temperature, a similar incidence of thyrotrophic imbalance in the SHR cannot be observed. Serum T_3 levels are low and serum TSH levels elevated. Under these circumstances it was possible to demonstrate a highly significant increase in the TSH cold-response in the SHR as compared with the WKY control rats. On the other hand, the TRH-induced TSH secretion is not altered. These results demonstrate that the reason for the thyroidal disturbance in the SHR evidently lies in the hypothalamic TRH neurons rather than in the anterior pituitary. It is noteworthy that we do not see any alteration in the MBH TRH levels. Similarly, it has also been impossible earlier to detect a measurable change in the hypothalamic TRH content after practically any physiological or pharmacological treatment[16].

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Identification of JH III as the principal juvenile hormone in *Locusta migratoria*

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Summary. JH titers in the hemolymph of nymphal and adult female *Locusta migratoria migratorioides* (R. and F.) were determined using a selective mass spectroscopic detection technique. Only JH III could be found in either stage, with no detectable JH I (or II). Titers observed were 10–1000-fold lower than those found via a recently reported radioimmunoassay procedure.

Many species of insects representing several orders have been investigated in recent years to ascertain the structural nature of their juvenile hormones (JH). Among the Orthoptera only JH III (C_{16}JH) has been identified to date[1,2], in an admittedly small sampling of species. However, Baehr et al.[3] recently reported the existence of a JH I (C_{18}JH) 'immunoreactive substance' in nymphal hemolymph of *Locusta migratoria migratorioides* (R. and F.), identified and quantified by means of a radioimmunoassay (RIA) technique. These researchers, moreover, interpret this result to be a representation of authentic JH I titer in *Locusta*. Simultaneously, workers at the University of Utrecht, using gas chromatography-electron capture detection (GC-ECD) have found what is claimed to be JH III in the same species at comparable developmental stages[4,5]. As we have maintained a long-standing interest in JH identification and titer determinations[6,7], we in turn have examined hemolymph extracts from *Locusta* nymphs and female adults by a recently developed technique[8] using combined gas chromatography/mass spectroscopy (GC/MS) with selected ion monitoring (SIM), and report our findings on JH titers thereform.

Materials and methods. Insects were reared on grass and bran in a 15L-9D photoregime at 32 °C; under these conditions the IVth and Vth larval instars last 4–5 and 7 days respectively. The animals were staged for sampling

Comparative *Locusta* JH titer data obtained by GC/MS (A), GC-ECD (B[5]), and RIA (C[3])

Age/stage	Analytical method	JH[a,b]($\bar{X}_n$) I	II	III	n
24–48-h IVth instar nymphs	A	≤0.02	≤0.02	0.93	3
	B	<0.3	<0.1	1.1	2
	C	25.5	ND	ND	15
0–24-h Vth instar nymphs	A	≤0.02	≤0.02	0.35	2
	B	<1.7	<0.8	≤1.8	2
	C	30.0	ND	ND	21
10–11(18)-day female adults	A	≤0.03	≤0.02	41.3	3
	B	(<1.7)	(<2.0)	(48.0)	4
	C	ND	ND	ND	-

[a] Titers are expressed in ng/ml (A, C) or ng/g (B) of hemolymph [b] ND = not determined.

within 16 h after a molt. Hemolymph was harvested by piercing the integument behind the head and collecting the expressed fluid in a capillary tube. Extraction of hemolymph, sample purification, conversion of JH to the 10-hydroxy-11-d_3-methoxy derivatives (methoxyhydrins), and final JH analysis were done as described elsewhere[8].

Results. Our analytical technique for JH titer determinations exploits the electron impact (EI) mass spectroscopic fragmentation of the JH d_3-methoxyhydrins, which affords an intense base peak at m/z 76 (JH III and internal standard, IS) or m/z 90 (JH II and I); by monitoring these 2 ions in the SIM mode (fig. a), we can achieve detection limits in biological samples of ca 0.01–0.02 ng JH per ml of hemolymph. By our method we find only JH III in all stages of *Locusta* examined (table), with no detectable JH I (or JH II, C_{17}JH) present (fig. b).

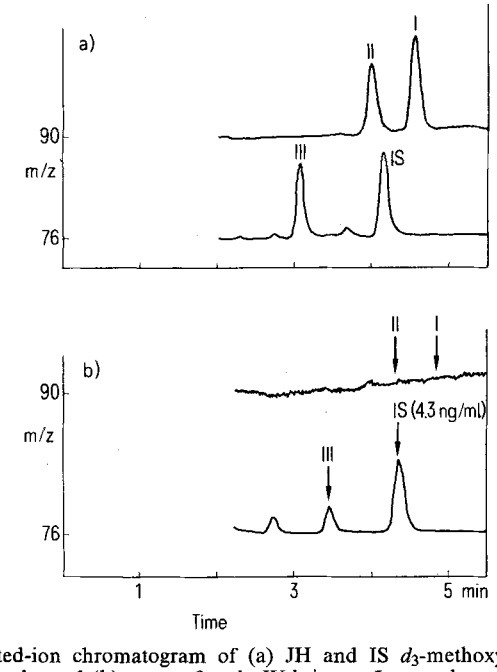
Qualitatively and quantitatively our data differ sharply from those obtained by RIA[3], and generally confirm the findings reported for the GC-ECD method[5,9] (table). Due to interfering compounds, threshhold sensitivities in the latter assay were rather poor for JH I and II (0.4–2.4 ng/g); consequently, the GC-ECD assay may have failed to detect levels of JH I and II comparable to those reported for JH III (0.9–2.3 ng/g) in *Locusta* nymphs. However, our current minimum detectable quantities of JH I and II approximate < 0.02 ng/ml, and we could detect neither.

Finally, from circa 2 ml hemolymph of 10–11-day-old *Locusta* adult females, we isolated approximately 100 ng of JH III as its underivatized epoxide, and obtained a confirmatory total ion EI mass spectrum, authenticating the suspected structure of the hormone.

Discussion. At this point we cannot state the reasons for the manifestly contradictory findings – in regard to both the identity and quantity of JH – between GC/MS(SIM) and GC-ECD vs the RIA determinations. A chromatographic peak-matching assay can well provide 'false positives' due to the presence of structurally similar coeluting contaminants. However, in a valid chromatographic assay the lack of a particular peak at a specific retention time quite conclusively demonstrates the absence of a chemical species. The Baehr group, on the other hand, reported low (~ 1%) cross-reactivity between JH I and JH III vs the 'JH I-specific' antibody used in their assay. Were they in fact measuring JH III titer only, their titer values reported as JH I (25–30 ng/ml) in nymphs would suggest actual levels of 2500–3000 ng/ml of JH III, a figure disproved by both GC/MS and GC-ECD data. Baehr et al. also found high (> 50%) cross-reactivity between JH I and its corresponding ester hydrolysis product, JH I acid; however, they used an alumina purification which reportedly removed the acidic metabolites. It would appear, then, that their data are not explicable by false positives generated by high amounts of carboxylic acid metabolites of JH present in hemolymph.

We believe that titer determination by RIA of one or more of the known JHs is currently flawed by insufficient ligand specificity and/or inadequate sample purification. These deficiencies would be especially troublesome in those many cases where more than one JH is produced simultaneously by an insect[6,10] and where there are dynamic fluctuations between the relative proportions of each homolog according to the stage of development[11,12]. In our view, it is imperative that present RIA methods for JH determinations include a penultimate liquid chromatographic separation of insect tissue extracts, in which well-resolved zones representing each JH are individually isolated before final assay with the appropriate JH-specific antibody (see Granger et al.[11] for an illustration of similar methodology).

In conclusion, definitive proof for the presence of a juvenile hormone other than JH III in Orthoptera has yet to be offered. By a specific mass spectroscopic detection method, we have shown the presence of JH III and – more significantly – the absence of JH I or II in *Locusta migratoria* juveniles and female adults.



Selected-ion chromatogram of (a) JH and IS d_3-methoxyhydrin standards, and (b) assay of early IVth instar *Locusta* hemolymph. JH III levels in this sample are ~ 1.1 ng/ml, while JH I and II are not detected at or above 0.02 ng/ml (0.003 ng = 2 × background). Analytical conditions are as described in Bergot et al.[7].

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