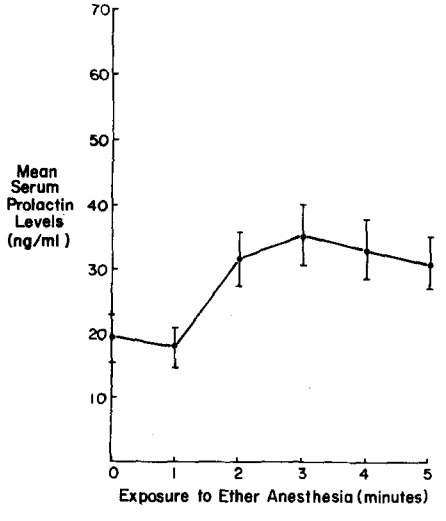
time 0 for 30 sec and thereafter exposed to an ether saturated nose-cone for the remainder of the blood collection period. Prior to placing the rat into the etherized container, caution was taken not to disturb the cage or rat in any manner. Blood was collected in 0.5 ml samples at 1, 2, 3, 4 and 5 min of exposure to ether. A separate 1 ml glass syringe fitted with a 21-gauge needle was used to withdraw blood from the cannula for each sample. After each blood sample, the cannula was filled with heparinized saline (0.06 ml, cannula volume). The total amount of blood withdrawn from the cannula of each rat was 2.5 ml. 24 h after taking the last blood sample, the animal was decapitated to obtain a basal (time 0) blood sample. Blood was allowed to sit overnight at 4 °C and the serum collected the following morning. Serum was frozen at −20 °C until assayed for immunoreactive prolactin by the method of NISWENDER et. al[9]. The significance of differences between mean serum prolactin levels was calculated by Student's *t*-test.

*Results and discussion.* Mean serum prolactin level was 19.0 ± 3.7 ng/ml at time 0. After exposure to ether for 1 min, serum prolactin values were 17.8 ± 3.3 ng/ml, a value not significantly different from time 0. Following 2 min of ether, the mean prolactin level showed an approximate 2-fold increase, to 35.6 ± 4.8 ng/ml ($p < 0.001$) and remained at a high level throughout the 5 min sampling period (Figure). Every rat, without exception, showed an increase in serum prolactin at 2 min of etherization, when compared with prolactin values at times 0 or 1 min.




The effect of continuous ether stress on immunoreactive serum prolactin levels. Animals were placed in an etherized container for 30 sec and exposed to an ether saturated nose cone for the remainder of the 5 min etherization. Each point represents the mean of 16–18 values. The '0' time represents 6 decapitation values. The vertical bars represent ± 1 standard error.

Other laboratories have demonstrated[2–6,10] an ether anesthesia-induced rise in rat serum prolactin, but the critical time in which this rise takes place was not the objective of these studies, consequently it has not been adequately evaluated. The results of this study, in which an intra-atrial cannula was implanted into male rats for blood collection, show that this rise occurs within 1 to 2 min of ether exposure. These are in accord with the study of TERKEL et al.[3] who reported a rise in serum prolactin in lactating rats after 1–2 min of ether exposure and WUTTKE and MEITES[11] who found no change in serum prolactin in normal female rats after $^1/_2$ min of etherization. Thus, if the blood sample can be drawn in the first min of etherization, a valid level of prolactin can be obtained. Such a practice should eliminate, at least in part, the well acknowledged intra-group deviation of serum prolactin values reported by us[12–15] and numerous other laboratories.

*Zusammenfassung.* Bei männlichen Ratten wurde das «Timing» der Prolactin-Stressreaktion gemessen und festgestellt, dass die Prolactin-Konzentration invariabel nach 2 min Äther-Stress ansteigt.

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## Thyroxine Formation by Fish Kidney Soluble Supernatant of *Anabas testudineus*

It has recently been reported from this laboratory[1] that head kidney soluble fraction (105,000 × *g* supernatant) from a teleostean fish demonstrated significant peroxidase activity, and this peroxidase could oxidise the iodide into tri-iodide, a property which is met in connection with the thyroid peroxidase[2,3]. That head kidney of teleost may possess thyroidal activity is again supported by the observation of large concentration of thyroid follicles in this area[4–8]. More clear cut evidence in this

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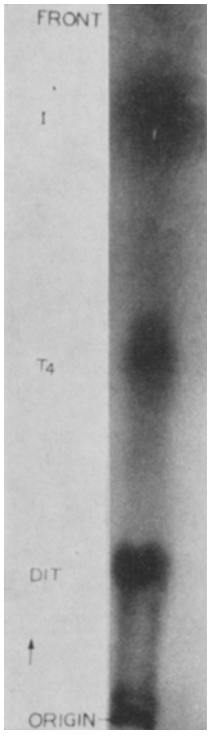
regard was provided by CHAVIN and BOUWMAN[9]. They injected $^{125}I$ i.p. and observed that head kidney of gold fish (*Carassius auratus*) could accumulate large amounts of radioiodine which was eventually incorporated organically in the thyroid hormone. Very recently, DASGUPTA and BHATTACHARYA[10] reported that soluble fraction ($105,000 \times g$ supernatant) of head kidney from a teleost could catalyze the synthesis of monoiodotyrosine (MIT) and diiodotyrosine (DIT). All these prompted us to investigate the synthesis of thyroxine by fish head kidney soluble fraction which had not been reported by any earlier worker.

*Materials and methods.* *Anabas testudineus* was purchased from the local market and kept in the aquarium for at least 1 week. On each occasion, 4 properly acclimatized fishes were chosen for study and sacrificed. Head kidney from each specimen was carefully dissected out, weighed and homogenized in a Potter-Elvehjem homogenizer in 0.05 *M* sodium phosphate buffer, pH 6.5 to make a 0.5% homogenate. The homogenate was centrifuged in a Spinco Model L preparative ultracentrifuge at $105,000 \times g$ for 1 h and the supernatant was used as enzyme. All these steps were carried out at 0–4 °C. Formation of thyroxine ($T_4$) was assayed in an incubation medium contained 300 μmoles of sodium phosphate buffer pH 6.5; 150 μmoles of KI; 20μCi of Na-$^{131}I$; 120 μmoles of MIT; 3 mg of glucose; 12.5 g of glucose oxidase; suitable concentrations of head kidney $105,000 \times g$ supernatant fraction and glass-distilled water to make the final volume 3.0 ml. For each set of experiments, a control was always made where all other additions were same except the addition of boiled head kidney soluble fraction (boiled at 100 °C for 10 min in a water bath). Incubation was carried out at 37 °C and time period was varied. The reaction was stopped by the addition of 0.6 ml of 50% TCA and incubation mixture was then centrifuged at $700 \times g$ for 5 min. 240 μl aliquot of the supernatant was spotted on Whatman No. 3 chromatography paper. To prove the identity of the labelled $T_4$ formed during the incubation, marker $T_4$ (supplied by Sigma Chemical Co.) were spotted on one end of the chromatography paper. Chromatogram was developed in an ascending system for 22 h in N butanol: ethanol: $2NH_3$ (25:50:10) at room temperature in a chromatocab. After drying the chromatography paper, the portion contained standard $T_4$ spot was cut off and sprayed with 0.2% ninhydrin-acetone (w/v) solution and redried. The rest of the paper was then placed on Kodak X-ray film ($30 \times 25$ cm) in a closed dark chamber to allow exposure for 24 h. The X-ray film was developed with standard photographic solution and dried. X-ray paper indicating spots was placed over the chromatography paper for detection. The portion of the paper which corresponded exactly with the standard $T_4$ was then cut off and counted in a Nuclear Chicago solid Scintillation counter[11]. The regions contained DIT and unreacted $^{131}I$ (except $T_3$, which, however was not detected) were similarly detected and collected separately with the spot at the origin for calculating the distribution of $^{131}I$ in chromatography paper. The result was expressed as percent $^{131}I$ incorporated into $T_4$. The protein was measured according to the method of LOWRY et al.[12].




Radioautogram of paper chromatogram showing $^{131}I$ distribution in head kidney soluble supernatant catalyzed iodination of MIT. Standard thyroxine corresponded exactly with the region marked as $T_4$. Additions and assay were the same as already described and this chromatogram was the result of 1.5 mg of head kidney soluble fraction incubated for 90 min.

*Results and discussion.* It was reported earlier that peroxidase like myeloperoxidase[13] and chloroperoxidase[14] which could oxidise iodide when incubated with tyrosine or iodotyrosine results in the synthesis of thyroxine. Work from this laboratory showed clearly that $105,000 \times g$ supernatant of the head kidney of teleost could efficiently catalyse the peroxidation of iodide into triiodide[1]. Thus an attempt was made to observe the synthesis of thyroxine by *A. testudineus* head kidney soluble supernatant fraction. When this fraction was incubated for 90 min, a detectable amount of $^{131}I$ labelled thyroxine formed during the iodination of MIT. The chromatography was performed in the solvents already mentioned and the radioautogram obtained in such an experiment is indicated in the Figure. The added thyroxine marker always corresponded exactly with the band on the radioautogram. This band was easily detectable in 90 min sample but could not be observed in 70 min incubated sample. Darkness of the DIT spot indicates that a substantial amount of DIT was formed during the period of incubation, and this is expected as incubation medium contained MIT as substrate. Formation of DIT might have occured at the onset of incubation which then coupled together and resulted in the formation of thyroxine. There was no visible band corresponding to added $T_3$ marker.

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The Table shows that formation of labelled thyroxine gradually increased proportionately from 90 to 120 min and further increase in the labelled thyroxine synthesis was not observed in 130 min incubated sample. When the amount of head kidney soluble protein was increased to double and incubated for the same periods, the quantity of thyroxine formed in almost double quantity (Table), which indicates that formation of thyroxine is an enzymatically catalyzed process. This is again evident from the fact that when head kidney soluble supernatant was boiled at 100 °C (in a water bath, for 10 min), cooled and then incubated, no visible band was observed at the region of thyroxine and DIT. Besides, the quantity of head kidney soluble protein is a very important factor. When 1 mg of soluble supernatant was incubated for 90 min, no visible band corresponding to thyroxine marker was observed. Thus, the minimum requirement of head kidney soluble fraction to observe thyroxine formation at 90 min incubated sample was 1.5 mg/3.0 ml of incubation mixture.

Formation of thyroxine by head kidney soluble supernatant

| Head kidney soluble supernatant (mg) | Incubation time (min) | $^{131}I$ incorporated into thyroxine (%) |
|---|---|---|
| 1.5 | 90 | 0.6 |
| | 100 | 0.8 |
| | 110 | 1.1 |
| | 120 | 1.4 |
| | 130 | 1.4 |
| 3.0 | 90 | 1.2 |
| | 100 | 1.5 |
| | 110 | 2.1 |
| | 120 | 2.7 |
| | 130 | 2.7 |

As thyroid follicular structure are present in the head kidney of teleost fishes[4–8], and as the head kidney soluble supernatant of this teleost fish possesses a peroxidase which significantly oxidizes the iodide into triiodide[1], the present observation of the formation of thyroxine by the same fish and by the same fraction, give further support to the possible role of teleost fish head kidney in the biosynthesis of thyroid hormone.

*Zusammenfassung.* In der löslichen überstehenden Fraktion (105,000 × *g*) der Kopfniere des Knochenfisches *Anabas testudineus* wurde ein Enzym nachgewiesen, welches die Synthese des Thyroxins katalysiert. Die Menge des löslichen Nierenproteins und die Inkubationszeit stellen zwei limitierende Faktoren für die in vitro-Synthese des Thyroxins dar.

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## Juvenile Hormone Promotes Dominance Behavior and Ovarian Development in Social Wasps (*Polistes annularis*)

The females of a *Polistes* (Vespidae) colony have a dominance hierarchy organized with the queen (as the preeminent aggressor and egg-layer) in the α-position and the workers arranged linearly beneath her[1,2]. Even though all females are very similar externally[3], there is striking differential ovarian development which is correlated with position in the dominance hierarchy[2]. However, wasps of a colony retain their dominance status after being ovariectomized[4].

Many studies have shown the effects of gonadal hormones on vertebrate aggression and dominance hierarchies[5]. Juvenile hormone (JH), the endocrine product of the corpora allata, is the gonadotropin of most female insects[6] and topical application of JH can promote ovarian growth in *Polistes*[7]. JH is also known to affect mating behavior and pheromone production in many insects[8] and to alter caste structure and the time course of specific labors in honeybees[9]. However, this is the first report of an insect hormone affecting aggression or dominance interactions.

Colonies of *Polistes annularis* were collected in late May from one locality on Lake Travis near Austin, Texas. Each of the 16 nests was glued in the corner of a 15 cm × 15 cm plexiglass and screen observation cage. Nests were chilled at 4 °C prior to manipulation of the wasps. Wasps were provided with live caterpillars and water ad libitum during the experiment. On the day after capture the queens were determined by observers (R.H., T.K. and L.J.L.) according to behavioral criteria[1,2]. The next day 4 workers/cage were given a color-coded marking according to the treatment they were to receive and surplus workers were removed. Wasps ecdysing during the experiment were designated 'newly-emerged' and left in the cage. At noon daily for 4 days the cages were chilled and 10 µl of acetone containing 20 µg test substance (or oil) were applied to the abdomens of 3 workers/cage. The identities of the test substances were not revealed to the observers, but included JH III, 3 JH analogs, and 3 identical solutions of olive oil. Each cage was treated with 2 different hormones and oil. The JH and analogs were a gift of Zoecon Corp., Palo Alto, Cal., and were 90% pure, 80% of which was the active *trans, trans, cis*-isomer. On the 6th day wasps were sacrificed and the length of the 3 largest oocytes determined with an ocular micrometer. Additional colonies were established later as controls under the same conditions except that there were either no treatments or only acetone and oil were administered (to 3 wasps/cage). Behavioral observations of the interactions between colony members were made each day 2–3 h after treatment and on the day of sacrifice. From these observations the following system of ranking

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