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.

18 H. C. Browning, Proc. R. Soc., Lond. *131*, 65 (1942).
19 C. H. Bordereau, M. Hirn, J. P. Delbecque and M. De Reggi, C. r. Acad. Sci. Paris *282*, 285 (1976).
20 A. Faux, D. H. S. Horn, E. J. Middleton, H. M. Fales and M. E. Lowe, Chem. Commun. *4*, 175 (1969).
21 M. Lagueux, M. Hirn, M. De Reggi and J. A. Hoffmann, C. r. Acad. Sci., Paris *282*, 1187 (1976).
22 J. M. Legay, B. Calvez, M. Hirn and M. De Reggi, Nature *262*, 489 (1976).

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

Mary J. Packard and G. C. Packard

Department of Zoology and Entomology, Colorado State University, Fort Collins (Colorado 80523, USA), 31 May 1977

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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2 C. L. Ralph, Am. Zool. *16*, 35 (1976).
3 K. Hoffmann, Naturwissenschaften *61*, 364 (1974).
4 G. R. Lynch and A. L. Epstein, Comp. Biochem. Physiol. *53C*, 67 (1976).
5 R. J. Reiter, D. E. Blask and M. K. Vaughan, Neuroendocrinology *19*, 72 (1975).
6 R. J. Reiter, M. K. Vaughan, P. K. Rudeen and R. C. Philo, Am. J. Anat. *147*, 235 (1976).
7 F. W. Turek, C. Desjardins and M. Menaker, Science *190*, 280 (1975).
8 V. L. DeVlaming, M. Sage and C. B. Charlton, Gen. comp. Endocr. *22*, 433 (1974).
9 J. C. Fenwick, Gen. comp. Endocr. *14*, 86 (1970).
10 I. L. Levey, J. exp. Zool. *185*, 169 (1973).
11 G. C. Packard and M. J. Packard, Copeia *1972*, 695 (1972).
12 W. W. Tanner and J. E. Krogh, Herpetologica *31*, 302 (1975).

Number of male *Callisaurus draconoides* possessing recrudesced or regressing testes after receiving daily injections of melatonin or saline for 20 days

Treatment	Testicular state	
	Recrudesced	Regressing
Saline	7	4
Melatonin	2	8

at each injection. Food (*Tenebrio* larvae) was provided at regular intervals, and only data for healthy animals are reported here. At the end of 20 days, the lizards were killed by etherization, and their testes were removed, weighed individually, and fixed in formalin. Since there was no difference between experimental and control animals in combined testicular weights, reproductive condition of the lizards was assessed by histologic examination of the gonads. The left testis from each animal was embedded in paraffin and sectioned at 7 μm. Median cross sections were mounted on glass slides and stained with Mayer's hematoxylin and eosin-Y. Prior to examination of the slides, the label on each was masked so that all examinations were made without knowledge of the group to which a particular donor animal belonged.

Initial examination of the sectioned material revealed that some lizards possessed maximally recrudesced testes at the time of sacrifice, whereas others were undergoing gonadal regression. In maximally recrudesced testes, the seminiferous lumen was relatively packed with sperm, the seminiferous epithelium was well-developed, and all cell types (insofar as they can be distinguished) were abundant. Additionally, some spermatigonial mitoses were evident, and the interstitium was well-endowed with Leydig cells. Conversely, testes in regression contained fewer sperm, the seminiferous epithelium was reduced (with a concomitant reduction in the number of cells and of cell-types present), and many fewer Leydig cells were evident.

The testes were assigned to different categories on the basis of these apparent differences in histologic condition (table), and the proportional representation of experimental and control animals in these categories was assessed using Fisher's exact test[13]. This statistical procedure indicates that significantly ($p = 0.05$) more lizards injected with melatonin exhibited testicular regression than in the control group.

The samples used in this preliminary experiment were rather small, and so the results must be viewed with caution. Nevertheless, the low probability of obtaining the observed frequency distribution (table) by chance alone indicates that melatonin may exert an antigonadotrophic effect in male *Callisaurus draconoides* similar to that reported for other ectothermic vertebrates[8–10].

13 R. R. Sokal and F. J. Rohlf, Biometry. W. H. Freeman & Co., San Francisco 1969.

PRO EXPERIMENTIS

Analysis of abscisic acid and indole-3-acetic acid from fruits of *Vitis vinifera* L. by high pressure liquid chromatography

H. Düring

Federal Research Institute of Grape Breeding, D–6741 Siebeldingen (Federal Republic of Germany), 9 March 1977

Summary. Simultaneous quantitative analysis of the plant hormones abscisic acid and indole-3-acetic acid from fruits of *Vitis vinifera* ssp. was performed by thin layer chromatography and high pressure liquid chromatography. Hormonal activity was proved in a biological test.

Biogenous plant hormones are suggested to play an important role in developmental and ripening processes of fleshy fruits[1,2]. In grape berries, auxin-, gibberellin- and cytokinin-like substances have been detected mainly by their activities in biological tests[3–6], while abscisic acid (ABA) and ethylene were determined by gas-liquid-chromatography[7]. In recent years, high pressure liquid chromatography (HPLC) has been introduced in plant hormone analysis, especially for cytokinins[8–10] and abscisic acid[11]. In this study, a method is described by which 2 hormones, ABA and indole-3-acetic acid (IAA), were simultaneously extracted, identified and quantitatively determined by HPLC. In addition the growth regulating activity of the extracted IAA was confirmed by a biological test.

Material and methods. Berries of *Vitis vinifera* L., cv. Bacchus, were harvested from fieldgrown grapevines of the institute. Berries were cleaned by dest. H_2O and kept frozen at $-20\,°C$ until extraction. Frozen berries (20 g fresh weight) were homogenized in 60 ml dest. H_2O and extracted in hot water (98–100 °C) for 20 min. After filtration under vacuum and a reextraction of the residues, the acidified filtrate (pH 3.0 by 2 N H_2SO_4) was continuously extracted by ether for 20 h in a liquid-liquid extractor. The ether fraction was then extracted by a saturated $NaHCO_3$-solution (5 times) and dest. H_2O alternately (5 times). The acidified water fraction (pH 3.0 by 2 N H_2SO_4) was again continuously extracted by ether for 20 h. The ether fraction was evaporated and subjected to thin layer chromatography (TLC) (Kieselgel HF 254, 200 μm, Merck) using n-propanol : NH_3(25%) : H_2O 80:10:10 v/v according to Wakhloo[12]. The extracts, as well as synthetic IAA and ABA, were spotted on TLC-plates as 2–3 cm bands and developed in chambers under saturated atmospheric conditions. The zones seen under UV 254 were marked and partly identified by their R_f-values and by spraying Ehrlich reagent (1% p-dimethylaminobenzaldehyde in conc. HCl + 96% ethanol mixed 1:1). Spots from unsprayed plates were scraped off, eluted by ether and after evaporation diluted in 0.1 ml methanol for HPLC-analysis. Details are given in figure 1. Peaks were identified by comparing retention times of synthetic and natural compounds. In addition peak identity was confirmed by collection of HPLC-separated IAA using a fraction collect valve and subjection to the Avena straight growth test according to Larsen[13].