

# Comparison of Two Juvenile Hormone Radioimmunoassays

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Juvenile hormone from the hemolymph of adult worker honey bees of known age and behavioral status was extracted and analyzed by two different radioimmunoassays in two independent laboratories. The assays are different in hapten attachment, radiolabeled tracer, and the method by which bound and unbound hormone are separated. Despite these differences in the methods, hormone determinations were in excellent agreement at lower levels (0–50 ng/ml) but diverged as the hormone concentrations increased (> 50 ng/ml). The relative changes are in good agreement, with a correlation coefficient of 0.97. © 1993 Wiley-Liss, Inc.

**Key words:** honey bee, *Apis mellifera*, juvenile hormone, radioimmunoassay, hemolymph

## INTRODUCTION

Quantitative analysis of JH\* titers from biological samples can be achieved using several different techniques including bioassay [1], physicochemical analysis [2,3] and immunological analysis [4,5]. Each method has advantages as well as disadvantages. The relatively labile nature of the hormone and its exceedingly low concentration in the insect circulatory system make precise and accurate quantification difficult. A functional assay must be able to

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\*Abbreviations used: GC/MS = gas chromatography/mass spectrometry; JH = juvenile hormone; RIA = radioimmunoassay.

identify and measure picograms of hormone in extracts that may contain milligrams of contaminating lipid.

Although methods based on physicochemical characteristics of the JHs are the most accurate, such analyses are costly and time consuming. To overcome the logistic problems presented by the GC/MS methods, some investigators turned to the use of JH RIAs [4–9]. Despite the widespread acceptance of the RIA as an analytical tool for other insect hormones, the JH RIAs have been severely criticized for their lack of precision and accuracy [10,11]. Although earlier studies using the JH RIAs exposed many pitfalls in the method, more recently developed assays have addressed these problems. Indeed, the criticisms leveled at earlier assay procedures have led to the development of stringent criteria that exceed those used for other insect hormone radioimmunoassays.

In theory, all JH RIAs should yield similar results; however, differences in covalent attachment of JH to the immunogen, extraction of JH from the biological sample, and RIA protocol may lead to quantitative differences. In the present study, we compared the measurement of JH III in identical biological samples by two of these assays, that of Strambi et al. [6] and Goodman et al. [5]. Extracts from the hemolymph of adult worker honey bees were used to compare the two JH RIAs. JH is involved in the control of age-base division of labor among workers in honey bee colonies [12]. Previous studies have shown that low titers are associated with behavior within the nest such as brood care during the first to third week of adult life. Higher JH titers observed at about the third week are associated with the onset of foraging [3,13,14]. Precocious foraging is induced by treatment with JH [15], or JH analogs [16–19]. To test the efficacy of the present JH RIAs, honeybee hemolymph samples were split into two identical aliquots, coded, and sent to the respective RIA laboratories where they were assayed for JH III content.

## METHODS

### Insects

Bees were obtained from colonies maintained according to standard techniques at the University of Illinois Bee Research Facility, Urbana, Illinois. Bees of known age were obtained by removing combs containing developing pupae from a colony and placing them in an incubator (33°C). Emerging adults (N = 800) were marked on the abdominal dorsum with a paint spot (Testor's PLA) and introduced to a different colony with a population of about 40,000 workers, occupying two Langstroth hive bodies. This procedure permits precise staging of development without introducing undue stress.

### Hemolymph Collection and Preparation

Hemolymph was collected from well-staged adult bees [20] 1 day post emergence, 8–9 days post emergence (individuals involved in brood care, "nurses") and 21–22 days post emergence (foragers). After bees were chilled (0°C, 5 min) and immobilized with plasticine in a dissecting dish, hemolymph was collected using a micropipet. For each sample, hemolymph from individuals (2.5–15  $\mu$ l) was pooled from approximately 10–35 insects (48.5–154  $\mu$ l).

Hemolymph (40  $\mu$ l) from each sample was transferred to a vial containing 150  $\mu$ l of methanol [6]; the remainder was measured (7–114  $\mu$ l aliquots) and frozen in 5 ml conical-tipped vials. The methanol-hemolymph mixture was extracted 3 times with hexane. The hexane phase containing the JH was stored at  $-20^{\circ}\text{C}$ . The hexane-extracted samples were sent to Marseille while the frozen hemolymph was sent to Wisconsin. All samples were coded for blind analysis.

#### RIA Determination of JH Titers

Hemolymph was processed for RIA by either the method of Goodman et al. [5] or by the method of Strambi et al. [6]. Standard curves for JH III were generated using the 4 parameter logistic program of Rodbard et al. [21]. JH titers were interpolated from the curve and corrected for recoveries. Recoveries of JH were monitored using either an external [ $^3\text{H}$ ]-JH III standard [6,20] or an internal [ $^{14}\text{C}$ ]-heptachlor derivative [5].

### RESULTS AND DISCUSSION

With increasing use of RIA as the method of choice for determining JH titers, it is important that assays derived from different laboratories be cross-referenced with identical biological extracts. Simple comparisons of  $\text{ED}_{50}$ s and cross-reactivities derived from standard curves are insufficient criteria to evaluate RIAs since these evaluations do not reflect actual assay conditions. In both development and protocol, notable differences exist between the JH RIAs examined in this study. Theoretically, these differences should not lead to disparate results; however, in practical application deviations may occur. In assay development, the hapten for the Strambi assay was covalently attached to human serum albumin, through the epoxide end of the hormone. In contrast, the hapten in the Goodman assay was attached to bovine thyroglobulin via the C1 carbon, yielding an antiserum that displays different cross-reactivity and specificity patterns [4,5]. Sample preparation protocols are also different. In the Strambi assay JH is derivatized to a more polar molecule to eliminate cross-contamination with nonpolar lipids, while the Goodman assay employs TLC to remove cross-contaminants. Moreover, bound and unbound hormone are separated by different procedures; the Strambi assay utilizes dialysis while the Goodman assay employs ammonium sulfate precipitation.

Table 1 demonstrates that despite these major differences, both assays yield similar results. There is an age-dependent increase in JH titers as previously shown [14]. When newly emerged bees were titered, a paired t-analysis found no significant difference between the assays ( $P > 0.28$ ). Both assays also yielded statistically identical results for JH titers in nurse bees ( $P > 0.38$ ); however, comparison of forager JH titers indicates that they are significantly different ( $P < 0.05$ ). The basis for this statistical deviation is unclear, given that both assays have been validated by GC/MS [5,22]. Outliers greater than 6 S.D. from the mean were observed in both assays with a frequency of 7%. At present, the data bases for both RIAs are relatively small, thus making identification of outliers arbitrary; however, as data bases are enlarged, the use of statistical programs to assign misidentifications will be employed.

TABLE 1. Mean JH Titers for Adult Worker Honey Bees Obtained by Two Different RIAs\*

Bee age (days)	Strambi JH RIA (ng/ml $\pm$ S.E.)	Goodman JH RIA (ng/ml $\pm$ S.E.)
1	16.0 $\pm$ 2.1 (4) <sup>a,b</sup>	12.0 $\pm$ 2.9 (5) <sup>a</sup>
8-9 (Nurses)	28.4 $\pm$ 0.5 (5)	22.5 $\pm$ 5.0 (5)
21-22 (Foragers)	210.0 $\pm$ 28.7 (5)	113.6 $\pm$ 7.0 (4) <sup>b</sup>

\*Each sample (composed of hemolymph from 10-35 bees) was divided into identical aliquots. The JH titer of each sample was then determined by the Strambi [6] or Goodman [5] RIA.

<sup>a</sup>Number in parenthesis represents number of samples assayed. Each sample contains hemolymph pooled from 10-35 honey bees.

<sup>b</sup>One outlier sample ( $> 6$  S.D. from the mean) was deleted from the final computation.

Using the data from Table 1, a different type of comparison can be made, in which relative changes are compared. Plotted in this way (Fig. 1), the assays display a high degree of similarity with a correlation coefficient of 0.97 despite their divergence at higher hormone concentrations. The clinical endocrinology literature is replete with interassay comparisons [see references 23-25 as examples] citing poorer correlations than reported here. Moreover, many of these clinical comparisons were conducted in the same laboratory, while the present study was performed in two different laboratories.

Rapidly identifying trends in fluctuating hormone titers remains one of the most important functions for all radioimmunoassays. Our results indicate that the RIAs, despite their differences, yield comparable absolute values at lower concentrations (0-50 ng/ml) of JH but deviate when titers are higher than 50 ng/ml. Both assays yield excellent relative results that will make interlaboratory and interspecies comparisons of JH titers acceptable to the critical endocrinologist.

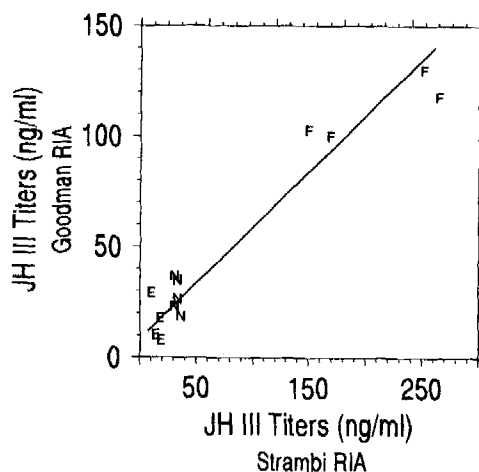


Fig. 1. A comparison of two different JH radioimmunoassays. Pooled samples of honey bee hemolymph were divided into identical aliquots and JH determined by either the Strambi [6] or Goodman [5] RIA. Comparisons are based on the data presented in Table 1. The correlation coefficient was 0.97 with a regression equation of  $Y = 4.58 + 0.47X$ . E = newly emerged bees; F = foragers; N = nurse bees.

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