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Precocene-induced collapse and resorption of corpora allata in nymphs of *Locusta migratoria*

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Summary. Precocene II causes an irreversible regression of corpora allata in young 4th instar nymphs of the migratory locust. Within 2 h after application, the cells collapse. Cell fragments are subsequently phagocytosed by haemocytes.

Precocene II (ageratochromene; 6,7-dimethoxy 2,2-dimethyl-chromene) is a compound isolated from the ornamental plant *Ageratum houstonianum*[2,3] which exhibits marked biological effects on several insect species. When administered to hemimetabolous larvae, a precocious metamorphosis follows, hence the name 'precocene'[3]. This effect closely resembles the effect of the extirpation of the corpora allata (CA). Indeed, there is some evidence, both physiological[3,4] and biochemical[5], that the CA cease to produce juvenile hormone (JH). In our laboratory, Pener et al.[6] found that the CA in morphogenetically abnormal, precocious 'adults' of *Locusta migratoria* which developed from precocene II-treated 4th instar nymphs, had atrophied. This raised the question whether the atrophy resulted from a direct toxic effect of precocene II on the glands, or from a more gradual process of regression due to lack of stimulation. In *Oncopeltus fasciatus,* signs of structural disintegration were found at 7 days after treatment[7]. We present here electron microscope data on CA of *L. migratoria* nymphs which indicate that precocene II acts in a matter of hours, possibly through a direct toxic action on the glands.

Material and methods. 4th instar nymphs of *Locusta migratoria migratorioides* R. & F., 16–24 h after the moult, were treated topically on the abdomen with 200 µg precocene II, dissolved in 2 µl acetone. Controls received only acetone. For incubation periods up till 18 h, the locusts were kept at 32–34 °C and 40–50% RH; for longer periods, in a climate chamber with a cyclically changing temperature and RH[6]. Locusts were sacrificed at intervals from 90 min to 15 days and corpora allata were dissected and processed for electron microscopy as described before[8].

Results. The ultrastructure of CA in normal *L. migratoria* nymphs has been described by Joly et al.[9]. Here, the observed deviations from the normal picture will be emphasized. Individuals showed some variability in response but the general pattern of structural changes is clear and can be summarized as follows.

In some of the glands fixed as early as 90 min after treatment, a condensation of the cytoplasm of most of the allatum cells occurred. The cytoplasm appeared more electron-dense due to a closer packing of ribosomes and other cytoplasmic constituents. The extracellular spaces which were relatively small in the controls (figure A) appeared enlarged at the same time (figure B).

After 2–3 h, the condensation process had proceeded in all individuals. The cytoplasm reached a high electron density, the chromatin in the nuclei became condensed as well, and the nuclear membrane lost its smoothness. The size of extracellular cisternae had further increased.

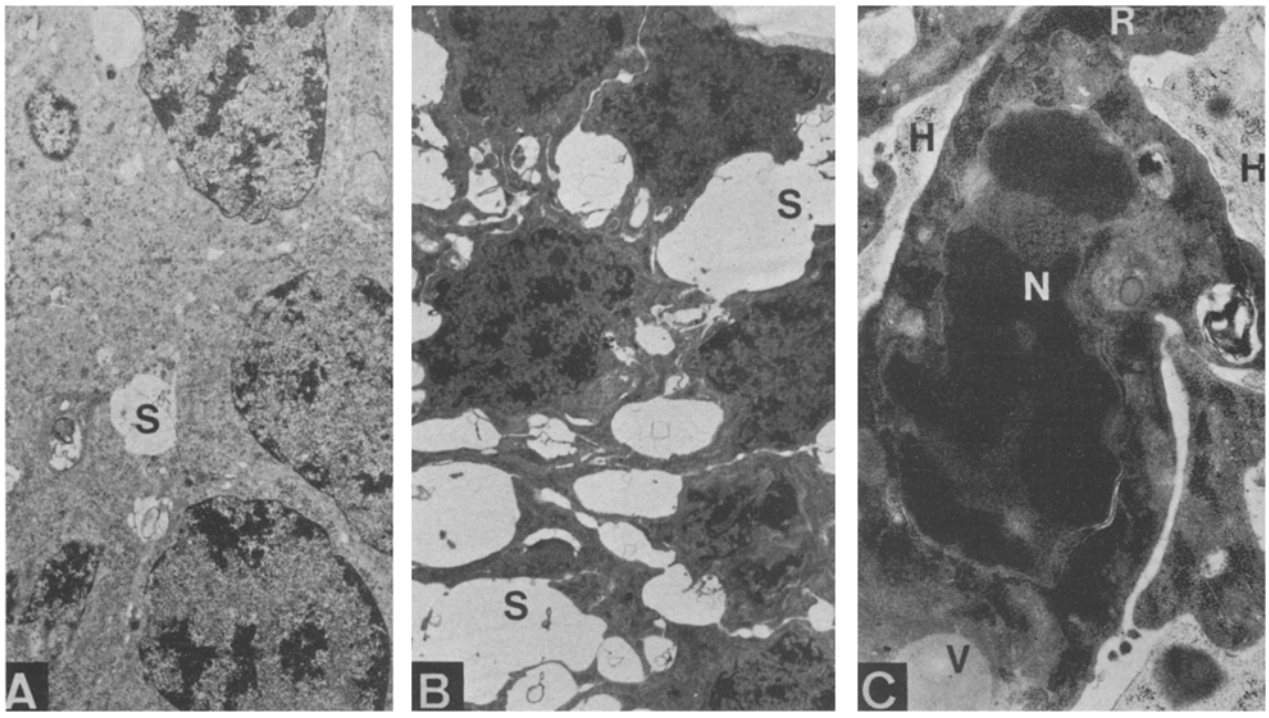
After 7 h the process of condensation resulted in a maximal shrinkage of the cells. Nuclei became small and of irregular shape, the chromatin coagulated and attained a very high electron density. The cytoplasm now reached maximal density, ribosomes were packed tightly in certain cytoplasmic areas (see also figure C). Mitochondria appeared shrunken and their membranes became vague. Microtubules remained intact and continued to support the cell's 3-dimensional shape; however, since little cytoplasm was left, the cells lost intimate cell to cell contact normally provided by desmosomes. Extracellular spaces had reached maximal dimensions. Preliminary observations indicate that the total volume of the gland had not markedly changed by this stage.

After 12 h, the structure of the gland cells had not changed much further. The nuclear membrane was not always discernable but occasionally a narrow perinuclear space was seen. The remains of the nucleus had a very irregular shape but fragments did not coalesce with the cytoplasm. The basement membrane surrounding the CA consisted of a loose texture of collagen filaments, and was interrupted at several places. Haemocytes, both granulocytes and plasmatocytes[10], penetrated and migrated deep in between the CA cells.

After 18 h, the haemocytes were quite numerous and had started engulfment and phagocytosis of cell fragments. The digestion of these fragments occurred very quickly. Details about the resorption process will be published elsewhere.

After 26 h, approximately 75% of total gland material had disappeared, probably by haemocyte action. The rather small condensed cell fragments that were left at this stage often contained a piece of nucleus. Cytoplasmic vacuoles of sizes up till 1 µm had developed; these contained a homogeneous, possibly lipoid, material of moderate electron density (figure C).

A small number of cells in the periphery of the gland responded in a different way to precocene II. These light cells contained clear and spheroid nuclei and did not condense as much as the predominate type of cell. Ultimately the nuclear chromatin became necrotic and pieces



Electron micrographs of corpora allata of *Locusta migratoria* fixed at different intervals after treatment of freshly moulted 4th instar nymphs with precocene II (2.5% glutaraldehyde fixation, 1% OsO_4 postfixation, sections contrasted with uranyl acetate and lead citrate). *A* Control CA with more or less spheroid nuclei, abundant cytoplasm and conspicuous but small extracellular spaces (S). *B* 90 min after treatment, electron dense cells with irregular nuclear and cellular boundary, little cytoplasm, and strongly increased extracellular spaces (S). × 6000. *C* 26 h after treatment, detail of electron dense cells with irregular nucleus and condensed chromatine (N), local aggregations of ribosomes (R) and vacuole (V); the cell is surrounded by haemocytes (H). × 20,000.

of cell material were phagocytosed and digested by haemocytes as well.

After 3 days, only a little gland material had remained. Misleadingly enough, the shape and size of the former CA had persisted in the spheroid body which now contained an assembly of haemocytes. The latter remained united for some more days but eventually dispersed, leaving a mass of trachea and axons carrying neurosecretory granules.

Discussion. The observations leave little doubt that the effect of precocene II takes place much more rapidly than was hitherto inferred[6,7]. It is not known, however, in which phase of structural disintegration the biochemical performance of the glands (i.e. JH biosynthesis) is inhibited or blocked. In general, the effect of toxic agents on physicochemical events in cells can be measured well before structural changes become apparent[11]. Precocene II apparently affects osmotic equilibria resulting in a dramatic extrusion of water from CA cells within a few hours. This does not necessarily result in cell death or cessation of all metabolic activity, as organized, structural changes such as the formation of vacuoles were still observed 26 h after treatment. In the rat, fragments of similarly condensed parenchymal cells (Councilman bodies), found in several types of liver damage, were also found to be capable of limited metabolic activity[12]. On the other hand, biosynthesis of JH III by CA of *Periplaneta americana* in vitro was largely blocked after pre-incubating the glands for 0.5–4 h in the presence of precocene II[5]. It therefore seems reasonable to assume that the short-term ultrastructural changes in *Locusta* are indicative of a collapse of CA cells and decreased capacity for JH synthesis.

The type of cell degeneration observed here seems not to have been reported in insects before and is quite distinct from cases of 'programmed' cell death summarized recently[13]. The changes rather resemble 'shrinkage necrosis'[14], also called 'apoptosis', a phenomenon of cell delection found in higher animals during specific stages of embryogenesis, after the administration of noxious agents, or under certain pathological conditions[15]. Under the present conditions, the effects of precocene II are irreversible. No other organs or cells have yet been reported to be affected by the chemical. An interesting parallel exists in the vertebrate literature where the chemical alloxan is known to destroy specifically the insulin-producing beta cells in the islets of Langerhans of the endocrine pancreas[12].

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