





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 Titters

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 Rødbard 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 Cl 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.





17. Robinson GE: Effects of a juvenile hormone analogue on honey bee foraging behavior and alarm pheromone production. *J Insect Physiol* 31, 277 (1985).
18. Robinson GE: Regulation of honey bee polyethism by juvenile hormone. *Behav Ecol Sociobiol* 20, 239 (1987).
19. Sasagawa H, Sasaki M, Okada I: Experimental induction of the division of labour in worker *Apis mellifera* L. by juvenile hormone (JH) and its analog. Int Cong Apimondia, 30th, Japan, Bucharest:Apimondia, pp 140–143 (1986).
20. Huang Z-H, Robinson GE, Tobe SS, Yagi KJ, Strambi C, Strambi A, Stay B: Hormonal regulation of behavioural development in the honey bee is based on changes in the rate of juvenile hormone biosynthesis. *J Insect Physiol* 37, 733 (1991).
21. Rodbard D, Munson PJ, DeLean A: Improved curve-fitting parallelism testing, characterization of sensitivity and specificity, validation and optimization for radioligand assay. In: Radioimmunoassay and Related Procedures in Medicine. International Atomic Energy Agency, Vienna, Vol 1, pp 469-517 (1978).
22. de Kort CAD, Koopmanschap AB, Strambi C, Strambi A: The application and evaluation of a radioimmunoassay for measuring juvenile hormone titers in Colorado beetle hemolymph. *Insect Biochem* 15, 771 (1985).
23. Uldall A, Jensen BS, Henriksen J: Kits for the diagnosis of infectious mononucleosis compared with the Paul-Bunnell test. *J Clin Chem Clin Biochem* 28, 423 (1990).
24. Vermes I, Bonte HA, Sluijs-Veer G, Schoemaker J: Interpretations of five monoclonal immunoassays of lutropin and follitropin: Effects of normalization with WHO standard. *Clin Chem* 37, 415 (1991).
25. Lindqvist U, Chichibu K, Delpech B, Goldberg RL, Knudson W, Poole AR, Laurent TC: Seven different assays of hyaluronan compared for clinical utility. *Clin Chem* 38, 127 (1992).