Precocene II has no anti-juvenile hormone effects in adult honey bees[1]

P. Fluri[1]

Federal Dairy Research Station, Apicultural Section, CH-3097 Liebefeld-Bern (Switzerland), November 10, 1982

Summary. In order to study further details of the function of JH in adult honey bees, precocene II was applied to workers and queens. Corpora allata ultrastructure, JH and vitellogenin titer (worker bees) as well as egg-laying activity (queens) showed no specific changes after precocene treatment. Doses of 80 µg caused high mortality in workers while queens tolerated 200 µg.

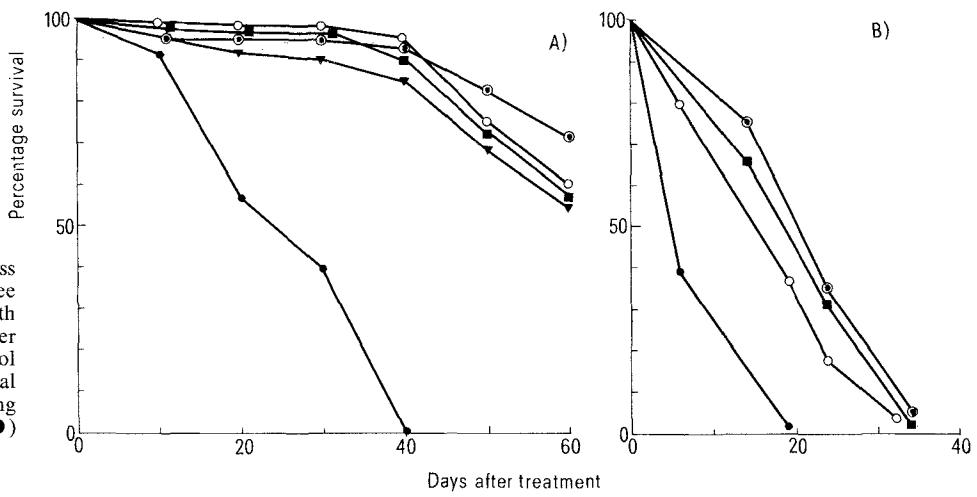
Division of labor in the honey bee *(Apis mellifera)* worker caste is characterized by distinct behavioral and physiological changes during the life of the individual[2-9]. In the last years more and more evidence has accumulated showing that juvenile hormones (JH) play a role in the regulation of several behavioral and physiological parameters of workers such as length of life[9,10] and vitellogenin titer[9-11]. In contrast to this it has not yet been possible to show that vitellogenin titer and egg-laying activity of honey bee queens are under control of JH[12-15].

Further details of the function of JH could be found by stopping the JH production in the corpora allata. As surgical allatectomy in worker bees is difficult to carry out[16], one might use instead the anti-JH chromene derivatives, the precocenes[17]. Anti-JH activity of these compounds has mainly been demonstrated in hemimetabolous insects[17-20]. It was shown, however, that precocene II causes atrophy of the corpora allata in the holometabolous honey bee queen larva[21]. In the present work we investigated whether precocene II has anti-JH effects in adult honey bee workers and queens.

Materials and methods. Adult worker bees (hybrids of the Nigra and Carnica race) were marked shortly after emergence on the thorax and introduced into free-flying colonies (2-comb observation hives and 10-comb Dadant hives). For precocene treatment the marked bees were collected quantitatively from the colonies. After cooling down to 7 °C, 2 µl acetone containing 10–80 µg precocene II or 2 µl acetone alone were applied topically on the abdomen. Before reintroduction into the colonies the bees were slowly warmed up to room temperature. Percentage survival was estimated by periodically counting the number of marked bees. Topical application to queens was done in the comb. Egg-laying activity was estimated by visual inspection of the open and sealed brood. For experiments with caged bees, 60 newly emerged insects were placed in cages 12/10/5 cm and fed with pollen and sugar solution. The biological anti-JH activity of our precocene II samples (EGA-Chemie) was controlled by application on larvae of *Oncopeltus fasciatus.* Haemolymph titers of JH and vitellogenin were measured as described previously[9]. Paraffin sections of van Leeuven-fixed[24] brains were stained according to Ewen[25] for light microscopy.

Results and discussion. Length of life. Topical application of 10–30 µg precocene II on the 6th day after emergence did not affect the survival of caged queenless bees (fig., A) or that of queenright ones in free flying colonies (fig., B). On the other hand, application of 80 µg precocene II resulted in high mortality of both caged and free flying bees (fig., A and B). This dose was toxic too, when 15-day-old worker bees from a free flying colony were treated (results not shown). In contrast to this, honey bee queens in free flying colonies showed no mortality even after topical application of 200 µg precocene II (results not shown). In analogy to this, queen larvae survived after application of 100–150 µg, while worker larvae tolerated at most 10–25 µg precocene II[21,22].

Hemolymph titers of vitellogenin and JH and histology of the corpora allata. 6 and 15-day-old worker bees in free flying colonies were topically treated with acetone containing 80 µg precocene II or acetone alone. 6 or 10 days later, the treated individuals (aged 12 or 25 days respectively) were collected for vitellogenin and JH titer measurement (hemolymph pools from about 80 bees of the same age) and histological preparation of the corpora allata (5 bees of the same age). The relative vitellogenin titer was measured in mm^2 peak surface, the JH content in galleria units (GU) per ml hemolymph (1 GU = about 10 pg JH III, Calbiochem). Both parameters were not significantly different in 12-day-old (treatment on the 6th day) or 25-day-old (treatment on the 15th day) control and precocene treated bees. The vitellogenin titers were 52–61 mm^2 in the 12-day-old control and precocene treated bees and 29–32 mm^2 in the 25-day-old ones. These data are in line with vitellogenin titers of untreated bees reported previously[9] showing decreasing concentrations with increasing age. The JH titers were 1200–1350 GU/ml in the 12-day-old control and precocene treated bees and 1700–2100 GU/ml in the 25-day-old ones. This age-dependent increase of JH content



Percentage survival of queenless caged (A) and queenright free flying (B) honey bees treated with precocene II on the 6th day after emergence. Untreated control (○); 2 µl acetone per individual (⊙); 2 µl acetone containing 10 µg (▼), 30 µg (■), 80 µg (●) precocene II per individual.

was reported previously[9]. Investigation of the corpora allata ultrastructure with light microscopy (12-day-old bees treated on the 6th day and 25-day-old bees treated on the 15th day) revealed no degenerative changes after precocene treatment. From these experiments it can be concluded that precocene is not acting as anti-JH or as chemical allatectomizer in adult bees.

Egg-laying activity. The queens of 5 free flying colonies were treated topically with 30–200 µg precocene II. Weekly inspection of the brood area revealed no reduction of egg-laying activity in comparison with the acetone controls during a period of 6 weeks after treatment. Also, compared with the controls, no size reduction was observed in the precocene treated colonies. Thus, it can be concluded that the egg viability was not affected by precocene application on the queen.

Mode of action of precocene II in the adult honey bee. Under natural conditions long life span worker bees have low JH titers[9] while short life span is correlated with increased JH titers[10]. Thus the life shortening effect of high precocene doses cannot be interpreted as an anti-JH action. However, it cannot be decided from our experiments whether the life shortening effect of the high precocene dose is due to an anti-feeding action of the drug, followed by disturbance of endocrine processes[22] or results from a general toxic effect. Furthermore it is not clear why queens can survive at much higher doses of precocene II than workers. Our results, together with those of other authors[23], might mean that the honey bees' corpora allata lack the enzymic capability for oxidizing precocene molecules to highly reactive epoxides which are supposed to exert a cytotoxic action on these glands.

1 The author thanks Dr A. Buehler for doing the histological part of the work, Dr H. Wille and Dr L. Gerig for valuable discussions and Dr S. Bogdanov for critical reading of the manuscript. The investigation was supported by the Swiss National Science Foundation grant No. 3.188.77.

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0014-4754/83/080919-02$1.50 + 0.20/0

Hyperglycemic effect in the rabbit induced by $ACTH_{4-10}$

R. Drouhault, A.M. Courtes and B. Dufy[1]

Laboratoire de Neurophysiologie, Université de Bordeaux II, INSERM U.53, Domaine de Carreire, Rue Camille Saint-Saëns, F-33077 Bordeaux cedex (France), June 21, 1982

Summary. In the rabbit, a single i.v. administration of $ACTH_{4-10}$ (130 µg/kg) induces hyperglycemia. As $ACTH_{4-10}$ also induces hypocalcemia and inhibits insulin secretion, we suggest that hypocalcemia, inhibition of insulin secretion, and hyperglycemia, are closely related.

Studies of the relationship between structure and activity of adrenocorticotropin (ACTH) have shown that this hormone possesses different biological properties according to its various target cells. Since they share the same receptor in the adrenal gland the sequence $ACTH_{1-24}$ (synacthen) has the same biological action as the whole molecule.

It is known that the peptidic sequences necessary for the binding and/or the biological action are not always the same. The N-terminal sequence 1–10 of ACTH seems to be essential for the biological action, but the 11–24 sequence is necessary for the binding of the hormone on the adrenal target cells[2–5]. As a consequence of this observation numerous authors have reported that the 11–24 sequence, which is biologically inactive, can present an antagonistic effect towards the whole molecule[4,6,7]. According to Beloff-Chain et al.[8] the 18–39 sequence of corticotropin like intermediate peptide (CLIP) should stimulate insulin secretion. On the other hand earlier studies have shown that in the rabbit $ACTH_{4-10}$ and in some instances α, β MSH or related pituitary peptides induce: a) hyperemia of adipose tissue[9], b) increased concentration of free fatty acids (FFA) in adipose tissue, blood plasma, liver and kidney[9], c) lipemia[10,11], d) acute nonkenotic metabolic acidosis[12,13], e) hypocalcemia with hypophosphatemia in rabbit but not in rats[14].

In addition to these effects we show, in this paper, that $ACTH_{4-10}$ induces hyperglycemia and hypocalcemia, and inhibits insulin secretion.

Material and methods. Our experiments were performed on rabbits and rats. 'Fauve de Bourgogne' rabbits weighing 3000–3500 g, and Sprague-Dawley rats weighing 250–300 g, were kept without food for 24 h before the experiment but