Changes in ecdysone levels in the spider *Pisaura mirabilis* nymphs (Araneae, Pisauridae)

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Summary. RIA of ecdysones are made on nymphal extracts of *Pisaura mirabilis* during the intermoulting cycle. The hormone level is low during exuviation and the greatest part of the intermoulting period but increases notably before and after ecdysis. The main hormonal peak or pre-ecdysial peak is due to β-ecdysone, characterized by combined TLC/RIA.

Changes in ecdysone level in the hemolymph or in homogenates of whole animals during post-embryonic development are now well-known for a great number of insects[2-9] and several crustaceas[10,11]. As for the class of Arachnids, the situation is quite different since physiological studies have been scarce up to now; the existence of ecdysones has yet to be revealed since no hormonal analyses has been made on these animals.

Ecdysterone injected into *Pisaura* nymphs has already yielded some information: it induces the early appearance of preparatory moulting phenomena, and ecdysis itself[12-14]. This activating effect of injected ecdysterone is variable, depending upon the period of injection[15]. This suggests that the effects of exogenic ecdysterone are related to the fluctuations of endogenic hormone level during the intermoulting period. In order to investigate this problem, ecdysone analyses were made during one whole nymphal stage by radioimmunoassays. Several analyses were also made on adult individuals of both sexes.

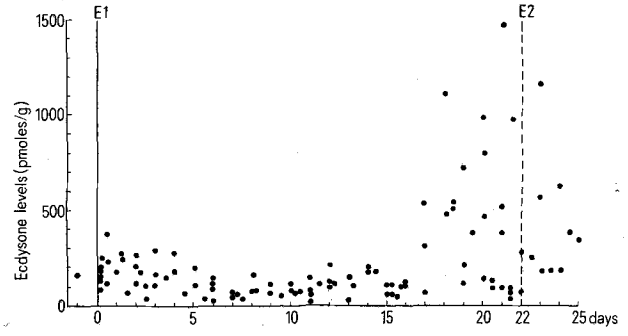
Material and methods. Pisaura nymphs, captured in early January, are bred in the same conditions as described elsewhere[13]. The rank of the nymphal stages of the experimental and control animals was determined by the technique based on 'trichobothriotaxic schedules'[16]. The spiders reach the nymphal stage 8 and weigh 30–70 mg before the analyses are made. The mean duration of the nymphal intermoulting period was evaluated from the control animals and did not significantly differ between the 2 sexes at the nymphal stage 8, so the results obtained for males and females were grouped (duration of the nymphal stage 8: 22 days ± 4, n = 16).

Radioimmunoassays (RIA): The spiders to be used for the quantitative analyses are sonicated in pure methanol; after centrifugation, in order to discard the proteins, the supernatant is evaporated until dry under reduced pressure. The dry pellet is suspended in a volume of citrate buffer allowing a satisfactorily adjusted dilution. Ecdysones are analyzed by RIA according to the method of De Reggi, Hirn and Delaage[17]. The results are expressed as pmoles of ecdysterone-equivalent per g of fresh weight. Generally speaking, the term ecdysone is used to designate the ecdysteroid compounds which react positively in the RIA of ecdysones.

Combined TLC/RIA: In some cases, the RIA active material is characterized by combining thin-layer chromatography with the RIA. 20 µl of the previous methanolic extracts are chromatographed on a silica gel plate in the solvent system chloroform-methanol (80:20, v/v) during 2 periods of 1 h. The gel is divided according to the position of the standards, then scraped off and suspended in methanol. After sonication, the gel is discarded and the solvent is evaporated. Lastly, the RIA activity of each fraction is measured.

Results. The RIA analyses of nymph 8 extracts of *Pisaura mirabilis* show that ecdysone are constantly present during the intermoulting cycle.

The fluctuations in hormone level are visualized in the figure; they follow several phases: a) During exuviation E1, the level of ecdysones is relatively low (mean: 150 pmoles/g); in this case, the analyses were made on individuals sampled exactly during ecdysis. b) From exuviation to about the 5th days of intermoulting period, the hormone level seems to increase up to 300–400 pmoles/g. c) From the 5th to the 17th day, no very high



Evolution of the level ecdysones during the intermoulting cycle (nymphal stage 8) in the spider *Pisaura mirabilis*. Ordinate: ecdysone level in pmoles/g fresh wt. Abscissa: length of nymphal-8-stage in days. E1: exuviation 1 (precise limit nymphal stages 7 and 8). E2: exuviation 2 (theoretical limit between nymphal stages 8 and 9).

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values are observed. d) From the 17th to the 25th day, a main peak appears which culminates at 1000–1500 pmoles/g.

The dispersion of values between the 16th and the 25th day of the intermoulting period is due to the important individual variability in duration of the intermoulting cycle, which produces a shift of the main peak. The low values recorded at the beginning of the stage show that the level of ecdysone drops before ecdysis. Our analyses show that the existence of a peak of moderated intensity, occurring before the main peak from the 12th to the 14th day of the intermoulting period, is not to be disregarded. The combination of TLC with RIA for each of the fractions separated by chromatography reveals that the RIA active material is exclusively localized in the zone corresponding to β-ecdysone. No α-ecdysone can be detected using the same dilution.

As far as adults are concerned, the RIA yields the following values: a) Adult males (n = 6) ecdysone level: mean of 50 pmoles/g; b) adult female (n = 2) ecdysone level: 500 pmoles/g.

Discussion. RIA of total extracts of nymphs of *Pisaura mirabilis* shows fluctuations in ecdysone level during the intermoulting period. Evident similarities become apparent when our results are compared with those obtained for other arthropods: The hormone level is relatively low during exuviation as well as during resting period of the tegument, as determined by Browning[18] (from the 5th to the 16th day of the intermoulting cycle in this case); this level increases slightly during the postmoulting period and strongly during the premoulting period before dropping drastically before ecdysis. As is now well established for insects, the possible existence of several preecdysial peaks is not to be disregarded.

The ecdysone peaks during the pre- and postmoulting periods seem to be related to pre- and post-exuvial cuticular synthesis. From a quantitative point of view, the hormone level in the Arachnids studied is around 100 pmoles/g (i.e. about 50 ng/g) at the lowest and around 1000–1500 pmoles/g (about 500–750 ng/g) in the main peak. The hormonal form, characterized by combined TLC and RIA, is β-ecdysone (ecdysterone). These ecdysone levels are comparable to those obtained by identical or different techniques from total extracts of insects[2,3,5,7,19] or crustaceas[10,20].

Important quantities of ecdysones are revealed in adult females spiders, which parallels other published results of adult females insects[19,21] and is probably in relation to the maturation of ovocytes[22].

Nymphal intermoulting stages of *Pisaura mirabilis* are difficult to determine precisely at homogenization for no morphological criteria comparable to those used for insects and crustaceas are available. Histological studies of the tegument during the intermoulting cycle are presently under way and should give some information as to the localization of apolysis in particular, which would greatly contribute to the interpretation of our results.

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Antigonadotrophic effect of melatonin in male lizards (*Callisaurus draconoides*)[1]

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Summary. Results of a preliminary experiment indicate that injections of melatonin elicit testicular regression in male lizards having maximally recrudesced gonads at the outset of study.

Melatonin, an indoleamine synthesized and secreted by the pineal gland[2], is reported to have both pro- and antigonadotrophic effects when administered to adult mammals, depending upon the treatment level used and the mode of administration[3–7]. Conversely, a limited number of studies of ectothermic vertebrates indicate that exogenous melatonin elicits only antigonadotrophic effects in these animals[8–10]. We here report results of a preliminary experiment further documenting the antigonadotrophic action of melatonin in lower vertebrates.

Adult male lizards (*Callisaurus draconoides*), collected in May 1975 on the Mojave Desert, were maintained in an outdoor enclosure[11] at the Desert Research Institute's laboratory in Boulder City, Nevada. The animals therefore were exposed to natural photothermal conditions for the duration of the study. All lizards were approaching peak reproductive condition at the time of capture[12].

Experimental animals were injected with 5 µg of melatonin/day for 20 days, and controls received injections of saline. The average b. wt of the lizards was 17.9 g, and so each was given approximately 0.3 µg of melatonin/g b. wt

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