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EFFECTS OF JUVENILE HORMONE ANALOGUE ON ECDYSIS PREVENTION INDUCED BY PRECOCENE IN *RHODNIUS PROLIXUS* (HEMIPTERA: REDUVIIDAE)

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Precocene II, added to the meal of fourth-instar larvae of Rhodnius prolixus *(25 μg/ml of blood), induced an increase in the duration of the molting cycle. This effect was related to the decrease of both the nuclear area of the prothoracic gland cells and the mitotic activity in epidermal cells.*

Juvenile hormone analogue applied topically (60 μg/insect) together with Precocene II treatment avoided atrophy of the prothoracic glands and induced a higher number of epidermal mitosis accelerating the time of subsequent ecdysis.

A possible relationship between juvenile hormone and production of ecdysone is discussed.

Bowers et al. (1976) showed that two-chromene derivatives (Precocene I and II), extracted from the plant *Ageratum houstonianum*, induce in certain hemipteroid bugs symptons of juvenile hormone (JH) deficiency causing younger larvae to molt to precocious adults, whereas treatment of adults results in lack of oocyte development. Further studies showed that precocenes inhibit the *corpus allatum* (CA) growth (Bowers & Martinez-Pardo, 1977; Pener, Orshan & De Wilde, 1978; Masner et al., 1979; Schooneveld, 1979). *In vitro* studies showed a direct action of precocenes on CA (Pratt & Bowers, 1977; Müller et al., 1979) inhibiting the production of JH. Recent data suggest that the CA metabolizes precocenes to cytotoxic 3, 4-epoxy-precocenes, probably due to the high content of mono-oxygenase activity present therein for JH biosynthesis (Brooks, Pratt & Jennings, 1979; Pratt et al., 1980; Soderlund, Messeguer & Bowers, 1980).

It has recently been shown that in *Rhodnius prolixus* Precocene II (PII) exercised a powerful morphogenetic effect as well as a prevention of the molting (Tarrant & Cupp, 1978; Azambuja, Garcia & Ribeiro, 1981). The latter effect might be counteracted by ecdysone (Azambuja, Garcia & Ribeiro, 1981) and explained by some histological alterations found in prothoracic glands (PG) (Azambuja, Garcia & Furtado, 1981).

We now report that, in *Rhodnius prolixus*, the PG atrophy as well as the prevention of the molting induced by PII could be avoided by JH analogue treatment.

MATERIAL AND METHODS

Insects: fourth-instar larvae of *Rhodnius prolixus* were used throughout this study. Insects were reared and maintained as described before (Garcia & Garcia, 1977; Garcia, Guimarães & Prado, 1978). In all experiments animals of similar size and age were used.

Human blood and other reagents: citrated human blood was used. Precocene II (PII) was kindly supplied by Dr. W.S. Bowers (New York State Agriculture Experimental Station, Cornell University, Geneva, N.Y., USA). Methyl-epoxy-farnesoate (juvenile hormone analogue, JHA) was a generous gift from Dr. B. Gilbert (UFRJ, Brazil). All other reagents were of analytical grade.

Precocene and juvenile hormone treatment: following ecdysis, the insects were starved for 20-30 days and then fed on a special feeding apparatus (Garcia et al., 1975). PII dissolved in ethanol was added to the blood meal at dose of 25 μg/ml. JHA was diluted in heptane 60 μg/μl) and 1 μl was applied on the abdominal tergites one day after feeding. Control experiments showed that ethanol in the concentration used (0.05% in the blood) and heptane (1 μl/insect) did not disturb *Rhodnius* rearing. Intake of blood was determined by the difference in body's weight just before and after feeding. The amount of PII consumed was estimated from the weight of ingested blood. All experiments were carried out at 28°C and 50-60%RH.

Histological processes: (a) Nuclear area determination: the prothoracic glands were dissected in *Rhodnius* saline (5.8g NaCl, 1.8g KCl, and 1.2g $CaCl_2$ /liter) and fixed in acetic Bouin's solution for 16-20 hr, embedded in agar-paraffin. Sections (5 μm) were cut and stained with Heidenhain's azan. For each nucleus sample, the two largest diameters at right angles to each other were determined and the nuclear area was observed at X300 magnification and drawn on graph sheet paper under a camera lucida. The area thus

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estimated was expressed in arbitrary units. (b) Mitotic indices: samples of dorsal epidermis from the fourth to the sixth abdominal segments were fixed in Carnoy's for 2-3hr and stained in haematoxylin. The tissues were then dehydrated in ethanol, cleared in xylol and mounted in Canada balsam. Mitotic bodies were counted in three separate regions of each epidermal sample. Each region comprised aprox. 1,000 cells. Five to seven replicate samples were assayed for each reported time period.

RESULTS

Precocene II, juvenile hormone analogue and ecdysis prevention: the effects of JHA treatment counteracting the ecdysis prevention induced by PII was studied in the following experiment: four groups of 60 insects each were used (Table I): Group 1 – control-insects receiving blood with solvent; group 2 – each insect receiving topically 60 μg of JHA one day after feeding; group 3 – each insect taking in 25 μg of PII/ml of ingested blood, group 4 – receiving 25 μg of PII/ml of blood and 60 μg of JHA per insect. We observed that both the control insects (Group 1) and those receiving blood plus 60 μg JHA/insect (Group 2) molted between 14-18 days after feeding while in the insects receiving 25 μg of PII/ml (Group 3), this period extended until 25 days, and only 60% of the insects underwent ecdysis (Table I); 80% of such animals were adultiforms. In Group 4, fed on blood with PII plus JHA treatment, there was 98% of molting with the intermolting period ranging 14 to 20 days (Table I). Such insects, as expected, did not show any adultoid characteristics.

TABLE I

Effects of juvenile hormone analogue on the intermolting period in 4th-instar larvae of *Rhodnius prolixus* treated with Precocene II

Groups	Treatments		Blood ingested	Intermolting period	Percent moulted	
	PII μg/ml	JHA μg/insect	mg	(range)	Ist 5 days	Total
1	–	–	102.1 ± 6.5^{x}	14-18 days	100	100
2	–	60	97.1 ± 3.9	14-18 days	98	100
3	25	–	91.1 ± 4.9	15-25 days	35^{xxx}	60^{xx}
4	25	60	95.6 ± 5.6	14-20 days	85^{xxx}	98^{xx}

Used 60 insects per group; ^{x}Mean ± SE; ^{xx} & ^{xxx} significant and highly significant differences, applied X^2 test.

Precocene II, juvenile hormone analogue and prevention of prothoracic gland cells atrophy: the above results could be explained by histological alterations in the PG (Azambuja, Garcia & Furtado, 1981). If this was the case, we would be able to induce the effect of PII and PG and reverse this effect by a combined treatment with JHA. This suspicion was confirmed by the study of the histology of the PG's development of the treated and control insects. We then decided to measure the nuclear area which has been used by many workers as an index of the activity of PG cells (Wigglesworth, 1952; Joly et al., 1973; Furtado, 1977). Groups of 20-30 insects each were either fed on blood or blood plus PII (25 μg/ml) and then treated with JHA (60 μg/insect) or not. An increase nuclear area in the control and PII plus JHA groups was obtained by plotting the nuclear areas against days after feeding (Fig. 1); these groups were not significantly different from each other ($p > 0.01$). However, these values were significantly different from the nuclear areas of the PII-treated insects ($p < 0.025$, a X^2 test was applied). As a whole these observations indicate that JHA avoided the atrophy of the PG cells induced by PII.

Effects of Precocene II and juvenile hormone analogue on epidermal mitosis: in order to test whether the foregoing findings indicated an altered production of ecdysone by PG, we measured the epidermal mitotic activity as an objective measure of the onset of molting. Groups of 30-40 insects each were fed on blood or blood plus PII (25 μg/ml) and then treated with JHA (60 μg/insect). As shown in Fig. 2, by day 5 the mitotic index in the PII plus JHA-treated bugs was as high as in the control group, whereas in PII-treatment alone it was significantly slower than in the other two groups. Consequently, the period between the onset of mitosis and the deposition of the new cuticle was larger in the PII-treated group than in insects preparing for a larval-larval molt (control and PII plus JHA-treated groups).

DISCUSSION

The main findings in this paper are concerned with the effect of JHA in reverting the prevention of ecdysis induced by PII in *Rhodnius prolixus*. Previous works showed, in *Rhodnius*, that PII caused a prevention of molting (Tarrant & Cupp, 1978; Azambuja, Garcia & Furtado, 1981; Azambuja, Garcia & Ribeiro, 1981). This effect was attributed to a drastic atrophy of the PG cells (Azambuja, Garcia & Furtado, 1981). However, when JHA was applied combined with PII treatment the molting cycle was initiated and

completed more rapidly, as judged by the increase in the nuclear area of the PG cells and by the abreviation of the onset of mitotic activity and ecdysis itself. Wigglesworth (1940) had already shown that in supernumerary larval molt the period between the onset of mitosis and the beginning of new cuticle deposition was considerably shorter than in *R. prolixus* undergoing metamorphosis.

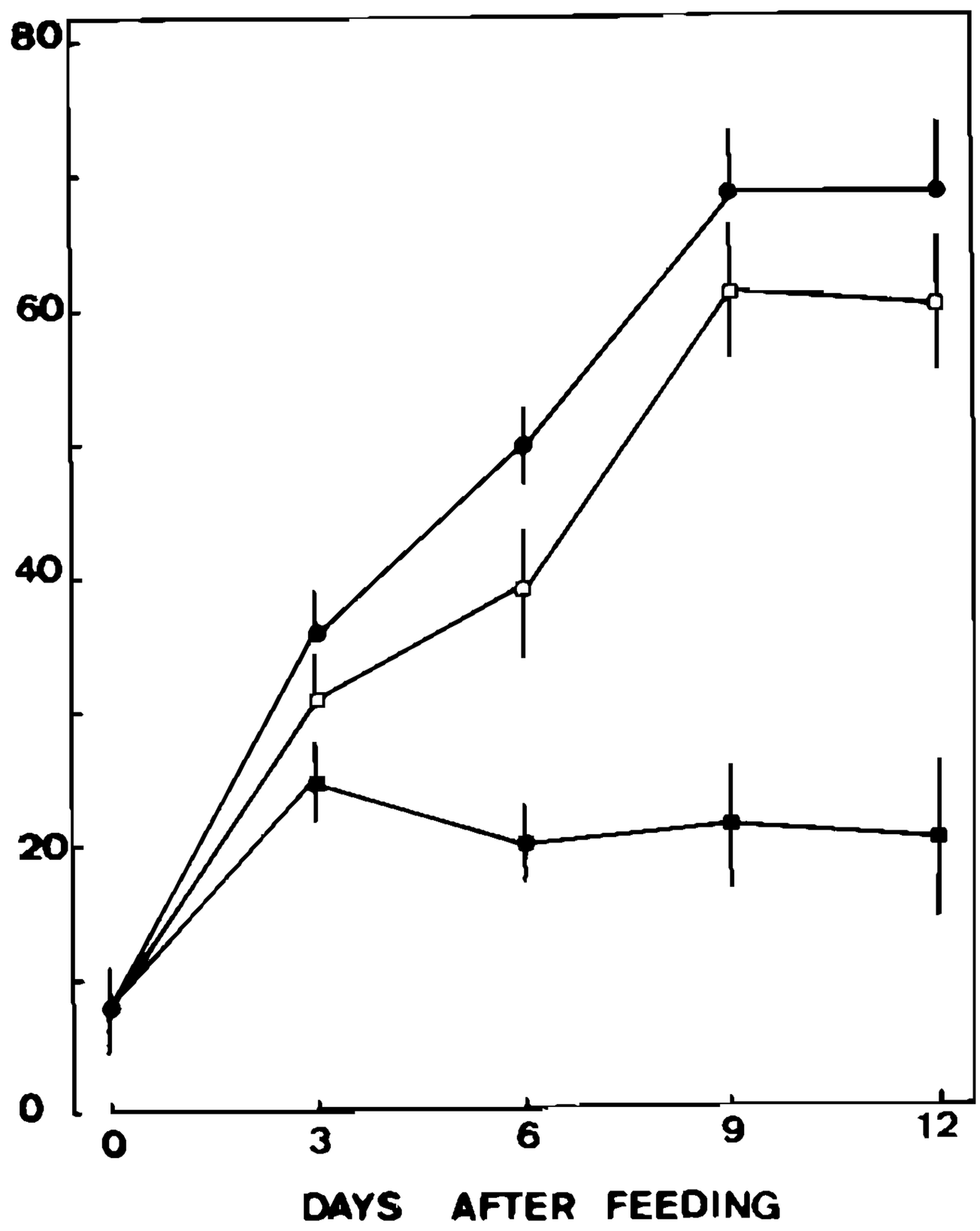

Fig. 1: effects of Precocene II and juvenile hormone analogue on nuclear area (arbitrary) units of the prothoracic gland cells during the fourth-instar larvae of *Rhodnius prolixus*. ●—● Control insects (60 µg JHA/insect); □—□ Precocene II (25 µg/ml) plus 60 µg JHA/insect; ■—■ Precocene II treatment (25 µg/ml). Each point represents the mean nuclear area of prothoracic gland cells from five to seven insects; bars indicate standard errors.

Mitosis is not thought to be a direct response to ecdysteroid level but occurs only in cells which have previously undergone activation (Wigglesworth, 1964). Mitosis activity therefore occurs at the end of the sustained plateau of the ecdysteroid titre. Since there is a relationship between both the level of ecdysteroids in haemolymph and mitotic activity (Smith & Nijhout, 1981) and the mitotic activity we observed was coincident with the ecdysteroid level of *Rhodnius* reared in similar conditions (Baehr, Porcheron & Dray, 1978) we conceive that JHA induces PG cells to secrete ecdysone. However, it is not known in *Rhodnius* whether JH is required for stimulation of PG. Several authors showed that PII induced atrophy of the CA inhibiting JH production (Pratt & Bowers, 1977; Pener, Orshan & De Wilde, 1978; Müller et al., 1979; Unnithan, Nair & Bowers, 1977) and extending the molting cycle period in different insects (Bowers et al., 1976; Tarrant & Cupp, 1978; Pener, Orshan & De Wilde, 1978; Masner et al., 1979; Azambuja, Garcia & Ribeiro, 1981). On the other hand, it has been known that under certain conditions JH can exert prothoracicotropic activity on PG: JH is capable of provoking brainless saturniid pupae to initiate growth (Williams, 1959; Gilbert & Schneiderman, 1959), and numerous compounds with juvenilizing effects could also activate the PG of brainless individuals of a number of lepidopterous insects (Krishnakumaran & Schneiderman, 1965). This effect of JH was confirmed by Cymborowski & Stolarz (1979) in *Spodoptera*, and Safranek, Cymborowski & Williams (1980) in *Manduca*. Recently, Smith & Nijhout (1981) showed that JHA accelerated the onset of ecdysone secretion in *Oncopeltus*. Furthermore, Baehr (1975) observed that there was ecdysis delay in allatectomized fourth-instar *Rhodnius* that could be corrected by injection

of ecdysone. In fifth-instar *Rhodnius* JH accelerated the ecdysis process causing a supernumerary larval molt (Wigglesworth, 1934, 1936; Barrett, 1974). Until now it was believed that the supernumerary larval molts induced by JH are due to the survival of the PG (Sláma, Romanuk & Sorm, 1974).

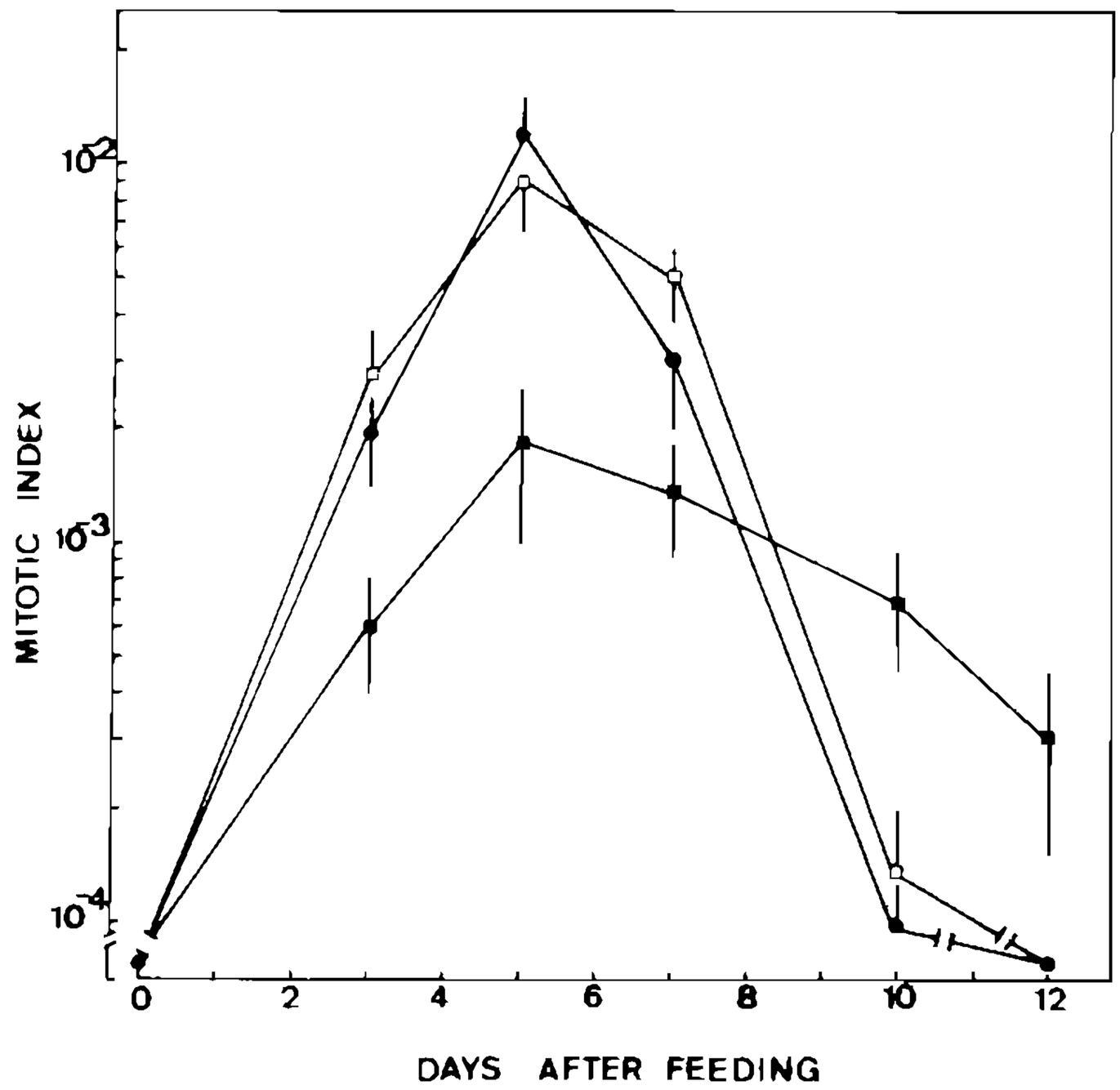

Fig. 2: effects of Precocene II and juvenile hormone analogue on mitotic activity during the fourth-instar larvae of *Rhodnius prolixus*. ●–● Control insects (60 μg JHA/ insect); □–□ Precocene II (25 μg/ml) plus 60 μg JHA/insect; ■–■ Precocene II (25 μg/ml). Each point represents the mean mitotic index of cuticles from five to seven insects; bars indicate standard errors.

In summary, we may conclude that PII causes atrophy of PG cells, alters the mitotic activity and extends the molting cycle period in *R. prolixus*. Whether PII acts directly on PG or through the CA-brain interaction or on general metabolism is still undecided and presently under investigation.

RESUMO

Adicionado ao sangue alimentar na dose de 25 μg/ml o Precoceno II causou um aumento no período de intermuda em ninfas de 4º estádio de *Rhodnius prolixus*. Este atraso da muda foi relacionado com a diminuição da área dos núcleos das células das glândulas protorácicas e com a queda da atividade mitótica das células da epiderme do inseto.

Um análogo de hormônio juvenil aplicado topicamente (60 μg/inseto) junto com o tratamento oral com Precoceno II preveniu a atrofia das glândulas protorácicas e induziu um aumento no número de mitoses nas células da epiderme, diminuindo o período de intermuda nestes insetos.

A possível relação entre a ação do hormônio juvenil e a produção de ecdisona pelas glândulas protorácicas é discutida.

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