti JH_2 in the presence of increasing amounts of unlabelled JH_2 (■).
Inhibition of labelled JH_3 binding to anti JH_3 in the presence of increasing amounts of unlabelled JH_3 (▲).

The cross reactions for the three JH's were as follows :

	JH_1 (p. 100)	JH_2 (p. 100)	JH_3 (p. 100)
Anti JH_1	100	7.3	0.8
Anti JH_2	26	100	12
Anti HJ_3	0.3	0.9	100

The specificity of the antisera raised against JH_1 and JH_3 was satisfactory, but that of the antiserum to JH_2 could be improved. An increased sensitivity of all three assays would mean that the volume of biological sample required could be reduced.

Attemps to improve RIA sensitivity and specificity. — A study of the cross reactions of the antiserum directed against JH_1 showed that the JH_1-histamine complex had a cross-reaction of 210 p. 100 as compared with the JH_1 (Baehr *et al.*, 1976). The greater affinity for the iodinated tracer than for the JH_1 resulted in an RIA of lower sensitivity than that which could be achieved using a tracer of lower affinity but with the same specific radioactivity. The presence of a carboxy amino link in C_1 in the immunogen explained the strong affinity of the antibodies for this structure, which was also found in the JH-His complex.

The use of heterologous tracers improved the sensitivity and specificity of the RIA to some extent (see table 1).

TABLE 1

Variations of RIA sensitivity with combinations of the different incubation systems.

A = anti JH_1, A′ = JH_1, A″ = JH_1-His-^{125}I,
B = anti JH_2, B′ = JH_2, B″ = JH_2-His-^{125}I, C = anti JH_3, C′ = JH_3,
C″ = JH_3-His-^{125}I

Antisera	Cold standard	Iodinated tracer	50 p. 100 sensitivity B/B_0 (ng/ml)	B_0 p. 100 $\left(\frac{B-T}{AT}\right)$	Antiserum initial dilution
A	A′	A″	0.75	33	100 000
A	B′	A″	10.17	33	100 000
A	C′	A″	93.80	33	100 000
B	B′	B″	1.00	31	100 000
B	A′	B″	3.80	31	100 000
B	C′	B″	7.93	31	100 000
C	C′	C″	2.60	33	100 000
C	A′	C″	866.00	33	100 000
C	B′	C″	288.10	33	100 000
A	A′	B″	0.47	33	50 000
A	A′	C″	0.79	34	10 000
B	B′	A″	3.60	40	100 000
B	B′	C″	1.00	30	30 000
C	C′	A″	2.42	35	25 000
C	C′	B″	2.60	38	35 000
B	A′	A″	9.13	37	50 000
C	A′	A″	50.95	45	25 000
B	C′	A″	31.58	35	50 000
C	B′	A″	27.75	40	25 000
A	B′	B″	1.58	31	50 000
C	B′	B″	30.34	42	35 000
A	C′	B″	15.56	34	50 000
C	A′	B″	44.55	46	35 000
B	A′	C″	50.21	40	30 000
A	B′	C″	6.03	34	10 000
A	C′	C″	16.61	36	10 000
B	C′	C″	7.14	42	30 000

JH_1 assay : homologous assay sensitivity (B/Bo = 50 p. 100) was about 0.75 ng/ml, which fell to 0.47 ng/ml using JH_2-His-^{125}I (anti JH_1 + JH_1 + JH_2 His ^{125}I). In these

conditions, cross-reactions with JH_2 and JH_3 were 29 and 3 p. 100, respectively, as compared with 7.3 and 0.8 p. 100. The use of the JH_3-His-^{125}I tracer did not improve sensitivity or specificity.

JH_2 assay : homologous assay sensitivity (B/Bo = 50 p. 100) was about 1 ng/ml, which was not improved when other combinations were used. The system (anti JH_2 + JH_2 + JH_3-His-^{125}I) did not alter sensitivity (1 ng/ml), but improved specificity for JH_1. The cross-reactions with JH_1 and JH_3 were 1.9 and 14 p. 100, respectively, as compared with 26 and 12 p. 100.

JH_3 assay : no alteration significantly impoved either sensitivity or specificity.

The modification of the JH_1 assay (using the JH_2-His-^{125}I tracer) may be used in the routine method, since it definitely increased sensitivity. Although there was a loss of specificity in regard to the other JH's, the system could be applied to assay JH_1 after separation from JH_2 and JH_3 by high-performance liquid chromatography (HPLC) (see below).

The use of the JH_3-His-^{125}I tracer for the assay provided satisfactory sensitivity, increased the cross-reaction with JH_1, and caused a negligeable loss in the cross-reaction with JH_3.

None of the other combinations showed any advantages ; unfortunately, a sensitive system was not found in which the three JH's were similarly recognized.

Application of juvenile hormone RIA to biological samples.

RIA is a rapid technique which lends itself to the routine analysis of large numbers of biological samples. Extraction and purification techniques should be effective without being too laborious. Many techniques have been tested ; only those giving the most satisfactory results are reported here.

Sampling and extraction of JH's.

— Haemolymph : a known volume of haemolymph was taken up in a calibrated capillary tube, and 100 to 200 µl were placed in at least twice their volume of methanol/diethyl ether (1:1, v/v). To prevent the action of esterases this operation must be carried out as rapidly as possible. A known amount of tritiated JH_1 (9,000 dpm, 13 Ci/mM) was added to each sample to measure the recovery of JH which was extracted, purified and redissolved in a buffer prior to assay. The samples were stored in the methanol/diethyl ether mixture at — 30 °C.

The juvenile hormones were extracted by hexane (8 volumes of hexane per volume of methanol/diethyl ether/sample) ; this mixture was homogenized supersonically for 1 min and then centrifuged at 3 500 g for 20 min at 4 °C. The upper phase containing the JH's was removed ; recovery was between 96 and 100 p. 100. The lower phase contained the ecdysones, which could also be assayed by RIA.

— Whole insects or insect organs : the samples were homogenized in a mixture of diethyl ether/methanol 1:1, v/v) (2 volumes of solvent for 1 volume of sample) and were then treated as described above for the heamolymph.

Extract purification.

Schooley (1977) has reviewed several techniques for the purification of JH's from biological extracts. The following methods were tested in these experiments : cold

precipitation of lipids ; thin layer chromatography ; chromatography on celite column, aluminium oxide column, silica gel column, and Sephadex LH-20 gel column, and high performance liquid chromatography (HPLC). When possible, the same extract was assayed after purification by these different techniques. Preliminary results showed that the first three methods did not separate JH's from other substances which interfered with the assay (negative dilution test), and gave only low recoveries. These three were therefore discarded.

Chromatography on Sephadex LH-20 gel (gel permeation) : several solvent systems were used (benzene/acetone 1/1 ; benzene/methanol 1/1 ; methanol alone). Although the technique was laborious, it was the best method for purifying lipid-rich samples.

Chromatography on aluminium oxide column : the column consisted of Pasteur pipettes 5.7 mm in diameter packed with 3 cm of aluminium oxide powder (activity, 2-3 Merck) and then with 3 cm of disodium phosphate. The extracts, redissolved in 2 ml of hexane, were placed on the column and eluted with 1 ml of hexane/benzene, 1/1, then with 3 ml of benzene. The JH's were eluted in the benzene fraction with a recovery of about 95 p. 100. In these conditions, the JH acid and diol forms were eluted by more polar solvents than benzene. The column blanks were less than 10 pg/column.

Chromatography on silica gel : the column consisted of Pasteur pipettes 5.7 mm in diameter with a 5 cm silica gel stationary phase (Merck, granulation less than 0.063 mm). The sample, in 2 ml of hexane, was placed on the column and eluted with 2 ml of hexane/diethyl ether 95/5, 2 ml of hexane/diethyl ether 90/10 and 5 ml of benzene/diethyl ether 85/15. The JH's were eluted in the last phase. JH break down products (acids and diols) were eluted by the more polar solvents. Recovery was about 95 p. 100 and the column blanks were less than 10 pg/column.

High-performance liquid chromatography (HPLC) : it was necessary to purify the extracts before injecting them onto the column. Aluminium oxide or silica gel columns are well adapted to this purpose.

Normal phase columns (30 cm $\times$ 0.7 mm μPorasil Waters Associates) and reverse phase columns (30 cm $\times$ 0.7 mm C_{18} μBondapaks, Waters Associates) were tested. In normal phase, the JH's were eluted in 9-11 min (hexane/diethyl ether, 96/4, 1.5 ml/min), and it was difficult to separate them in those conditions. In reverse phase (methanol/water, 4/6, 1.5 ml/min) the JH_3 was eluted in 8 min, the JH_2 in 10 min and the JH_1 in 13 min. Recovery was 93 p. 100 in both systems, and the blancks were below 5 pg/ml of elution.

Results were obtained rapidly using the aluminium oxide column or silica gel column chromatography. Their effectiveness was validated by comparing the results of JH_1 RIA (homologous assay) of an aliquot of the same extract either without purification or after purification on aluminium oxide column alone, purification on aluminium oxide column plus normal phase HPLC or purification on aluminium oxide column plus normal and reverse phase HPLC. After correcting the results for recovery, similar values were obtained for 13 samples assayed after the three different types of purification. The amounts measured in crude extracts were much higher than those measured in purified extracts.

Redissolution in the buffer for RIA.

Whatever purification technique was used, the JH's were collected in an organic phase and the solvents had to be evaporated before redissolving the extract in the buffer for RIA. The JH's dissolved poorly in the buffer and recovery was generally low ; it could be improved by using one of the following techniques :

— extract taken up in methanol (1/3 of the final volume), shaken and buffer is added (2/3 of the final volume). The RIA was unaffected by this amount of methanol (which equalled 1/9 of the final RIA incubation volume) ;

— extract taken up in buffer and supersonically treated for 1 min, then shaken for 1 hr at 37 °C.

After either of these procedures, total recovery (extraction, purification on aluminium oxide or silica gel column, redissolution in the buffer) was between 70 and 80 p. 100.

Discussion.

The endogenous levels of JH reported in the literature are very variable. This is probably due to the many different methods of extraction used. The concentrations reported are of the order of ng/g or of ng/ml of haemolymph, which are compatible with the synthetic capacity of the corpora allata *in vitro* (Pratt and Tobe, 1974). Radioimmunoassay (limit of detection, 10 to 20 pg) is useful in the assay of JH's in biological samples ; the specificity of the antibodies is such that the different JH's may be quantitatively measured individually.

The three JH's may be assayed (each assay carried out in duplicate) using only 200 µl of haemolymph, and the volume of haemolymph required may be further decreased by reducing the incubation volumes.

Up to 150 samples may be assayed by one person in one week. These assays have already been used to study the variations of JH levels during growth and reproduction in some insect species (table 2) and for direct measurement of the JH's in a culture medium (table 2).

As compared to biological assays, RIA has the advantage of being specific and rapid, and requiring only small amounts of biological material. As compared with physico-chemical techniques, such as electron capture or mass spectrometry, RIA is as sensitive (see Schooley, 1977), less expensive and more rapid, also requiring smaller amounts of biological material.

In addition to the dilution test (serial dilution assay of the same biological sample) and the addition test (recovery of a known quantity of JH from a biological sample), which were positive, the validity of RIA was demonstrated by comparing the JH levels in the haemolymph of normal insects and those treated with Procecene II. The larvae of *Locusta migratoria* treated with Precocene II (100 µg/animal in 100 µl of acetone) during the first 24 hrs of the fourth stage, moult into adult-like forms with an atrophied corpara allata (Pener, Orshan and de Wilde, 1978 ; Schooneveld, 1978, 1979). Radioimmunoassay of the JH levels in these Prococene treated fourth stage larvae revealed

TABLE 2

Minimal and maximal content of JH *immunoreactive material in total extracts* (TE) (in pg/animal), *haemolymph extracts* (HE) (in ng/ml of haemolymph) *or the culture medium* (CM) (in pg synthesized/gland/hr) *of various orders of insects*

Insect species	Instar		JH_1	JH_2	JH_3	Références
Locusta migratoria	4	HE.....	7.5-28.2	ND *	7.0-25	Baehr *et al.*, 1979 for JH_1 only
	5	HE.....	0.9-34.5	ND	0.4-59	JH_2 and JH_3 in progress
Schistocerca gregaria	4	HE.....	0.2-7	ND	2.5-15	Papillon, Porcheron, Baehr, 1979
rearing to 33 °C	5	HE.....	0-1.2	ND	0.4-3.8	in preparation
Schistocerca gregaria	4	HE.....	4.5-0.5	ND	0.8-7.4	in preparation
rearing to 28 °C	5	HE.....	0-2	ND	0-3	in preparation
Rhodnius prolixus	4	HE.....	0-60	0-16	0-18	Baehr, Porcheron and Dray, 1978
	5	HE.....	0-24	0-16	0-17	
Labidura riparia	non vitello.	HE.....	0	0	2	
	vitellogenic	HE.....	0	0	25-9	Caussanel *et al.*, 1979
Aspis mellifera	adult worker	HE.....	1-9	3-7	31	in progress
	4 queen	TE.....	330	ND	2 160	Lensky, Baehr and Porcheron, 1978
	5 worker	TE.....	60	ND	10	Lensky, Baehr and Porcheron, 1978
Locusta migratoria	5	CM	3-6	7.5-39	77-860	Caruelle, Baehr and Cassier, 1979
Manduca sexta	5	CM	38	ND	ND	Granger *et al.*, 1979 (in preparation)

* ND : non determined.

that in the 3 days following Precocene application, the JH levels dropped to almost zero (table 3).

TABLE 3

Juvenile hormone immunoreactive material in control and Precocene-treated Locusta migratoria *fourth instar larvae.* JH levels are expressed in ng/ml of haemolymph. For each JH titre determination, the haemolymph of 5 to 7 insects was pooled. (In this experiment, the JH levels of control animals were lower than in normal animals (Baehr *et al.*, 1979) because the controls, as the treated animals, were kept in a dark room to prevent Precocene breakdown)

	Time after Precocene application											
	6 h			24 h			48 h			72 h		
	JH_1	JH_2	JH_3	JH_1	JH_2	JH_3	JH_1	JH_2	JH_3	JH_1	JH_2	JH_3
Control	2.44	0.88	1.83	1.72	1.56	1.72	1.42	1.69	1.69	1.59	2.16	4.16
Precocene-treated	2.10	5.50	7.50	1.23	1.02	2.87	0.73	0.99	0.31	0.62	0.25	0.31

The disappearance of the immunoreactive substance in chemically allatectomized insects argues in favor of RIA validity which, however, cannot be extended to other, cases. Since insect material is heterogeneous, the appropriate purification technique removing the substances binding aspecifically to the antibody or the tracer, must be selected in each case.

Reçu en mai 1979.
Accepté en juin 1979.

Résumé. Trois dosages radioimmunologiques (RIA) sensibles et spécifiques ont été mis au point pour le dosage de chacune des hormones juvéniles (JHs) naturelles d'insectes. Les immunogènes ont été préparés en couplant la JH à la sérum albumine humaine. Des traceurs iodés ont été obtenus en couplant une molécule d'histamine à la JH puis en fixant une molécule d'iode 125 par molécule d'histamine.

Les RIA sont effectués dans du tampon phosphate 0,02 M contenant 0,1 p. 100 de BSA ; la limite de détection des dosages est de 20 pg pour chaque JH. La sensibilité des RIA (B/Bo = 50 p. 100) pour la JH_1, la JH_2 et la JH_3 sont respectivement de 0,75 ng/ml, 1 ng/ml et 2,6 ng/ml. Les antisérums dirigés contre la JH_1 croisent à 7,3 p. 100 avec la JH_2 et 0,8 p. 100 avec la JH_3, les antisérums dirigés contre la JH_2 croisent à 26 p. 100 avec la JH_1 et 12 p. 100 avec la JH_3 et les antisérums dirigés contre la JH_3 croisent à 0,3 p. 100 avec la JH_1 et 0,9 p. 100 avec la JH_2. Diverses méthodes de préparation des échantillons biologiques pour les RIAs sont décrites et discutées.

References

BAEHR J. C., PRADELLES P., LEBREUX C., CASSIER P., DRAY F., 1976. A simple and sensitive radioimmunoassay of Insect Juvenile Hormone using an iodinated tracer. *FEBS Letters*, **69**, 123-128.

BAEHR J. C., PORCHERON P., DRAY F., 1978. Dosages radioimmunologiques des ecdystéroïdes et des hormones juvéniles au cours des deux derniers stades larvaires de *Rhodnius prolixus* (Stal) Insecte hémiptère Reduviidae. *C. R. Acad. Sci. Paris, Sér. D*, **287**, 523-526.

BAEHR J. C., PORCHERON P., PAPILLON M., DRAY F., 1979. Haemolymph levels of juvenile hormone, ecdysones and protein during the last two larval stages of *Locusta migratoria*. *J. Insect Physiol*, **25**, 415-421.

CARUELLE J. P., BAEHR J. C., CASSIER P., 1979. Synthèse d'hormones juvéniles *in vitro* pour les *Corpora allata* de larves du 5^{e} stade de *Locusta migratoria migratorioides* (R. et F.) (Insecte, Orthopteroïde). *C. R. Acad. Sci. Paris, Sér. D*, **288**, 1107-1110.

CAUSSANEL C., BAEHR J. C., CASSIER P., DRAY F., PORCHERON P., 1979. Corrélations humorales et ultrastructurales au cours de la vitellogénèse et de la période de soins aux œufs chez *Labidura riparia* (Insecte, Dermaptera). *C. R. Acad. Sc. Paris, Sér. D*, **288**, 513-516.

GIESE C., SPINDLER K. D., EMMERICH H., 1977. The solubility of Insect Juvenile Hormone in aqueous solutions and its adsorption by glassware and plastics. *Z. Naturforsch.*, **32C**, 158-160.

GRANGER N. A., BOLLENBACHER W. E., VINCE R., GILBERT L. I., BAEHR J. C. DRAY F., 1979. *In vitro* biosynthesis of Juvenile hormone by the larval Corpora allata of *Manduca sexta* : quantification by radioimmunoassay (in preparation).

LAUER R. C., SALOMON P. H., NAKANISHI K., ERLANGER B. F., 1974. Antibodies to Insect C-16 Juvenile hormone. *Experientia*, **15**, 558-560.

LENSKY Y., BAEHR J. C., PORCHERON P., 1978. Dosages radioimmunologiques des ecdysones et des hormones juvéniles au cours du développement post-embryonnaire chez les ouvrières et les reines d'abeille (*Apis mellifera* L. var. *ligustica*). *C. R. Acad. Sci. Paris, Sér. D*, **287**, 821-824.

PAPILLON M., PORCHERON P., BAEHR J. C., 1979. Taux hémolymphatiques d'hormones juvéniles (JH_1 et JH_3) d'ecdystéroïdes et de protéines au cours des deux derniers stades larvaires de Schistocerca gregoria : effects de la température. *Experientia* (in press).

PENER M. P., ORSHAN L., DE WILDE J., 1978. Precocene II cause atrophy of *Corpora allata* in *Locusta migratoria*. *Nature*, **272**, 350-353.

PRATT G. E., TOBE S. S., 1974. Juvénile hormones radiobiosynthetised by *Corpora allata* of adult female Lucusts *in vitro*. *Life Sci.*, **14**, 575-586.

SCHOOLEY D. A., 1977. Analysis of the naturally occuring juvenile hormones their isolation, identification and titer determination at physiological level, 241-288. In TURNER R. B. *Analytical biochemistry of insects*. Elsevier Sc. Publ. Comp. Amsterdam, Oxford, N. Y.

SCHOONEVELD H., 1978. Precocene-induced *Corpus allatum* degradation in the migratory locust, *Locusta migratoria*. *Eighth int. Symp. Comparative Endocrinology*, Amsterdam.

SCHOONEVELD H., 1979. Precocene induced collapse and resorption of *Corpora allata* in nymphs of *Locusta migratoria*. *Experientia*, **35**, 363-364.