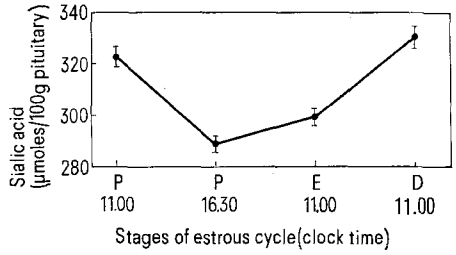
each day between 10.00 and 11.00 h and again when necessary at 16.30 h. Only those rats which exhibited at least 2 consecutive 4-day cycles were used. 10 rats in each group were sacrificed by decapitation on the days of proestrus, estrus, and diestrus (but not metestrus) at 11.00 h or 16.30 h. The pituitary glands were dissected out quickly, posterior lobe was discarded and the anterior lobe weighed individually to the nearest 0.2 mg on a torsion balance, homogenized in cold 0.1 N H_2SO_4, hydrolyzed at 80 °C for 1 h and the sialic acid was estimated by the thiobarbituric acid method of WARREN[13]. The p-values were calculated using student's t-test.

The concentration of sialic acid in the pituitary gland during different phases of estrous cycle is shown in the Figure. The highest concentration of sialic acid in the pituitary was seen on the morning of the day of proestrus. By 16.30 h on the same day, the concentration decreased significantly ($p < 0.01$) to the lowest level observed during the cycle. The sialic acid level remained low at estrus to the value close to that observed during the afternoon of proestrus. Thereafter, a marked increase in the sialic acid concentration was evident by 11.00 h on the day of diestrus.



Changes in the pituitary sialic acid concentration during the estrous cycle of rats. P, proestrus; E, estrus; D, diestrus. Each point represents 10 pituitaries.

Variations in the concentration of pituitary sialic acid observed are similar to the fluctuations in the pituitary FSH concentration in female rats during different phases of estrous cycle. CALIGARIS et al.[1] and NEGRO-VILAR et al.[4] observed a sharp decline in pituitary FSH concentration between 09.00 h and 17.00 h on the afternoon of proestrus. Also a drop in the pituitary FSH and LH levels is seen between proestrus and estrus, reflecting the discharge of an ovulation-inducing surge of gonadotrophins on the afternoon of proestrus[3,14,15]. Thereafter, FSH levels increased until the next proestrus. Since sialic acid is absent in rat LH[12], the changes in the pituitary sialic acid concentration of the two sexes are more closely correlated with FSH levels than LH. Thus the close correlation in the changing levels of pituitary FSH and sialic acid during different phases of estrous cycle suggests that sialic acid levels in the pituitary gland may be taken as an indicator of FSH content.

Zusammenfassung. Bei der Ratte wurde der Sialinsäuregehalt des Hypophysenvorderlappens während des Prooestrus, Oestrus und Dioestrus bestimmt. Da das Luteinhormon der Ratten keine Sialinsäure enthält, können die Schwankungen des Sialinsäuregehaltes als Mass für die FSH-Konzentrationen genommen werden.

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[13] L. WARREN, J. biol. Chem. *234*, 1971 (1959).
[14] N. B. SCHWARTZ and D. BARTOSIK, Endocrinology *71*, 756 (1952).
[15] N. B. SCHWARTZ, Am. J. Physiol. *207*, 1251 (1964).
[16] The authors are grateful to Prof. M.R.N. PRASAD and Prof. C.M.S. DASS for providing all facilities and encouragements.

Termination of Diapause by Juvenoids in Two Species of Ladybirds (Coccinellidae)

The juvenoids or juvenile hormone analogues are known to initiate egg development in diapausing adult insects[1,2]. For example, methyl 10,11-epoxyfarnesoate induces reproduction in beetles *Hypera postica*[3] and *Oulema melanopus*[4], and methyl farnesoate was reported to stimulate previtellogenesis in *Pterostichus nigrita*[5]. The present study compares activities of 18 juvenoids[6] and describes their effects on diapausing adults of beetles *Semiadalia undecimnotata* (Schneider) and *Coccinella septempunctata* Linnaeus (Coccinellidae).

The life-cycle of aphidophagous coccinellids includes a long period of diapause that occurs usually in the adult stage. In the thermo- and xerophilous *S. undecimnotata*, the main distribution area of which lies in Southern Europe and Asia the diapause lasts from late July or early August to late April or May. The beetles migrate to permanent hibernation quarters where they form large aggregations. Although in the open the beetles remain inactive in spite of the high temperatures of late summer and early autumn, they can easily be activated under long-day conditions (18 h photophase) and high temperature (e.g. 20–23 °C) when provided with appropriate food, i.e. certain aphids[7]. To prove that activation was initiated by the application of juvenoids, the diapause promoting photoperiod of 12 h light and 12 h dark was used in the present experiments.

In *C. septempunctata*, which is the most common coccinellid in Central Europe, most of the beetles diapause in small groups for 7–8 months. The diapause is rather stable. Exposure of beetles to a long photophase before October activates only 15–20% of diapausing specimens. In the course of diapause development in subsequent months the endogenous inhibition of reproduction gradually ceases and the beetles start to respond more

[1] W. S. BOWERS, *Naturally Occurring Insecticides* (Ed. M. JACOBSON; Marcel Dekker, New York 1971), p. 307.
[2] K. SLAMA, A. Rev. Biochem. *40*, 1079 (1971).
[3] W. S. BOWERS and C. C. BLICKENSTAFF, Science *154*, 1673 (1966).
[4] R. V. CONNIN, O. K. JANTZ and W. S. BOWERS, J. econ. Ent. *60*, 1752 (1967).
[5] H. EMMERICH and H. U. THIELE, Naturwissenschaften *56*, 641 (1969).
[6] The juvenoids I, II, VI, VII, VIII, XII, XIII, XIV, and XVIII were kindly provided by Drs. M. ROMAŇUK, V. JAROLÍM, P. BERAN, Z. ARNOLD and Prof. F. ŠORM of the Institute of Organic chemistry and Biochemistry, ČSAV, Prague; the remaining juvenoids were obtained through the courtesy of Dr. J. B. SIDDALL of the Zoecon Corporation, Palo Alto, California. All juvenoids were mixtures of isomers; the aliphatic compounds contained about 80% of the 2-*trans* isomers.
[7] I. HODEK, Acta ent. bohem. *67*, 218 (1970).

readily to long photophase[8]. Our experiments were conducted in September. At that time, the pre-diapause preparatory phase had been completed and the diapause had just begun.

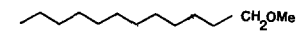
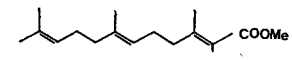
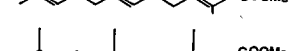
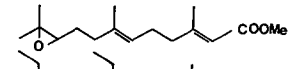
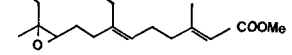
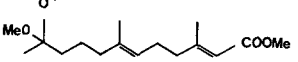
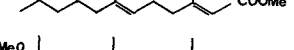
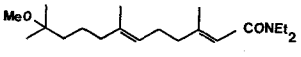
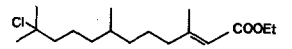
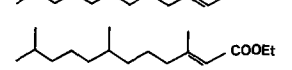
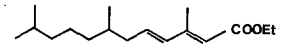
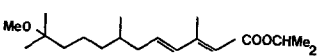
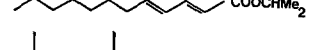
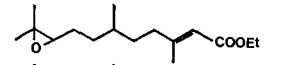
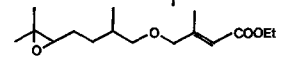
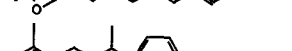
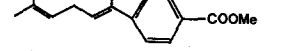
The beetles used in this study were collected in their hibernation quarters in the vicinity of Louny in Central Bohemia (*S. undecimnotata*) and in Southern Moravia (*C. septempunctata*) at the beginning of September. They were divided in groups of 5 males and 5 females each and kept thereafter in 250 ml plastic containers at 20 °C and 12 h photophase. Each insect of experimental groups received topically 1 µl of acetone solution containing the juvenoid. Both the control and experimental groups were supplied with drinking water and fed on the pea aphid, *Acyrthosiphon pisum*, and the black bean aphid, *Aphis fabae*. Freshly cut horse bean seedlings with aphids were provided in 3-day intervals. The experiments were terminated 1 month after treatment when the affected insects had deposited some eggs. The females were then dissected and the developmental stage of their ovaries classified as follows: +++, ripe eggs were present in all examined beetles and some oviposition occurred; ++, two oocytes were present in each ovariole (groups of insects classified with this score sometimes included individuals with only one oocyte in each ovariole and also individuals with mature eggs); +, most females had one oocyte in each ovariole; —, no oocytes present.

The results of testing juvenoids on *S. undecimnotata* are summarized in the Table. The Table shows that 100 µg of several aliphatic compounds induced egg deposition whereas the most active aromatic compound XV produced only the effect ++. 3 active aliphatic substances were studied in more detail. Compound IX was found to be the most active one since it induced reproduction at a dose of 10 µg per insect and stimulated considerable ovarian development at the dose of 1 µg/insect. Corresponding doses of VII failed to elicit egg deposition, although the compound stimulated ovarian development. 10 µg of XII were almost inactive.

The development of a sample of 256 eggs, deposited by the females treated with 100 µg of VII or with 10–100 µg of IX was followed until the adult stage. No substantial difference between the effects of the 2 compounds was detected. The hatchability of eggs varied between 60% and 100%, usually being approximately 95%. Mortality of larvae and pupae was 30–85%; and the average was about 40%. Death occurred at various times. The adults which emerged appeared normal both externally and internally and the ratio between sexes was 1:1.

Compound VII was also tested on *C. septempunctata*. Application of 100 µg/insect induced ovarian development and egg deposition. The hatchability of the 78 eggs collected was about 55% (the loss includes those eggs destroyed by hatched larvae) but the mortality of larvae and pupae was only 20%.

The capacity of juvenoids to induce egg deposition in coccinellids may have some practical significance. Recently attempts have been made to use coccinellids as predators of aphids in glasshouses. The laboratory production of coccinellids is rather laborious and expensive and thus the use of beetles collected from the hibernation aggregations would be much more economical[9]. Application of juvenoids may enable us to use diapausing beetles as source of voracious larvae at any time during the year.

Compound		Effect	
No.	Structure	Development of ovaries	Egg deposition
I		—	—
II		—	—
III		++	—
IV		++	+
V		—	—
VI		±	—
VII		+++	+
VIII		+++	—
IX		+++	+
X		+++	+
XI		—	—
XII		+++	+
XIII		—	—
XIV		—	—
XV		++	—
XVI		+	—
XVII		±	—
XVIII		—	—

+++, ripe eggs; ++, two oocytes; +, one oocyte; —, no oocyte.

Zusammenfassung. Die Wirkung von 18 Juvenilhormon-Mimetica auf Marienkäfer, *Semiadalia undecimnotata* und *Coccinella septempunctata*, wurde verglichen. Rund die Hälfte der Substanzen bewirkte Eireifung, 5 Substanzen auch Eiablage. Von den abgelegten Eiern entwikkelten sich ca. 50% zu normalen Käfern.

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[8] I. Hodek, Čas. české Spol. ent. *59*, 297 (1962).
[9] B. Gurney and N. W. Hussey, Ann. appl. Biol. *65*, 541 (1970).
[10] The technical assistance by Miss O. Černá is acknowledged.