ENDOCRINOLOGICAL AND BIOCHEMICAL STUDIES ON THE FEMALE REPRODUCTIVE PHYSIOLOGY OF THE RED COTTON BUG, DYSDERCUS KOENIGII (PYRRHOCORIDAE, HETEROPTERA-HEMIPTERA)

BY
P. S. VIMALA DEVI, M.Sc.

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CERTIFICATE

This is to certify that Mrs. P. Sarvamangala Vimala Devi has carried out the research work embodied in the present thesis under my supervision and guidance for the full period prescribed under the Ph.D. Ordinances of this University. I recommend her thesis entitled 'Endocrinological and Biochemical Studies on the female reproductive physiology of the red cotton bug, Dysdercus koenigii (Pyrrhocoridae, Heteroptera-Hemiptera)' for submission for the degree of Doctor of Philosophy in this University.

P S Ramamurty
(P.S. RAMAMURTY)
Supervisor

P S Ramamurty
Dean
School of Life Sciences

Prof P S RAMAMURTY,
Dean School of Life Sciences
University of Hyderabad,
HYDERABAD 500 134 (India)

SCHOOL OF LIFE SCIENCES
UNIVERSITY OF HYDERABAD
HYDERABAD 500 134

DECLARATION

I, P. Sarvamangala Vimala Devi, hereby declare that the research work embodied in the present thesis has been carried out by me under the supervision and guidance of Prof. P.S. Ramamurty for the full period prescribed by the Ph.D. Ordinances of the University.

I declare that no part of this thesis was earlier submitted for the award of a research degree of any other University.

P.S. Ramamurty
(P.S. RAMAMURTY)
Supervisor

P. S. Vimala Devi
Signature of the candidate

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P.S. Vimala Devi

(P.S. Vimala Devi)

School of Life Sciences
University of Hyderabad
Hyderabad 500134(India)

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ABBREVIATIONS USED

BH	Brain hormone
CA	Corpus allatum
JH	Juvenile hormone
JH-III	Juvenile hormone-III
MNSC	Median neurosecretory cells
NSC	Neurosecretory cells
P-I	Precocene-I
P-II	Precocene-II
PF	Paraldehyde Fuchsin

CHAPTER I

General Introduction

The oocytes of insects attain prodigeous dimensions during a short phase of their vitellogenic growth, especially due to the deposition of massive amounts of proteid yolk materials. The existing belief is that, there is very little yolk protein synthesized within the egg cells, because light and electron microscopic studies showed a conspicuously poor development of the usual cell organelles associated with biosynthetically active secretory cells. Hence proteid yolk is believed to be exogenous in origin either from the enveloping follicle epithelial cells and/or by incorporation of blood protein(s). During oogenesis, copious amounts of vitellogenin(s), the female specific protein(s), are synthesized in the fat body and discharged into the haemolymph. This protein is sequestered by the developing oocytes and deposited as vitellin which represents the major protein of the yolk system (De Wilde and De Loof, 1973a; Wyatt and Pan, 1978; Engelmann, 1979; Gong and Zhai, 1979; Hagedorn and Kunkel, 1979). The number of vitellogenin(s) could vary from one to few, out of the many haemolymph proteins present and they are sequestered selectively by the developing oocytes (Telfer, 1960, 1961; De Loof and De Wilde, 1970a,b; Kunkel and Pan, 1976; Ferenze, 1978; Harry et al., 1979;

could also be involved in biosynthesis of yolk proteins. These, together with the blood protein, are built into proteid yolk spheres (Ono et al., 1975; Heubner et al., 1975a,b; Bast and Telfer, 1976; Benozzati and Basile, 1978; Borovsky and Van Handel, 1980; Jowett and Postlethwait, 1980; Brennan et al., 1981; Irie and Yamashita, 1983). Further, the autoradiographic analysis of the egg follicles of Hyalophora cecropia suggested that this follicle cell protein serves as the trigger for the subsequent pinocytotic uptake of vitellogenin (Anderson and Telfer, 1969). Thus, the present state of our knowledge on the origin of proteid yolk indicates that it has probably a dual origin, one from the haemolymph (vitellogenin) and the other from the follicle epithelium.

To date several studies exist on the hormonal control of ovarian development in insects. Regulation of vitellogenesis is brought about by endocrine mechanisms which are apparently quite varied in different groups of insects (Engelmann, 1968, 1970; De Wilde and De Loof, 1973b, Doane, 1973; Wyatt and Pan, 1978; Hagedorn and Kunkel, 1979). It is now evident that the reproduction of insects is controlled by at least three endocrine

glands: the cerebral neurosecretory cells, corpora allata and the prothoracic glands. The corpora allata (CA) secrete juvenile hormone (JH), which regulates the maturation of reproductive organs. Among the several JH-controlled individual processes associated with reproduction the important ones are vitellogenin synthesis, yolk deposition, pheromone production and accessory gland development.

Five closely related juvenile hormones (JH) have been reported, namely, JH I,JH II, JH III, JH O, and 4-methyl JH I - all of them occur in the tobacco hornworm Manduca sexta (Judy et al., 1973a; Bergot et al., 1980, 1981). Recent studies carried out in several laboratories, to identify and characterise JH from exopterygote insects, have shown that JH III is the most commonly prevalent and naturally occurring hormone in exopterygota (Judy et al., 1973b; Muller et al., 1974; Trautman et al., 1974a,b, 1976; Tobe and Stay, 1977, 1979; Lanzrein et al., 1978; Roseler and Roseler, 1978; Caussanel et al., 1979; Loher et al., 1983), including the Hemipterans (Rankin and Riddiford, 1978). Hormonal regulation of oocyte maturation occurs at two critical sites. One is the fat body, in which macromolecules are

synthesized for eventual incorporation into the oocyte; the other is the follicle epithelium, through which molecules are passed selectively for incorporation into maturing oocyte by pinocytosis. The synthesis of vitellogenin has been shown by numerous investigators to occur in the fat body and to be under the control of JH secreted from corpora allata, under appropriate physiological and/or environmental stimuli (Coles, 1965; Daufer et al., 1970; Engelmann, 1971; Wyatt, 1972; Doane, 1973; Chen et al., 1976, 1979; De Wilde and De Loof, 1977; Kelly and Telfer, 1977; Handler and Postlethwait, 1977, 1978; Hagedorn and Kunkel, 1979; Postlethwait and Handler, 1979; Jowett and Postlethwait, 1980; Rankin and Jackle, 1980; Reid and Chen, 1981; Engelmann, 1982; Zhai et al., 1984). The rate of synthesis of vitellogenin is influenced by several factors, such as JH titre (Engelmann, 1971; Pan and Wyatt, 1976), availability of protein precursors and possibly also by the presence of mature eggs in the ovaries. The last mentioned condition may cause partial inhibition of CA (Hagedorn and Kunkel, 1979). Egg-maturation was also reported to be largely independent of CA in honey bees, because even allatectomised young as well as old queens continued to lay eggs uninterruptedly (Van Laere, 1974; Engels and Ramamurty, 1976; Ramamurty and Engels, 1977b). These authors

suggested that in honey bee queens, there is the possibility of other known insect hormones such as ecdysone and/or brain hormone playing a gonadotropic role. In Aedes aegypti vitellogenin synthesis in the fat body was definitely shown to be induced by ecdysone which is produced in the ovary after the blood meal(Fallon et al., 1974; Hagedorn et al., 1975).

In certain insects the protocerebral neurosecretory cells (NSC) were shown to be indispensible for oogenesis. They perform their function either by activating the CA resulting in the stimulation of protein synthesis in the fat body, which is a pre-requisite for proteid yolk formation, or by producing a true gonadotropic hormone (De Wilde and De Loof, 1973b). In locusts, the haemolymph protein concentration is controlled by the brain hormone (BH) released from the neurosecretory cells, while the JH is involved in regulating the transport of blood protein across the follicle epithelium and its incorporation into the maturing oocytes by a process of micropinocytosis (Highnam et al., 1963). The neurosecretory cells were reported to play an important role in the oogenesis of Schistocerca (Hill, 1965), Leptinotarsa (De Wilde and De Boer, 1969), Calliphora (Thomsen and Moller, 1963;

Thomsen and Thomsen, 1970), Phormia (Orr, 1964a,b) and Sarcophaga (Wilkens, 1967, 1968, 1969). From her experimental studies on Calliphora, Thomsen concluded to an influence of NSC on protein metabolism, but according to Wilkens (1969) and Engelmann et al.,(1971), in Sarcophaga, the CA regulates vitellogenic protein synthesis whereas NSC provide a gonadotropic hormone. As stated already, definitive evidence is available to indicate that ecdysterone controls vitellogenin synthesis in Aedes aegypti (Hagedorn et al., 1975). There are only few insect species where NSC do not appear to be necessary for oogenesis. Oogenesis can be induced in isolated abdomens of Rhodnius (Wigglesworth, 1936, 1948) and Leucophaea (Chambers and Brookes, 1967), merely by implantation of CA or by injection of synthetic JH (Bell and Barth, 1970). In these insects, the CA hormone (JH) seems to regulate protein metabolism associated with vitellogenesis (Engelmann, 1970), and the NSC need not activate the CA.

The juvenile hormone is also known to influence various morphological and biochemical events which are associated with follicle epithelium differentiation during oocyte maturation in different insect species.

It induces morphological alterations in the follicle epithelium such as changes in the shape of the cells, enlargement of the nucleus and development of conspicuous intercellular spaces in the follicle epithelium that facilitate inward migration of yolk precursors (Masner, 1968; Jalaja and Prabhu, 1976, 1977; Abu-Hakima and Davey, 1977a,b; Koeppe et al., 1980a). Furthermore, it has been shown that JH stimulates the DNA synthesis in the ovary, mainly in follicle epithelial cells (Koeppe and Wellman, 1980; Koeppe et al., 1980a; Koeppe, 1981). Apart from this, JH is also known to regulate the DNA dependent RNA synthesis in the follicle epithelial cells (Sahota, 1973; Koeppe and Wellman, 1980). Another follicle cell process that appears to be regulated by JH, at least in Periplaneta, is the protein synthesis, more precisely the induction of the specific follicle cell protein (Bell and Sams, 1974; Sams and Bell, 1977).

In consonance with its multifarious regulative functions, the corpora allata (CA) of various insects undergo cyclical juvenile hormone synthesis during the first gonotropic cycle and this is correlated with cyclical changes in CA volume and cell numbers (Schooneveld et al., 1977; Tobe and Stay, 1977; Kramer, 1978; Lanzrein

et al., 1978; Szibbo and Tobe, 1981). A similar change in volume was observed during the first ovarian cycle in Dysdercus cingulatus (Jalaja and Prabhu, 1977), Dysdercus similis (Judson et al., 1979), Dysdercus koenigii (Tiwari and Srivastava, 1979).

Bowers and his associates have successfully isolated two chromene derivatives from the common bedding plant Ageratum houstonianum (Bowers, 1976; Bowers et al., 1976), which induced precocious metamorphosis in Oncopeltus fasciatus, Lygaeus kalmii and Dysdercus cingulatus. Because of their ability to induce precocious metamorphosis, Bowers (1976) named the active compounds as precocene I (P-I) and precocene II (P-II), the latter being more potent than the former. In addition, these compounds also possess an indirect antigonadotropic influence and inhibit the ovarian development in some species of Heteroptera, Coleoptera, Diptera and Orthoptera (Bowers, 1976; Bowers et al., 1976; Unnithan et al., 1977; Pener et al., 1979; Masner et al., 1979; Landers and Happ, 1980; Unnithan et al., 1980; Wilson et al., 1983). In many insect species the corpus allatum was reported to be the main target for P-II because JH and its analogues could compensate for the inhibitory effect of P-II in Oncopeltus fasciatus (Bowers

et al., 1976; Masner et al., 1979), Locusta migratoria (Pederson, 1978), Schistocerca gregaria (Unnithan et al., 1980) and Diploptera punctata (Feyereisen et al., 1981). Ultrastructural studies clearly revealed that P-II selectively destroyed the secretory cells of the CA (Unnithan et al., 1977; Liechty and Sedlak, 1978; Schooneveld, 1979a,b; Feyereisen et al., 1981). In vitro experiments suggest that P-II inhibits the CA directly (Pratt and Bowers, 1977; Muller et al., 1979; Pratt et al., 1980; Feyereisen et al., 1981; Feldlaufer and Bowers, 1982). The transection of the nervous connection between the brain and CA in Oncopeltus fasciatus does not alter the susceptibility of CA to P-II in vivo, indicating a direct action of P-II on the CA, rather than involvement of a neural mechanism of CA regulation (Bowers and Aldrich, 1980). However, P-II fails to inhibit ovarian development in another Pyrrhocorid bug Pyrrhocoris apterus, which appears to be caused both by a low P-II sensitivity of the CA itself and an unknown 'anti-precocene mechanism' outside the CA (Hodkova and Socha, 1982). This shows that the sensitivity of different species to precocene is highly variable. Similarly, P-II has no anti-JH effect in the larval forms (Rembold et al., 1979) as well as in adults of honey bees (Fluri, 1983).

Recent studies on the mode of action of precocenes have shown that CA cells are selectively destroyed ('Chemical allatectomy') by the accumulation of a reactive intermediate of precocene, formed *in situ* (Brooks *et al.*, 1979; Pratt *et al.*, 1980). In adult insects, P-II thus induces destruction of CA which results in deficiency of JH.

Despite the numerous physiological investigations on the Hemiptera, surprisingly, only few data are available on vitellogenin synthesis. Similarly, works dealing with the hormonal regulation of vitellogenesis are also meagre and inconclusive.

In Hemiptera, the classical work by Wigglesworth (1936) on *Rhodnius* showed that the CA is essential for yolk deposition and this was further confirmed by Vanderberg (1963) and Baehr (1973). Contradictory findings were reported in the same species by Davey and his coworkers (Davey, 1967; Patchin and Davey, 1968; Pratt and Davey, 1972) who claimed that removal of CA did not totally prevent the formation of mature eggs. However, Coles (1965) clearly demonstrated that the yolk protein synthesis as well as the uptake is regulated by corpora allata in *Rhodnius*. Perassi (1973) suggested the involvement of

two different hormones (neurosecretory system and corpora allata) in the protein synthesis and its uptake by the maturing oocytes of Triatoma infestans. In a related species of Triatoma protracta, allatectomy prevented yolk deposition and the topical application of JH-III stimulated a dose-related de novo synthesis of vitellogenin in allatectomised females (Mundall and Engelmann, 1977). In Oncopeltus (Johansson, 1958) and Dindymus (Friedel, 1974), an active corpus allatum is essential for egg maturation whereas the extirpation of MNSC does not prevent vitellogenesis. Extirpation and reimplantation studies have revealed that both MNSC and CA are essential for vitellogenesis in Dysdercus cingulatus, although only CA was shown to play a direct gonadotropic role in the female (Jalaja and Prabhu, 1977).

Kelly and Telfer (1977) reported the existence of two vitellogenins (A and B) in the haemolymph of Oncopeltus fasciatus, and that the synthesis of vitellogenin B is JH- dependent whereas A is not. On the other hand, Rankin and Jackle (1980) suggested that vitellogenin A is the precursor of vitellogenin B. According to these authors synthesis of the precursor is ecdysteroid-mediated while the uptake and perhaps the processing of the precursor

into the final product may be under JH control in the same insect, just mentioned above.

From the foregoing account it is clear that we do not yet have a comprehensive and unified picture of the endocrine mechanism (s) controlling vitellogenin synthesis in the fat-body of Heteroptera, except perhaps in a few species (Coles, 1965; Kelly and Telfer, 1977; Mundall and Engelmann, 1977; Rankin and Jackle, 1980). In the present study, therefore it was thought fit to undertake a detailed study of the endocrine mechanism involved in the synthesis of precursor yolk proteins (vitellogenins) in a plant bug Dysdercus koenigii. In addition, a biochemical study was made on the quantitative changes occurring in the DNA, RNA and protein content of the fat body and ovary as well as the protein content of the haemolymph during the first reproductive cycle. Since it is well known that actinomycin-D inhibits the DNA-dependent RNA synthesis it was thought desirable to investigate the effect of this antibiotic on the RNA metabolism in the fat body of Dysdercus koenigii. Using P-II as chemical allatectomiser, in the present study an attempt was made to investigate the role of CA in the regulation of fat body growth and its protein synthesis

during egg-maturation. Ultrastructural changes in the CA cells caused by P-II treatment, have also been described.

THE INVESTIGATION OBJECT

Dysdercus koenigii (Hemiptera: Pyrrhocoridae) is commonly known as the red cotton bug. It is a serious pest of cotton plant chiefly and thrives well also on the alternate host plant, Hibiscus esculentus.

Rearing method and life-cycle:

The insects were reared in a culture room at 26 ± 1°C, RH-70 ± 5%, 14:10 LD period. These insects were maintained on soaked cotton seeds in glass troughs. Additional water requirement was provided by keeping water bottles containing 0.05% L-ascorbic acid. Transfer to fresh culture troughs was done on every third day. At this time, the eggs were collected and kept in petridishes with wet cotton swab in a corner to maintain the humidity and left for hatching. The eggs hatch in about 5-6 days. These newly hatched nymphs were transferred to another jar with fresh, soaked cotton seeds. The post-embryonic development in this bug passes through five nymphal instars and the duration of first to fourth instars is around 3-4 days. The fifth instar has a slightly longer duration ranging between 5-6 days, after which they moult

into adults. The adults start mating 2-3 days after their emergence and the mating continues up to egg-laying. Almost immediately after cessation of the prolonged copulation, the females start laying eggs on 7th or 8th day after emergence. The first reproductive cycle lasts for 7-8 days. This is followed by more reproductive cycles under laboratory conditions. Males have a longer life-span than females.

Insects used for the experiments:

Newly emerged adult males as well as females were collected at regular intervals and maintained separately. These were used at different times, according to the experimental requirements.

CHEMICALS

Acrylamide, actinomycin-D, bovine serum albumin (fraction V), brilliant blue R, DNA (calf-thymus), JH-III (cis-10,11-epoxy-3,7,11-trimethyl-trans-trans-2,6-dodecadienoic acid methylester), N N'-methylene-bis-acrylamide, POPOP (1,4-bis[2-(5-phenyloxazolyl)]benzene), PPO(2,5-diphenyloxazole), RNA (yeast), TEMED (N,N,N',N'-tetra

methylethylenediamine), trizma base[Tris(hydroxymethyl)-amino methane] were purchased from Sigma Chemical Company, St.Louis, MO, USA. Isotopes H^3-leucine (3,300 mCi/mmole) and H^3-uridine (13,800 mCi/mmole) were purchased from Bhabha Atomic Research Centre, Trombay, India. Hyamine hydroxide (tissue solubilizer) was supplied by Beckman Company, England. Precocene II (6,7-dimethoxy-2,2-dimethyl-2-H-1-benzopyran) was obtained from Ega-Chemie, W. Germany. Ilford K_2 emulsion was purchased from Ilford Ltd., England. Chemicals for electron microscopy were supplied by Taab Laboratories, England. Stains were purchased from Serva Chemical Company, Hiedelberg, W. Germany. All other chemicals used were obtained from commercial sources in India and were of analytical grade.

METHODS

Preparation of homogenate:

The fat body and ovaries were dissected out and freed from the surrounding tissues. All the subsequent operations were carried out at 0-4°C, unless otherwise specified. Tissues taken from several insects were pooled, weighed and homogenized in cold distilled water to make 1%

to 10% homogenates as required, in a glass homogenizer with a teflon pestle (Potter Elvejhem type). The aliquots were used for the extraction of nucleic acids and estimation of proteins.

Extraction of nucleic acids:

The nucleic acids were extracted according to the procedure of Schmidt and Thannhauser (1945) slightly modified, as suggested by Munro (1966).

One ml of 10% homogenate (w/v) was mixed with 1.25 ml of 10% ice cold TCA and centrifuged to remove acid soluble compounds. The sediment was washed once with 1.25 ml of ice cold TCA. After the removal of acid soluble compounds, the sediment was extracted twice with 2.5 ml of 95% ethanol and the extract was removed by centrifugation. An ethanol-ether (3:1) wash was given to the sediment to remove the lipids present. The lipid-free pellet was suspended in 1 ml of 1 N Potassium hydroxide and incubated for 2 h at 37°C. This incubation with 1N Potassium hydroxide was sufficient to hydrolyse the RNA of the ovary and fat body. DNA and protein were then precipitated by the addition of 0.2 ml of 6N hydrochloric

acid and 1.3 ml of 5% TCA and allowed to stand in ice for 10 min and centrifuged. The supernatant fraction was collected separately to estimate RNA content. The sediment was suspended in 1.25 ml of 5% TCA at 90°C for 15 min with occasional shaking. The mixture was centrifuged and the supernatant was collected in a test tube. Now, the sediment was washed with 0.75 ml of 5% TCA and both the supernatants were taken for estimation of DNA.

Extraction of RNA by the method of Fong and Fuchs (1976):

The fat body was dissected out and homogenized in 1 ml of cold 95% ethanol containing 10% potassium acetate (w/v). The homogenate was centrifuged at 27,000 g for 15 min at 4°C and the pellet was washed with 3 ml of cold 2% perchloric acid (PCA). Hydrolysis of RNA in the pellet was accomplished by adding 1 ml of 0.5 N Potassium hydroxide and incubating at 37°C for 24 h. After the incubation, the hydrolysate was acidified by the addition of 0.27 ml of 7% PCA. The hydrolysate was then centrifuged and the supernatant was used either for RNA estimation or for radiolabelled counting using Bray's mixture (see further below).

Estimation of DNA and RNA:

DNA was estimated by diphenylamine method (Burton, 1956). For estimation of DNA, 1 ml of DNA extract was mixed with 2 ml of diphenylamine reagent and heated for 10 min in boiling water. The intensity of blue colour developed, was read at 600 nm in a Systronics spectrophotometer. The amount of DNA present in a sample was determined from a standard curve using calf-thymus DNA as standard. RNA was estimated by the orcinol reaction (Schneider, 1957). For estimation of RNA, 1 ml of RNA extract was diluted to 2.5 ml with 5% TCA and heated for 30 min after adding 2.5 ml of orcinol reagent in a boiling water bath. The intensity of the resultant green colour was then read at 700 nm. A standard curve was prepared using purified yeast RNA as the standard.

Diphenylamine reagent:

This was prepared by dissolving 1 g of purified diphenylamine in 100 ml of glacial acetic acid and 2.75 ml of concentrated sulphuric acid.

Orcinol reagent:

One gram of purified orcinol was dissolved immediately before use in 100 ml of concentrated hydrochloric acid containing 0.5 g of Ferric chloride.

Preparation of standards:

DNA (calf-thymus) and RNA (yeast) were first dissolved in water at a concentration of 2 mg/ml. Then a portion of this solution was diluted with 5% TCA and heated for 15 min at 90°C. The volume of solution thus obtained was made up with 5% TCA in such a way that the final concentration was adjusted to 200 µg/ml.

Protein estimation:

To a fraction of the homogenate, an equal volume of cold 10% TCA was added and kept at 0-4°C for 10 min. The sample was then centrifuged and the pellet thus obtained was washed twice with cold 5% TCA, followed by a wash with an ethanol-ether (3:1) mixture. The pellet was dissolved in 0.1 N sodium hydroxide and used for protein estimation by the method of Lowry et al., (1951).

An aliquot of the sample was made up to 1 ml with distilled water. To this, 5 ml of alkaline copper reagent was added (1 ml of 2% sodium potassium tartrate and 1 ml of 1% copper sulphate mixed with 100 ml of 2% sodium bicarbonate in 0.1 N sodium hydroxide) and the contents of the tube were mixed in a cyclomixer. After 15 min, 0.5 ml of Folin-Ciocalteau reagent was added and the contents were stirred immediately. The colour was allowed to develop and after 30 min, the optical density readings were taken at 670 nm against a blank developed with 1 ml of distilled water. The protein concentration of the samples were determined from a standard curve drawn, using 20-200 µg of bovine serum albumin (fraction V).

H^3-uridine incorporation into fat body RNA:

The insects were injected with H^3-uridine (13,800 mCi/m mole) at a dosage of 0.5 µCi/10 mg body weight and sacrificed after 1 h incubation. The fat body was dissected out, homogenized and RNA extraction was carried out by the method of Fong and Fuchs (1976) as already mentioned above. The samples were counted in Beckman liquid scintillation counter (Model LS 3133 P-efficiency 40%) using 10 ml of Bray's solution.

Incorporation of H^3-leucine into TCA-precipitable material:

Incorporation of H^3-leucine into TCA-precipitable material was determined at 1 h after the injection. The insects were injected with 1 µCi/100 mg body weight. After 1 h incubation, the haemolymph was collected with the help of microcapillaries and diluted with cold distilled water. Various organs like ovaries and fat body were dissected out and homogenized (2%) in cold distilled water. The samples were precipitated by the addition of an equal volume of cold 10% TCA. The precipitate was centrifuged at 2000 g for 10 min. The resulting pellet was washed twice with cold 5% TCA, twice with ethanol, followed by one wash with 3:1 mixture of ethanol-ether. The pellet thus obtained was dissolved in tissue solubilizer (hyamine hydroxide) and counted in Beckman liquid scintillation counter (Model LS 3133 P), using a vial containing 10 ml of toluene-based scintillation fluid (4 g PPO, 0.2 g POPOP in 1 lit of toluene).

Polyacrylamide gel electrophoresis:

Fat body and ovaries were dissected out from the adult insects and were homogenized in cold distilled water.

The haemolymph samples were collected from the clipped end of the antennae of the insects in a capillary tube and diluted with cold distilled water, according to the requirement. After protein estimation, the appropriate amounts of the same samples were used for electrophoresis.

Polyacrylamide gel electrophoresis was carried out at 4°C, using 7% gels in 0.1 M Tris-0.039 M glycine buffer (pH 8.3) at a current of 3 m amp per gel (Davis, 1964). Staining of the gels was performed with Coomassie brilliant blue and destaining was done as described by Weber and Osborn (1969).

Histological and autoradiographic techniques:

For histological studies, the tissues were dissected out in insect Ringer and fixed in Bouin's fluid. Paraffin sections were cut at 5-7 µm and stained routinely in iron alum haematoxylin-eosin.

For autoradiographic investigations, the insects were injected with H^3-leucine (2 µCi/20 mg body wt.) as the precursor of protein. After varied incubation periods ranging from 30 min to 8 h, the insects were sacrificed.

The fat body and ovary were dissected out and fixed for 3 h in Carnoy's fluid. Paraffin sections (6 µm) were processed for autoradiography, using Ilford K_2 emulsion. The exposure time varied between 3-5 weeks. The emulsion coated slides were then developed in Kodak D 19 B developer. Autoradiographs were mounted in Zeiss L-15 mounting medium and examined under phase optics.

Staining of neurosecretory cells:

The brain and retrocerebral complex were dissected out in insect Ringer and fixed in Bouin's fluid. The whole preparations of the brain and retrocerebral complex were stained with paraldehyde-fuchsin (PF) technique, as modified by Bajra and Tandan (1964).

Measurement of corpus allatum volume:

The corpora allata were dissected out and taken in a drop of insect Ringer on a slide. The length and width were measured by occulometer and allatal volume was calculated using the equation $V = \pi/6\ d^2L$, according to Goodman et al., (1968), where V is volume, d is diameter and L is length.

Electron-microscopic studies:

Electron microscopic studies were carried out on corpora allata of experimental (precocene-treated) as well as control (acetone-treated) insects. The CA were dissected out and fixed in 2.5% glutaraldehyde in sodium-cacodylate buffer (pH 7.4) for 2 h at 4°C. After 2 h of repeated buffer wash, the materials were post-fixed in 1% osmium tetroxide for 1 h. Following dehydration, the materials were embedded in epon through propylene-oxide. Ultra-thin sections were contrasted with uranyl acetate and lead citrate, according to Reynolds (1963) and studied with Siemens Elmiscope 102 at an accelerating voltage of 60 K.V.

Statistical analysis:

All the data obtained in the study were statistically treated and the significance of difference between any two values was calculated according to Student's t-test.

MATERIALS AND METHODS

Laboratory reared insects of various age groups (ranging from 0 day to 6 days) were used for these studies. The source of chemicals is given in Chapter II. The nucleic acids of the fat body and ovary were extracted according to the procedure of Schmidt and Thannhauser (1945), slightly modified, as suggested by Munro (1966). The estimation of protein content and H^3-leucine incorporation studies were carried out as mentioned in Chapter II.

OBSERVATIONS

Changes in the weight of fat body and ovary during the first reproductive cycle:

The results obtained from this study can be visualised from Table I. It is seen that the wet weight of the fat body increased steadily up to 5 days and declined thereafter. The wet weight of the ovary was found to be low in freshly emerged females, increased gradually throughout the gonotropic cycle up to 6 days.

However, the increase was more pronounced in the later half of the cycle, mainly due to hectic vitellogenic activity.

Changes in the DNA and RNA content of the fat body and ovary:

The DNA and RNA contents of the fat body on different days of the first gonotropic cycle is presented in Table 2. It could be seen that the total DNA content of the fat body steadily increased up to 4 days, remained constant in 5 days old insects but declined slightly in 6 days old females. It may be noted that there was a significant increase in DNA between day 3 to 4. However, when the DNA content was expressed per mg tissue, it was found to be low up to day 1, but increased in 2 days old insects and remained more or less constant thereafter.

The total RNA content increased gradually and reached a high value at 5 days. Thereafter, a slight decrease was observed in 6 days old insects. The concentration of RNA per mg of fat body tissue increased up to 4 days. However, there was a slight decline in the RNA concentration at 5 days but it remained more or less at the same level in 6 days old females (Table 2).

Table 3 shows the DNA and RNA content of the ovary as a function of advancing age of the insect during the first reproductive cycle. The total DNA content of the ovary increased gradually up to 2 days, remained at the same level in 3 days old insects, but increased once again in still older females to reach the peak value in 6 days old insects. However, when the DNA content was expressed per mg of the ovary, the values diminished continuously from 0 day onwards up to 6 days and this is mainly due to a significant increase in the ovarian weight which is associated with the accumulation of large amounts of yolk protein.

The total RNA content of the ovary increased steadily up to 2 days and remained constant in 3 days old females but increased significantly once again in the second half of the reproductive cycle and reached a peak value at 6 days. Nevertheless, as in the case of DNA content of the ovary, the concentration of RNA per mg of ovary declined steadily from 0-day to 6-days.

Changes in the protein content of the fat body, haemolymph and ovary:

Table 4 shows the changes in the total protein content and the protein expressed per mg wet weight of

fat body, ovary and haemolymph. In the fat body the total protein content increased steadily up to 4 days. Thereafter, a decrease could be observed up to 6 days. The protein, as expressed per mg of wet weight, also increased up to 3 days and thereafter it has dropped to a lower plateau.

The haemolymph protein content was found to be high in freshly emerged females but declined drastically at 1 day and remained more or less constant up to 3 days. Again in 4 days old females there was a significant increase (89%) in the protein concentration of haemolymph and this remained high, up to 5 days. But once again it declined drastically in 6 days old insects, as it is being used up by the developing oocytes.

The total protein content of the ovary increased throughout the reproductive cycle so as to reach a high value in the 6 days old insects. It is to be noted that the increase was more pronounced in the second half of the cycle. The protein content, when expressed per mg wet weight of the ovary, remained low up to 2 days but increased later up to 5 days and remained more or less at the same level in 6 days old insects.

H^3-leucine incorporation into the TCA-precipitable proteins of fat body, haemolymph and ovary during the first reproductive cycle:

The pattern of H^3-leucine incorporation into the TCA precipitable proteins of the fat body, haemolymph and ovary has been set forth in the Table 5. The rate of incorporation was fairly high in the fat body of 2 and 3 days old insects but declined thereafter. The rate of incorporation of H^3-leucine into the haemolymph protein increased from 2 to 4 days but declined significantly in 5 days and a further decrease was observed in 6 days old insects.

The rate of H^3-leucine incorporation into the ovarian protein was high up to 3 days, decreased slightly in 4 days which was followed by a drastic decrease in 5 days old insects and remained more or less the same in 6 days old insects. The rate of incorporation of H^3-leucine per mg tissue follows the same pattern as the rate of synthesis i.e.,incorporation per mg protein.

Electrophoretic studies:

The electrophoretic study of the haemolymph samples of the male and female insects revealed the appearance of two new female-specific protein bands on the 3rd day after adult emergence (Fig. 1,c). It may be noted that these bands are conspicuously absent in the haemolymph samples of males of the same age group (see Fig.1,d). Furthermore, these bands are not in evidence in 1 and 2 days old females and make their appearance only on day 3 of the female gonotropic cycle. These haemolymph proteins showed a resemblance in their mobility to the two protein bands present in the ovary on 4th day in a more accentuated manner, as shown in Fig. 2.

Histology of the ovariole:

The ovary on each side is composed of seven ovarioles. Each ovariole is distinguishable into the apical terminal filament, followed by the germarium and vitellarium. The germarium is comprised of a large number of trophocytes which are densely packed into polygonal configurations. A small number of prefollicular cells and differentiated oocytes are found towards posterior end of

the germarium. The central part of the germarium shows a hyaline trophic core (Fig.3). As in all the Hemiptera, the present species also possesses the meroistic-telotroph ovarioles with distinct trophic cords issuing from the trophic core to the developing oocytes in the vitellarium (Fig.4).

The vitellarium is composed of large number of developing oocytes arranged in a serial order (Fig.5). During the first reproductive cycle, usually 9-11 oocytes undergo maturation simultaneously. Previtellogenic oocytes are characterised by the presence of a yolk-free ooplasma, a centrally placed germinal vesicle and the follicular envelope made up of close-fitting columnar epithelial cells bearing oval shaped nuclei (Fig.5). The germinal vesicle usually has a large number of nucleoli evenly distributed in the nucleoplasm (Fig.6). The follicle epithelium rests on a basement membrane, external to which a double layered peritoneal sheath is present (Figs.5 and 7). During the early vitellogenic stage, the histology does not change appreciably, excepting for the appearance of small yolk globules at the oocyte cortex (Fig.6). One often finds binucleate condition in follicle epithelial cells (Fig.8). In active vitellogenic follicles, the follicle epithelial cells assume cubical outline

(Figs.9 and 10). The ooplasma is replete with densely packed yolk spheres of various dimensions (Figs.9 and 10) and the germinal vesicle is displaced to a lateral position (Figs.6 and 8).

Autoradiographic studies of protein metabolism with H^3-leucine in vitellogenic egg follicles:

To study the vitellogenic activity, 3,4 and 5 days old insects were injected with H^3-leucine and autoradiographs were produced in the manner already described in the Chapter II dealing with Materials and Methods. In 3 days old insects, with 2 h incubation, a moderately strong radioactive zone at the follicle epithelium-oocyte interface was seen to develop (Fig.11) although with this incubation time, the radioactivity was still not detectable deep inside the oocyte. It was only with 4 h incubation that the radioactive yolk spheres begin to be pinched off from the oocyte cortex. However, the labelled yolk spheres were found only at the periphery of the oocyte and did not yet move far inwards (Fig.12). As the incubation period was extended to 8 h, the radioactivity of the follicle epithelial layer diminished slightly.

Autoradiographs obtained with H^3-leucine clearly showed that the rate of incorporation of yolk protein is much higher and more rapid in the 4 days old insect in comparison to 3 days. With 2 h incubation itself, one could see intense labelling of the follicle epithelium accompanied by the release of radioactive yolk spheres from the oocyte cortex (Fig.13). As the incubation was extended to 4 h, there was further increased intensity in the labelling of the yolk spheres (Fig.14).

The incorporation pattern in 5 days old insects however, presents a strikingly different picture (Figs.15 and 16). The follicle epithelium was moderately labelled while the chorion which is in the process of its formation, tends to be strongly radioactive (Fig.15). Also, the oocyte cortex, which became loosened and separated off from the chorion was strongly radioactive, but still deeper parts of the oocyte cortex, including the large yolk spheres, remained more or less unlabelled. This is apparently due to the cessation of the transport activity of the labelled molecules from the haemolymph to the oocyte across the follicle epithelium occasioned by the interpolation of the chorion (Fig.16). As the vitellogenesis was fully accomplished at this stage, there was

no evidence of large scale movement of radioactive substances into the deeper parts of the oocyte (Fig.16).

Plate I

Fig.1. Comparison of the electrophoretic patterns of haemolymph proteins on 1st (a), 2nd (b) and 3rd (c) day females with haemolymph proteins of 3rd (d) day male. Note the appearance of two new female specific proteins (→) in c in the haemolymph of 3 day old insects.

Fig.2. Comparison of the electrophoretic patterns of haemolymph (a) and ovarian (b) proteins of the 4th day females. Note the presence of two corresponding proteins (→1,2) with similar electrophoretic mobilities. In the ovary both these bands are greatly accentuated.

PLATE I

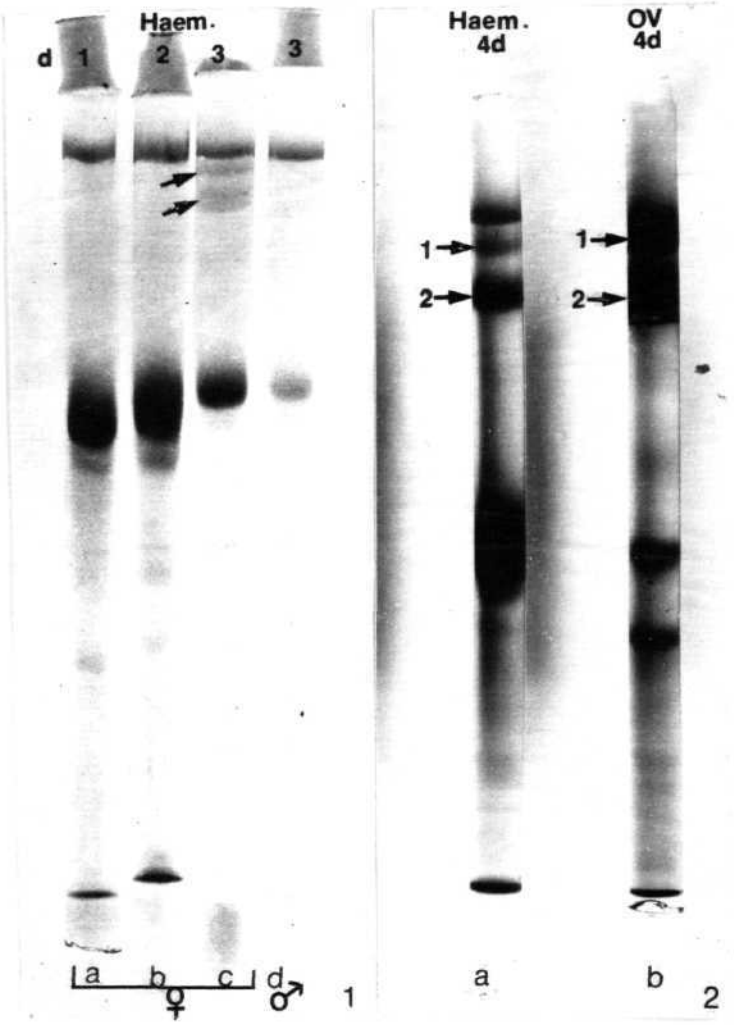


Plate II

Fig.3. Section of the germarium showing the densely packed trophocytes with their large polyploid nuclei (N) and well developed trophic core (TCo). Note the presence of large number of young oocytes (→) at the posterior end of the germarium. Bouin/Haemalum-Eosin.

x 375

Fig.4. L.S. of an ovariole showing the trophic cords (TC) reaching the oocyte. FE = Follicle epithelium. Bouin/Haemalum-Eosin.

x 480

Fig.5. L.S. of the vitellarium showing previtellogenic(I) and early vitellogenic(II) oocytes (OC) showing their columnar follicle epithelium (FE) which is bounded externally by a double layered peritoneal epithelium (PE). Bouin/Haemalum-Eosin.

x 320

Fig.6. An oocyte (OC) where the yolk formation has just commenced. Note the presence of finely distributed chromatin material in the germinal vesicle (GV). TC = Trophic cord, FE = Follicle epithelial cells. Bouin/Haemalum-Eosin.

x 480

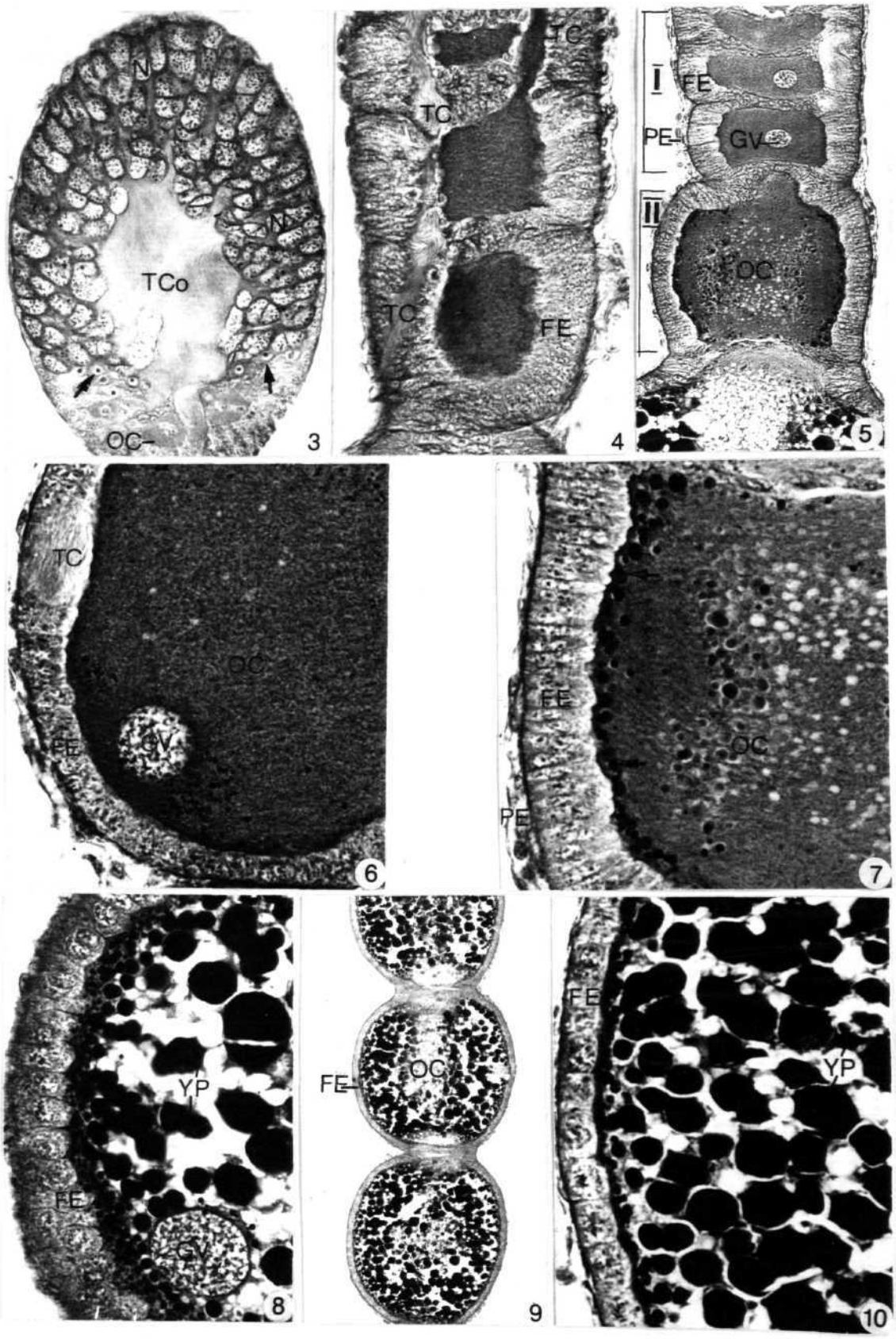
Fig.7. Section of an early vitellogenic oocyte, showing columnar follicle epithelial cells (FE), which is externally lined by a double layered peritoneal epithelial (PE) sheath. Note the presence of large number of small yolk droplets (→) at the periphery of the oocyte (OC). Bouin/Haemalum-Eosin. x 560

Fig.8. Active vitellogenic oocyte showing the accumulation of large number of darkly stained yolk platelets (YP). Note the presence of binucleate (→) follicle epithelial cells (FE) and eccentric position of the germinal vesicle (GV). Bouin/Haemalum-Eosin.

x 560

Figs.9 and 10. Section of late vitellogenic oocytes (OC) showing the advancement of vitellogenesis, with concomitant change in the shape of follicle epithelial cells (FE), which are now more or less cuboidal in shape. The ooplasma is filled with massive numbers of large yolk platelets (YP), Bouin/Haemalum-Eosin. Fig. 9 x 120
Fig.10 x 480

PLATE II


N
M
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7
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YP
GV
8
FE
OC
9
FE
YP
10

TABLE 1

Changes in the wet weight of the fat body and ovary during the first reproductive cycle.

Age	Fat body (mg)/Insect	Ovary (mg)/Insect
0 day (8)	1.97 ± 0.50	1.25 ± 0.10
1 day (9)	3.87 ± 0.60	1.89 ± 0.20
2 days (9)	4.47 ± 1.00	3.03 ± 0.60
3 days (7)	5.31 ± 1.10	3.18 ± 0.60
4 days (10)	9.13 ± 1.20	16.60 ± 4.20
5 days (9)	10.56 ± 0.70	26.78 ± 3.10
6 days (11)	7.11 ± 1.20	48.15 ± 2.80

The values represent means ± S.D. of the number of determinations given in parentheses. For each estimation, tissue from 15-20 insects were pooled together.

PLATE III

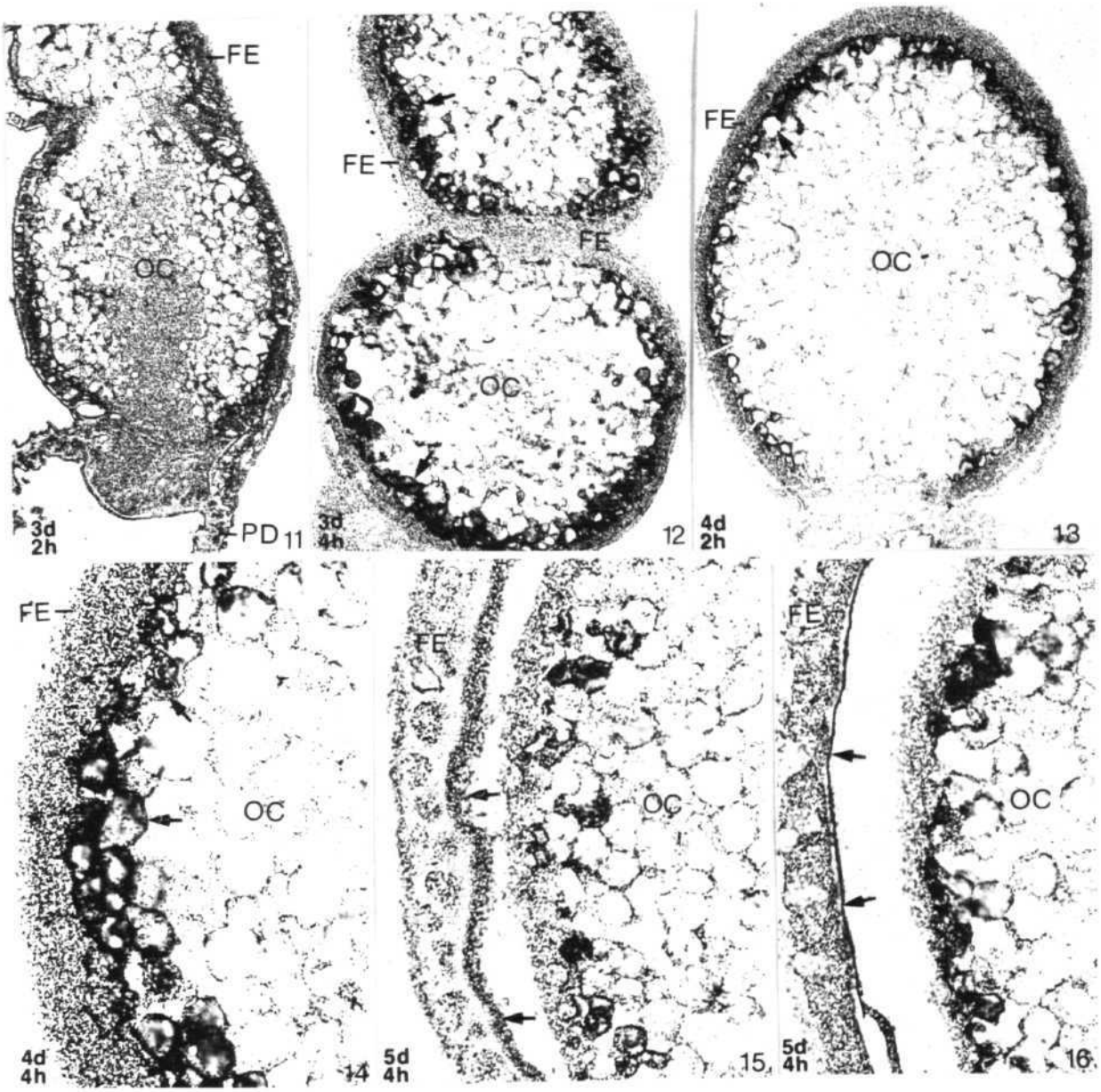

FE
OC
PD
3d 2h
11
FE
FE
OC
3d 4h
12
FE
OC
4d 2h
13
FE
OC
4d 4h
14
FE
OC
5d 4h
15
FE
OC
5d 4h
16

TABLE 3

Changes in the DNA and RNA content of the ovary during the first reproductive cycle.

Age	DNA (µg)		RNA (µg)	
	/Insect	/mg ovary	/Insect	/mg ovary
0 day (4)	4.46 ± 0.31	3.85 ± 0.21	17.68 ± 1.31	13.91 ± 0.56
1 day (4)	5.26 ± 0.82	3.35 ± 0.23	22.20 ± 1.22	12.64 ± 0.36
2 days (5)	10.42 ± 2.70	3.43 ± 0.32	34.60 ± 6.12	12.06 ± 0.91
3 days (5)	9.60 ± 0.41	3.08 ± 0.62	34.68 ± 4.04	10.42 ± 0.52
4 days (5)	25.53 ± 5.23	2.02 ± 0.29	87.96 ± 8.63	7.56 ± 1.34
5 days (5)	59.07 ± 6.21	2.17 ± 0.30	194.54 ± 27.81	7.49 ± 1.53
6 days (5)	89.02 ± 10.84	1.97 ± 0.27	339.07 ± 29.24	6.95 ± 0.53

The values represent means ± S.D. of the number of determinations given in parentheses. The tissue from 15-20 insects was pooled for each estimation.

Changes in the protein content of the fat body, haemolymph and ovary during various days of the first reproductive cycle.

Age	Fat body protein (µg)		Haemolymph protein (µg)	Ovary protein (µg)	
	/ Insect	/ mg tissue	/ µl	/ Insect	/ mg tissue
0 day (8)	59.00 ± 10.50	30.45 ± 5.30	42.26 ± 3.90	72.68 ± 11.30	58.17 ± 9.00
1 day (7)	149.10 ± 11.10	39.86 ± 4.00	26.56 ± 5.40	106.50 ± 8.80	57.31 ± 7.10
2 days (9)	199.50 ± 22.50	44.68 ± 5.00	25.73 ± 2.80	147.00 ± 7.90	59.31 ± 7.20
3 days (7)	267.50 ± 8.90	50.48 ± 1.60	29.43 ± 3.50	202.30 ± 23.40	64.63 ± 7.40
4 days (10)	445.40 ± 46.40	48.74 ± 5.80	55.62 ± 4.00	1698.00 ±161.40	102.67 ± 9.70
5 days (9)	397.52 ± 13.20	38.59 ± 6.00	48.67 ± 9.00	3666.00 ±140.90	135.34 ± 7.40
6 days (10)	284.70 ± 31.70	39.05 ± 7.10	29.67 ± 6.10	6322.00 ±213.10	129.86 ± 6.50

The values represent means ± S.D. of the number of determinations given in parentheses. The tissue from 15-20 insects was pooled for each estimation.

TABLE 5

H^3-leucine incorporation into the proteins of fat body, haemolymph and ovary during the first reproductive cycle.

Age	Fat body		Haemolymph		Ovary	
	CPM/mg tissue	CPM/mg protein	CPM/ml	CPM/mg protein	CPM/mg tissue	CPM/mg protein
2 days (9)	933 ±155	17,289 ± 2,347	1,31,143 ± 24,823	5,986 ± 730	642 ± 110	11,852 ± 1,794
3 days (8)	912 ±240	16,866 ± 1,640	3,90,640 ± 60,766	13,331 ± 4,641	956 ± 175	12,233 ± 1,641
4 days (9)	581 ± 43	11,522 ± 2,511	10,45,733 ± 1,60,229	34,724 ± 7,068	1022 ± 156	9,847 ± 1,900
5 days (9)	554 ± 45	9,227 ± 1,239	8,82,160 ± 1,98,507	15,538 ± 1,535	469 ± 108	2,910 ± 415
6 days (8)	287 ± 51	5,260 ± 1,077	1,73,280 ± 48,677	10,296 ± 2,796	241 ± 51	2,224 ± 284

The values represent the means ± S.D. of the number of determinations given in parentheses. The tissue from 3-5 insects was pooled for each determination.

CHAPTER IV

Effect of actinomycin-D on the fat body RNA content

MATERIALS AND METHODS

The source of chemicals and insects is given in Chapter II. Actinomycin-D was dissolved in water and diluted according to the requirements. Different dosages of actinomycin-D (0.1 µg, 0.25 µg, 1.0 µg and 2.5 µg/ Insect) were injected into the body cavity of insects of different age groups with 10 µl volume. The injections were given with the help of microsyringe piercing through the base of metathoracic leg. The control insects received an equal volume of the carrier. Both the control and experimental insects were sacrificed 24 h after treatment. The RNA and protein contents of the fat body were determined according to the method of Fong and Fuchs (1976), described in Chapter II.

OBSERVATIONS

Effect of actinomycin-D treatment on the fat body RNA content:

The effect of 24 h treatment with different dosages of actinomycin-D, on the weight of fat body in insects of various age groups is presented in Table 1. Two days old insects, when treated with actinomycin-D, did not show any variation in the weight of the fat body and it remained more or less the same in experimental as well as control insects. Injection of different dosages of actinomycin-D in 3 days old insects caused a decline in the fat body weight in a dose-dependent manner. However, a similar treatment given to the 4 days old insects did not reveal any effect on the weight of the fat body. When 5 days old insects were treated with actinomycin-D, the fat body weight was fairly high in experimental insects.

Table 2 shows the changes in the total RNA as well as the RNA expressed per mg of wet weight after actinomycin-D treatment. In 2 days old insects, the actinomycin-D treatment had no effect even with the highest

dose (2.5 µg/insect). Actinomycin-D at a dose of 0.1 µg/insect caused a slight decrease (20%) in the total RNA content in 3 days old insects and the effect was more pronounced with higher dosages. It is to be noted that 1 µg/insect dose produced a significant decrease (68%) in the total RNA content in 3 days old insects. The concentration of RNA per mg of fat body also showed a similar pattern. When 4 days old insects were treated with actinomycin-D at a dose of 0.1 µg/insect, it showed no effect on the total RNA content, which declined gradually with higher dosages. A similar effect was found when the RNA values were expressed per mg of fat body. The actinomycin-D treatment had more or less no effect in the 5 days old insects because the RNA values remained the same as in the controls.

The effect of actinomycin-D on the protein content is given in Table 3. Here also, the maximum effect was found in the 3 days old insects.

TABLE 1

Effect of 24 h actinomycin-D treatment on the weight of the fat body.

Age	Control	Actinomycin-D (μg) dosage/Insect.			
		0.1	0.25	1.0	2.5
2 days (5)	5.42 ± 0.91	6.11 ± 0.31	6.05 ± 1.12	6.15 ± 0.60	6.52 ± 0.31
3 days (7)	9.19 ± 1.83	8.04 ± 0.70	7.61 ± 1.22	7.23 ± 2.41	6.85 ± 1.30
4 days (7)	10.74 ± 0.41	9.17 ± 1.41	10.45 ± 1.90	10.83 ± 0.93	10.32 ± 2.12
5 days (3)	7.78 ± 1.01	9.85 ± 0.72	10.57 ± 0.70	9.13 ± 1.84	9.66 ± 1.52

All the values are the means ± S.D. of the number of determinations given in parentheses and expressed as mg fat body/insect. The fat body pooled from 4-5 insects was used for each determination.

TABLE 2

Effect of 24 h actinomycin-D treatment on the RNA content of the fat body.

Age	Control		Dosage of Actinomycin-D (µg) /Insect							
			0.1		0.25		1.0		2.5	
	/Insect	/mg tissue	/Insect	/mg tissue	/Insect	/mg tissue	/Insect	/mg tissue	/Insect	/mg tissue
2 days (5)	51.03 ± 5.38	9.67 ± 1.43	43.68 ± 8.90	7.70 ± 0.91	53.16 ± 11.12	9.11 ± 0.73	39.15 ± 6.84	5.90 ± 0.72	45.68 ± 5.44	6.98 ± 0.51
3 days (7)	124.79 ± 22.93	13.61 ± 2.42	104.15 ± 12.85	12.89 ± 2.80	54.07 ± 7.85	8.34 ± 0.35	36.53 ± 5.92	7.12 ± 0.31	46.07 ± 11.00	6.51 ± 0.34
4 days (7)	131.79 ± 17.26	14.87 ± 1.81	135.46 ± 16.48	13.65 ± 0.51	115.30 ± 15.35	10.66 ± 0.62	101.02 ± 10.41	9.42 ± 1.51	102.94 ± 12.43	8.90 ± 0.92
5 days (3)	113.07 ± 4.42	12.20 ± 3.32	108.66 ± 10.28	11.03 ± 1.10	109.81 ± 12.62	9.66 ± 1.23	106.19 ± 8.00	12.19 ± 2.84	115.80 ± 8.51	10.14 ± 0.91

All the values are the means ± S.D. of the number od determinations given in parentheses and expressed as µg of RNA. The fat body was pooled from 4-5 insects for each determination.

TABLE 3

Effect of 24 h actinomycin-D treatment on the protein content of the fat body.

Age	Control		Actinomycin-D (µg)/Insect					
			0.25		1.0		2.5	
	/Insect	/mg tissue	/Insect	/mg tissue	/Insect	/mg tissue	/Insect	/mg tissue
2 days(5)	233.61 ± 24.32	36.07 ± 10.73	236.60 ± 31.75	34.95 ± 4.31	229.43 ± 24.73	36.40 ± 2.04	214.86 ± 85.14	36.65 ± 4.11
3 days(7)	389.91 ± 56.82	51.69 ± 4.63	268.58 ± 29.00	31.83 ± 2.24	299.13 ± 18.41	42.97 ± 4.26	259.91 ± 14.34	37.16 ± 0.91
4 days(7)	402.14 ± 9.00	41.10 ± 2.13	301.29 ± 91.90	25.13 ± 5.92	328.44 ± 51.57	30.15 ± 1.64	373.41 ± 24.00	35.75 ± 0.80
5 days (3)	472.52 ± 38.15	50.98 ± 9.10	429.25 ± 41.61	39.06 ± 5.78	403.51 ± 64.03	41.29 ± 9.06	407.83 ± 17.54	39.77 ± 3.38

All the values are the means ± S.D. of the number of determinations given in parentheses and expressed as µg of protein. The fat body was pooled from 4-5 insects for each determination.

CHAPTER V

Effect of precocene-II on the neuroendocrine regulation of reproduction.

MATERIALS AND METHODS

The source of chemicals and insects used, was already given in Chapter II. Precocene-II was dissolved in acetone (5 mg/ml) and was applied topically to newly emerged females (within 10-15 min of emergence), at a dosage of 50 µg/insect which was found to be the optimal dose to bring about total inhibition of egg-maturation with the least mortality rate. The topical application was made on the dorsal surface of the abdomen below the wings, with the help of a microsyringe (TOP Company, Bombay). The control females were treated with an equal volume of acetone. All these insects were maintained in a glass container on soaked cotton seeds for the required duration together with an equal number of males. The methods used for extraction and estimation of RNA, H^3-uridine incorporation and determination of protein content were the same as described in Chapter II.

Precocene as well as acetone-treated 5 days old control insects were injected with H^3-leucine (2 µCi/20 mg body wt.) and incubated for 4 h. The ovaries were dissected, fixed at the end of 4 h and were processed for autoradio-

graphic studies. Paraldehyde-fuchsin staining was performed on the whole brains dissected out from 4 and 6 days old experimental and control insects to visualise the distribution of neurosecretory material. For electron microscopic studies, the corpora allata of precocene and acetone treated 6 days old insects were used. To study the recovery effect of exogenous JH-III on precocene treated animals, JH-III dissolved in acetone, was topically applied to precocene-primed 3 days old insects at two different dosages of 5 and 10 µg/insect. The precocene treated insects of the same age group were used as controls. The insects were sacrificed on day 8 and the protein content of the ovary was estimated, according to the method of Lowry et al. (1951).

In order to study the effect of exogenous JH-III on RNA metabolism in the fat body, JH-III was dissolved in acetone and applied topically to the 2 days old female insects in various dosages ranging from 5-15 µg. The insects were sacrificed 24 h after JH III treatment and the RNA of fat body was extracted by the method of Fong and Fuchs (1976) and estimated with the help of orcinol reaction.

OBSERVATIONS

Effect of precocene on the body weight and egg maturation:

Table 1 shows the changes in the body weight and ovarian weight in acetone and precocene treated insects. The acetone treated 6 days old insects have a much higher body weight (105 ± 12.05 mg/insect) in comparison to the precocene treated insects (60 ± 5.22 mg/insect) and this is more or less equal to the body weight found in 3 days old normal insects (62 ± 3.71 mg/insect). The ovarian weight of 6 days old acetone treated insects is fairly high and is nearly 15 times greater, as compared to the precocene treated insects of the same age group. The ovarian weight of precocene treated 6 days old insects is nearly equal to that of the 3 days old normal insects. This clearly indicates the deleterious effect of precocene on the ovarian weight.

Plate IV shows the effect of precocene treatment (50 µg/insect) on the general appearance of the insect. Acetone treated insects (Fig.1), usually possess a bulged abdomen which is largely due to the unhindered normal

ovarian development. On the other hand, precocene treated insects (Fig.2) show a narrow and flat abdomen whose dimensions and appearance are closely similar to the abdomen of 3 days old normal insects (Fig.3).

Dissected ovaries of 6 days old precocene treated females, remained considerably small and contained undifferentiated eggs, as compared to acetone-treated control insects, wherein the ovarioles showed the presence of a series of well differentiated mature oocytes (cf.Fig.4a and b). The ovaries of the precocene treated 6 days old insects appear to be about the same as those of 3 days old normal immature insects (cf.Fig.5a and b). This indicates that precocene has effectively blocked the normal ovarian growth and differentiation. Examination of histological preparations of the ovarioles showed that in the ovaries of control insects, there is a uniformly active vitellogenesis corresponding to the histological pictures of vitellogenic follicles shown in Figs. 8-10 (Plate II). However, in the ovaries of experimental insects, vitellogenesis was inhibited and the oocytes were devoid of yolk platelets and they remained fairly small in size. These gross morphological and histological observations on the ovaries were confirmed by tracer studies also, using

H^3-leucine as the precursor for yolk proteins. Control insects showed the appearance of several radioactive yolk spheres at the oocyte cortex in all the vitellogenic follicles. However, with 4 h incubation period, the labelled yolk droplets were mainly confined to the periphery of the oocyte and did not yet move far inwards (Figs.6 and 7). The follicle epithelium also showed moderate labelling (Fig.8). With the same incubation time, the incorporation patterns in precocene treated insects, however, presented a strikingly different picture (Figs.9,10 and 11). The follicle epithelium showed a moderate labelling but the ooplasm was practically devoid of labelled yolk droplets (Fig.10) and showed in some of the younger oocytes a diffuse non-specific radioactivity. The terminal follicle of acetone treated insects was fairly large and showed the presence of large number of proteid yolk globules (Fig.6) suggesting hectic vitellogenic activity, whereas in the precocene treated females even the terminal follicles remained smaller, with homogenous practically unlabelled ooplasm and with few lipid yolk droplets but no proteid yolk spheres at all (cf.Figs. 6 and 11).

Effect of precocene on the weight and the RNA content of the fat body:

Table 2 shows the effect of precocene treatment on the weight and the RNA content of the fat body, after different durations of treatment. The weight of the fat body was found to be low in precocene treated 1 day old insects. This increased gradually and reached its highest value in precocene treated 6-days old insects. On the other hand, in the control insects, the fat body weight was initially low up to 3 days but increased significantly (2 fold) at 4 days and remained more or less at the same level up to 6 days. The total RNA content of the fat body in precocene treated 1 day old insects was fairly low (50%) when compared with control insects. In precocene treated insects, a gradual increase was found up to 3 days, which remained nearly at the same level thereafter. However, in acetone control insects, it increased from day 2 to 3. Once again, a significant increase (2 fold) in total RNA content was observed in 4 days old control insects. Thereafter, a slight decrease was observed in 5 days old insects which remained nearly at the same level up to 6 days. Acetone control insects showed a gradual increase in the RNA content when expressed per mg tissue

up to 3 days, which shot up to a significant level (38%) at 4 days, but declined slightly in 5 days old females and remained nearly constant in 6 days old insects. In the case of precocene treated insects, the RNA content, when expressed per mg tissue, increased gradually from 1 to 3 days and declined gradually thereafter till 6 days.

Effect of precocene on H^3-uridine incorporation into the fat body RNA:

The effect of precocene (50 µg/insect) on the H^3-uridine incorporation into the fat body RNA is presented in Table 3. The rate of RNA synthesis was found to be high in 1 and 2 days old insects but declined gradually up to 6 days and it showed more or less the same pattern in experimental as well as control insects during the first gonotropic cycle. But the total RNA synthesised in the fat body varied conspicuously in experimental and control insects. The total RNA synthesis was fairly low (50%) in precocene treated 1 day old insects in comparison to controls, but it was found to be more or less the same in 2 and 3 days old experimental and control insects. However, the total RNA synthesis was significantly low

(approximately 40%) in 4 days old experimental insects in comparison with the controls and this is mainly due to the diminished quantity of fat body. Thereafter, the total RNA synthesis declined gradually up to 6 days in precocene treated insects. Even in controls, the total RNA synthesised, has declined significantly from day 5 to day 6.

Effect of precocene on the protein content of the fat body:

Protein content of the fat body in precocene treated and acetone control insects are set forth in Table 4. The total protein content of the fat body was fairly low in precocene treated insects throughout the first reproductive cycle as compared to the control insects and this difference was more pronounced in the second half of the cycle. When the protein content was expressed in terms of per mg tissue, it was found to be higher in 1 day old control insects than in precocene treated insects. However, the pattern remained more of less the same in 2 to 6 days old experimental and control insects.

Changes in the protein content of haemolymph after precocene treatment:

The data obtained on the effect of precocene treatment on the haemolymph protein content has been presented in Table 5. One day old experimental as well as control insects showed a high haemolymph protein content which declined in 2 days old insects. In precocene treated 3 days old insects, a further decrease was found but it again increased slightly on day 4 and remained nearly constant up to 6 days. On the other hand, 3 days old control insects showed more or less the same protein as in 2 days old insects. However, it shot up significantly (2.5 fold) in 4 days old insects and remained more or less the same in the 5 days old insects but decreased drastically in 6 days old controls.

Effect of precocene on the neurosecretory cells of the brain:

Two groups of median neurosecretory cells occur medio-dorsally in the pars intercerebralis of the protocerebral lobe of the brain. They consist of 9 cells on each side of the pars intercerebralis and they are

prominently stainable with paraldehyde-fuchsin technique (PF). In precocene treated 4 days (Fig.14) and 6 days (Fig.15) old insects, the cells are intensely stainable with purple colour. They have a large amount of cytoplasm filled with abundant neurosecretory granules with a relatively inconspicuous nucleus (Figs.14 and 15). On comparing with the same kind of preparations of acetone treated control insects of the same age group, it is seen that the cells are faintly stained with PF and the cytoplasm of cells shows the presence of only a small quantity of neurosecretory material (Figs.12 and 13). They bear conspicuously visible cell nuclei. These illustrations convincingly demonstrate that precocene is interfering with the release mechanism, leading to their massive accumulation in the perikarya of the neurosecretory cells. In the acetone controls there is a rapid turnover of the neurosecretory material.

Effect of precocene-II on the volume of corpus allatum (CA):

In acetone treated insects the CA showed remarkable fluctuations in its volume during the first reproductive cycle (Table 6). The CA volume increased gradually from 1 to 4 days in control insects but declined thereafter,

whereas, the CA volume did not show such marked fluctuation and remained at a low level in precocene-treated insects throughout the first reproductive cycle. In general, the CA of the experimental groups of insects of all ages, revealed lower values of their volume as compared to acetone treated controls. This indicates that precocene has a definite deleterious effect on the allatal growth.

Electron microscopic observations on the corpus allatum:

How the volume changes in CA caused by precocene treatment, reflect themselves in the ultrastructural organisation of the allatal cells was investigated with the electron microscope.

Acetone treated control insect:

The gland cells rest externally on a basement membrane of moderate thickness (Fig.16). The cell nuclei are branched. In the cytoplasmic space, several inclusions are noticeable. These include rough endoplasmic reticulum, mitochondria, golgi vesicles and numerous free ribosomes (Fig. 17).

The rough endoplasmic reticulum is often present in form of stacks or cisternae (Fig.17). The ribosomes may sometimes form dense aggregates and exhibit polysomal configuration (Fig.18). Typical Golgi lamellae found in the vertebrate cells are not present here. But few vesicular type of Golgi bodies are seen scattered in the cytoplasmic space, sometimes very close to the rough endoplasmic reticulum (Fig.17). The cytoplasm shows the presence of large number of evenly distributed mitochondria of ordinary size with well marked cristae (Fig. 18). The cytoarchitecture reveals the picture of all cell organells associated with active secretory cells.

Precocene treated insect:

Ultrastructural studies revealed a number of degenerative changes in the allatal cells of precocene treated insects. The basement membrane became loose, disorganised and detached from the cellular layer (Fig.19). Mitochondria tend to become aggregated but they retain, by and large, their internal organisation (Fig.20). Cisternae like organisation of rough endoplasmic reticulum is also not visible any more. The most conspicuous feature is the presence of large number of intracellular

vacuoles some of which contain electron dense material (Fig.21). These may be the autophagic vacuoles and/or the multivesicular bodies associated with primary lysosomes. Such structures are not detectable in the CA cells of acetone treated controls.

Effect of exogenous JH on precocene treated insects:

The precocene primed (50 µg/insect were topically administered two dosages of JH-III at 5 and 10 µg/insect on the 4th day after precocene treatment. As shown in Table 7, when 5 µg JH was applied, there was a 3 fold increase in ovarian weight and a 4 fold increase in total ovarian protein, in comparison with that of precocene control insects. However, 10 µg JH had less pronounced effect than 5 µg treatment.

Effect of exogenous JH on fat body RNA of normal insects:

The effect of JH on the fat body RNA in 2 days old insects, after 24 h treatment, can be seen from the data given in Table 8. The weight of the fat body increased significantly (43%) in 5 µg JH treated insects, as compared to the acetone treated controls. In those

insects treated with 10 and 15 µg JH the weight of the fat body remained more or less the same as in acetone controls. The total RNA content of the fat body per insect increased by about 64% in 5 µg JH treated insects when compared to the controls, whereas only a slight increase was observed in case of 10 and 15 µg JH treated insects. The RNA concentration (/mg fat body) also followed the same pattern as that of total RNA content.

Plate IV

Figs.1-3. Macrophotographs of 6 days old females show the bulged abdomen due to ovarian maturation in acetone treated control Fig.2 illustrates the effect of precocene on the general body appearance. Note the presence of a flat abdomen in precocene treated 6 days old insects which resembles closely that of the 3 days old normal immature insects (Fig.3).

x 60

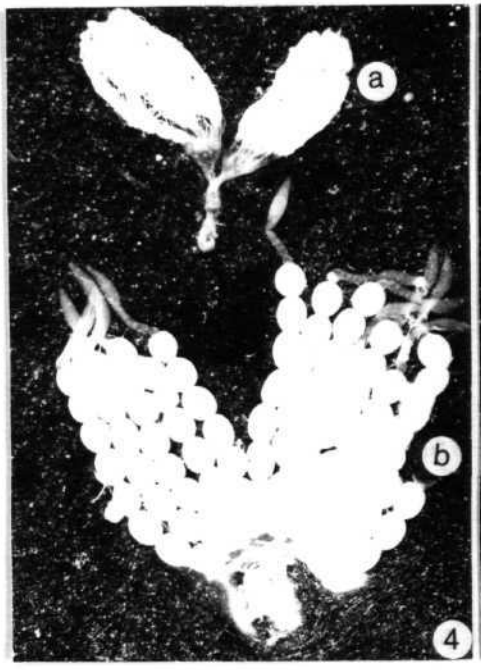
Fig.4. Shows a macrophotograph of the female internal reproductive systems of precocene-treated (a) and acetone treated (b) 6 days old insects as they appear in the dissected condition. Note the presence of well developed mature oocytes in acetone controls, while the ovaries in (b) appear degenerate.

x 75

Fig.5. Illustrates the comparison of internal reproductive system of precocene treated 6 days (a) old insects with 3 days old normal insects (b). They appear roughly to be equal.

x 75

PLATE IV



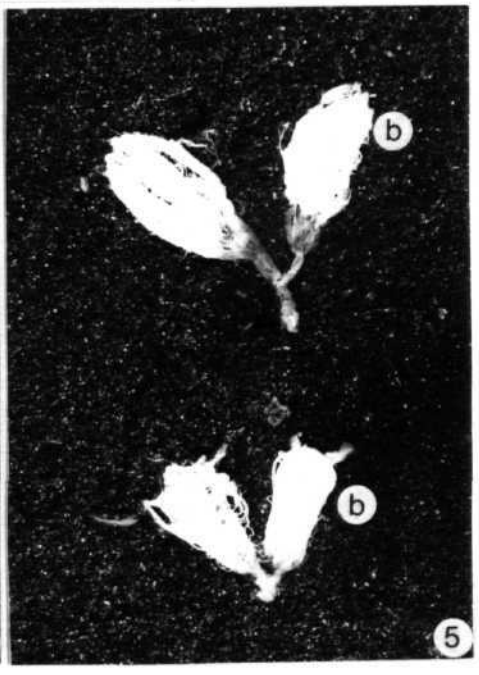
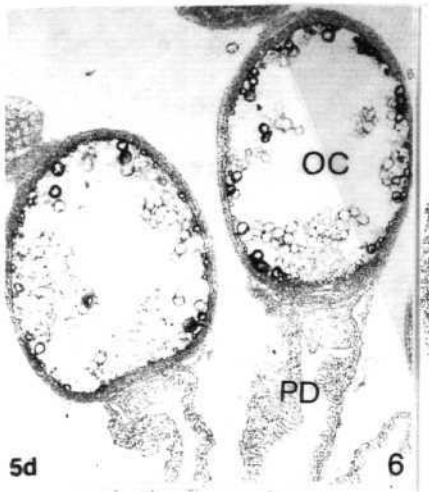


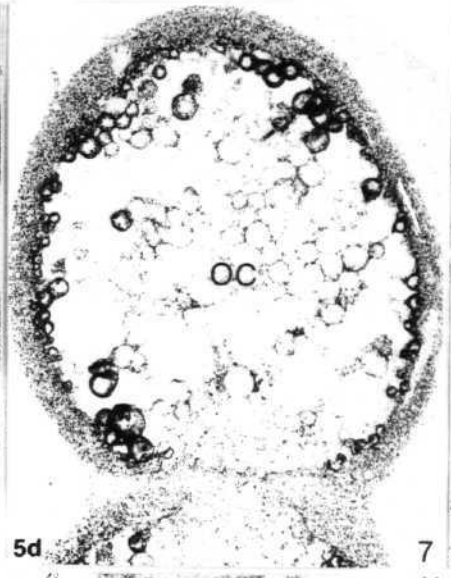
Plate V

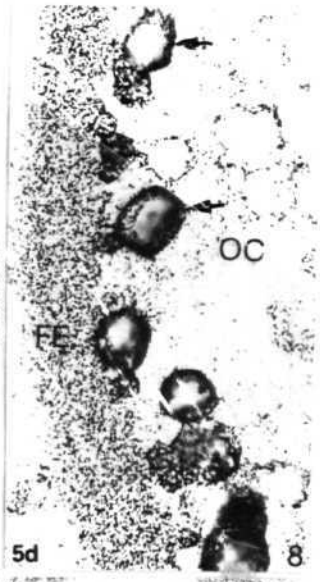
Figs.6-11. Illustrate the pattern of H^3-leucine incorporation in acetone (Figs.6-8) and precocene (Figs.9-11) treated 5 days old insects with 4 h incubation. Note the large number of labelled yolk droplets (→) at the cortex of the terminal (Fig.6) and other developing oocytes (Figs.7 and 8) in acetone treated 5 days old insects. On comparing the acetone autoradiograms with those of precocene (Figs.9-11), it is seen that there is a total absence of radioactive yolk spheres at the follicle cell/oocyte interface, suggesting the cessation of vitellogenin deposition in the oocyte (OC). PD = pedicel.

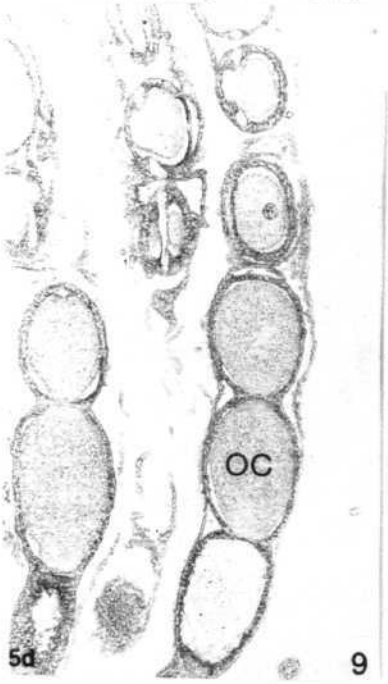
Figs.6 and 9 x 160
Figs.7 and 10 x 320
Fig. 8 x 480
Fig.11 x 375

PLATE V









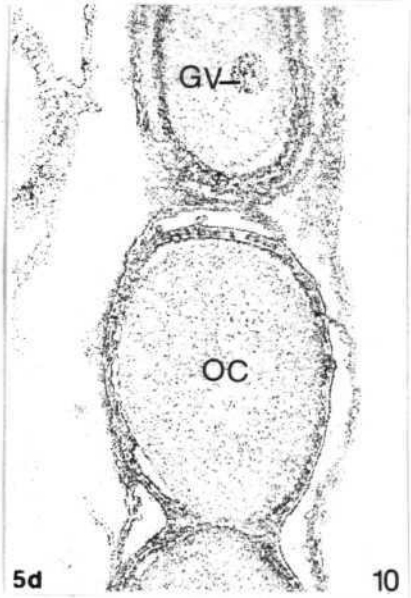


Plate VI

Figs.12-13. While preparations of brain showing the median neurosecretory cells of acetone treated 4 (Fig.12) and 6 (Fig.13) days old insects, and precocene treated 4 (Fig.14) and 6 (Fig.15) days old insects. Note the small amount of neurosecretory colloids in perikarya of acetone treated insects (Figs. 12 and 13) indicating a rapid turnover while the precocene treated (Figs.14 and 15) insects show the accumulation of large amount of colloid in the perikarya probably due to the inhibition of release. N = Nucleus, CY = Cytoplasm. Bouin/Aldehyde Fuchsin.

x 520

PLATE VI

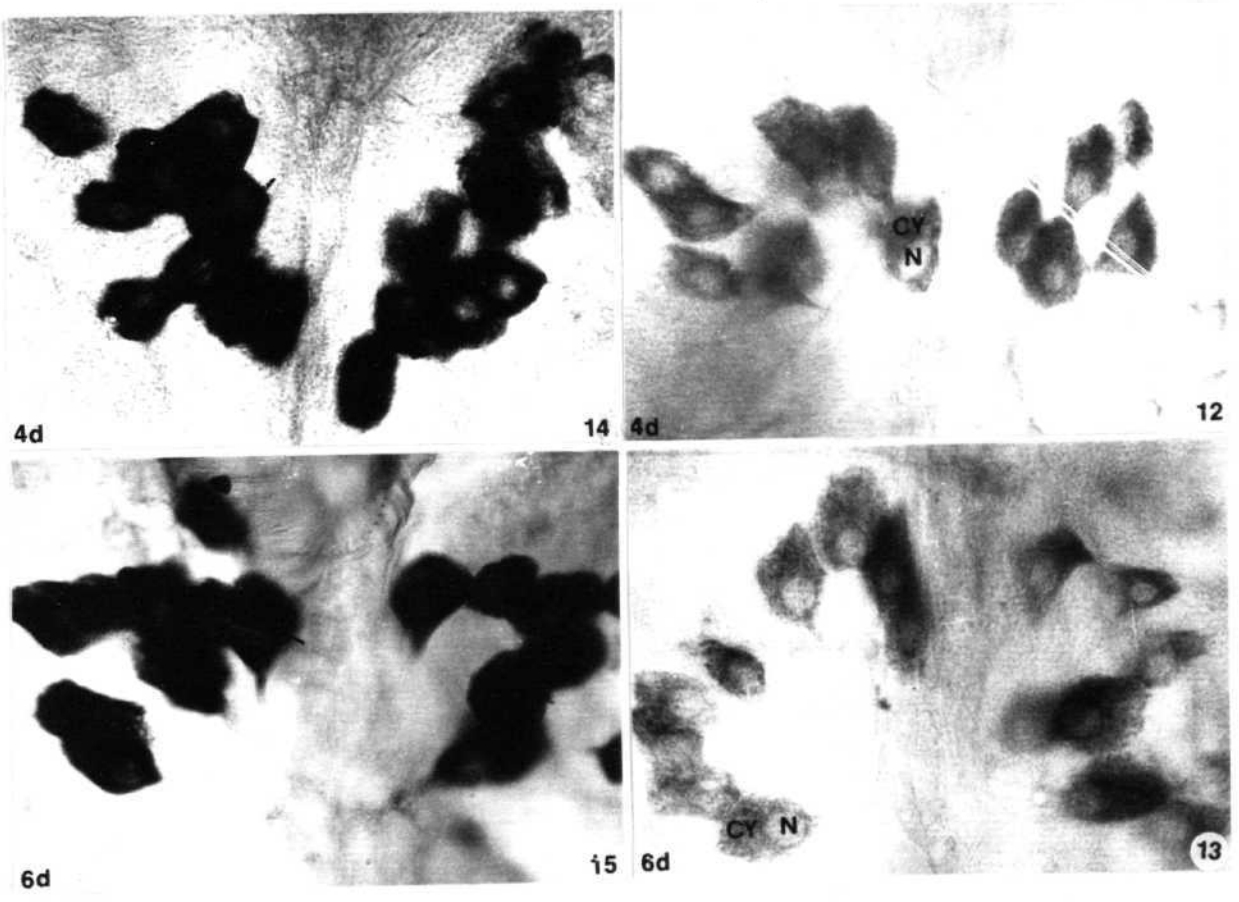
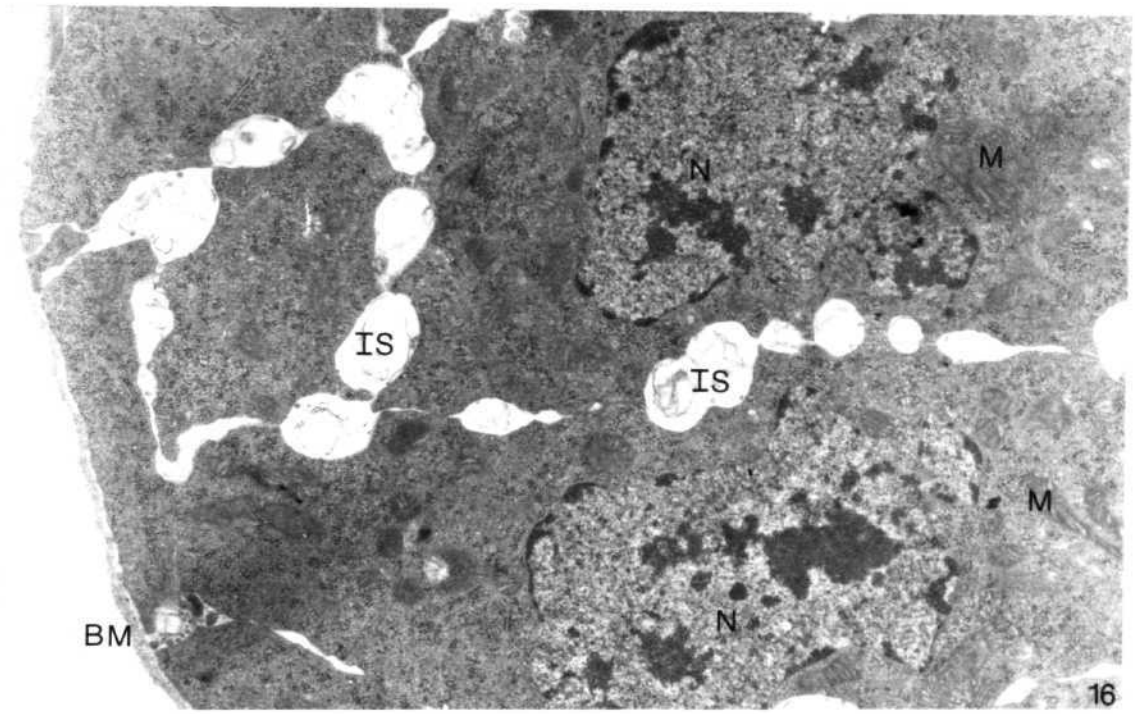


Plate VII

Figs.16 and 17. Electron micrographs of the corpus allatum of acetone treated 5 days old insects, depicting the gland cells closely adhering to the basement membrane (BM). The cells show intercellular spaces (IS) in Fig.16, mitochondria (M) in Figs.16 and 17, nucleus (N) with several nucleoli in Fig.16. The rough endoplasmic reticulum exists as stacks of cisternae in close association with Golgi vesicles (GV) (Fig.17).

PLATE VII



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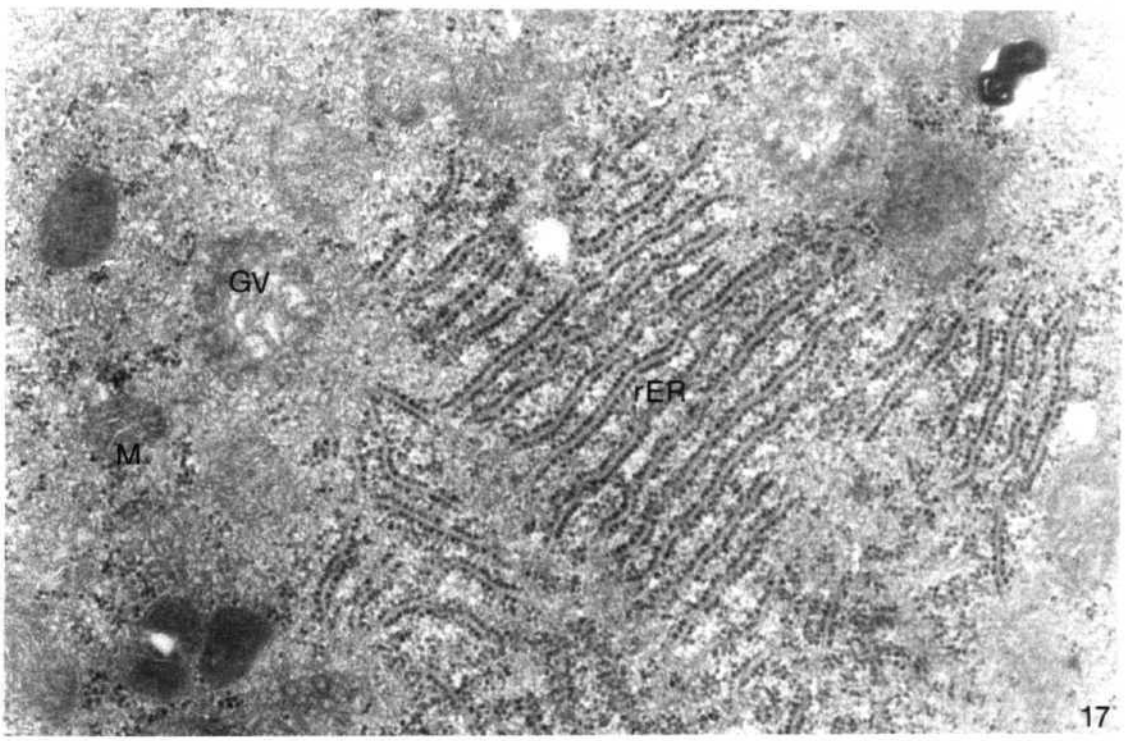

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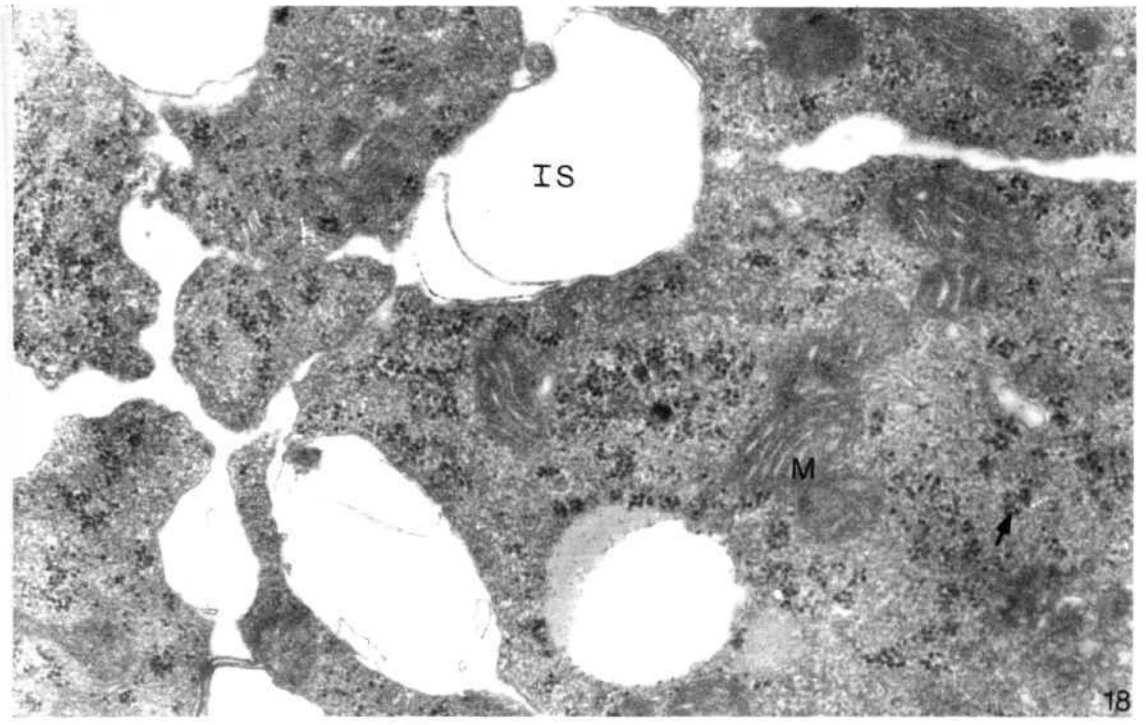
Plate VIII

Fig.18. Electron micrograph of gland cells of the corpus allatum of acetone treated 5 days old insects. Picture shows the presence of intercellular spaces (IS), mitochondria (M) and numerous polysomes (→).

Fig.19. Electron micrograph of gland cells of the corpus allatum of precocene treated 5 days old insects, showing the detached basement membrane (BM) which becomes disorganised, clustered mitochondria (M). The cisternae-like organisation of rough endoplasmic reticulum (rER) is no longer in evidence.

PLATE VIII



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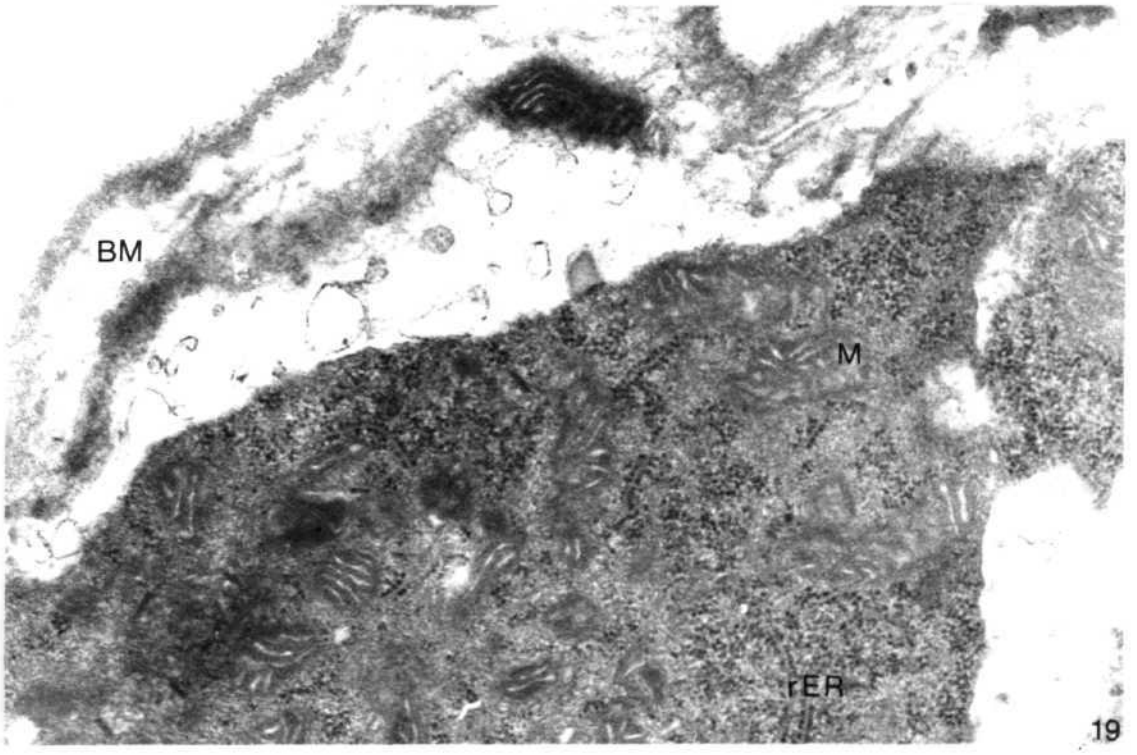

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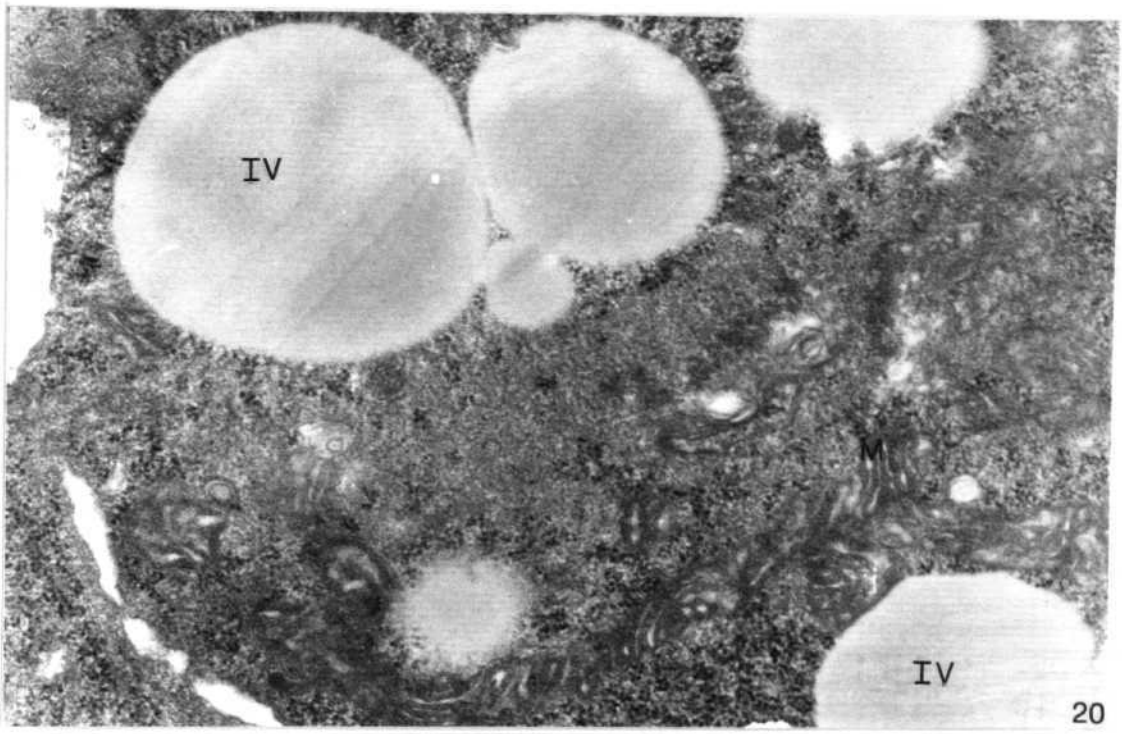
Plate IX

Figs.20 and 21. Electron micrographs showing the presence of large number of intracellular vacuoles (IV) which appear in the corpus allatum gland cells after precocene treatment. Note the presence of clusters of mitochondria (M).

PLATE IX



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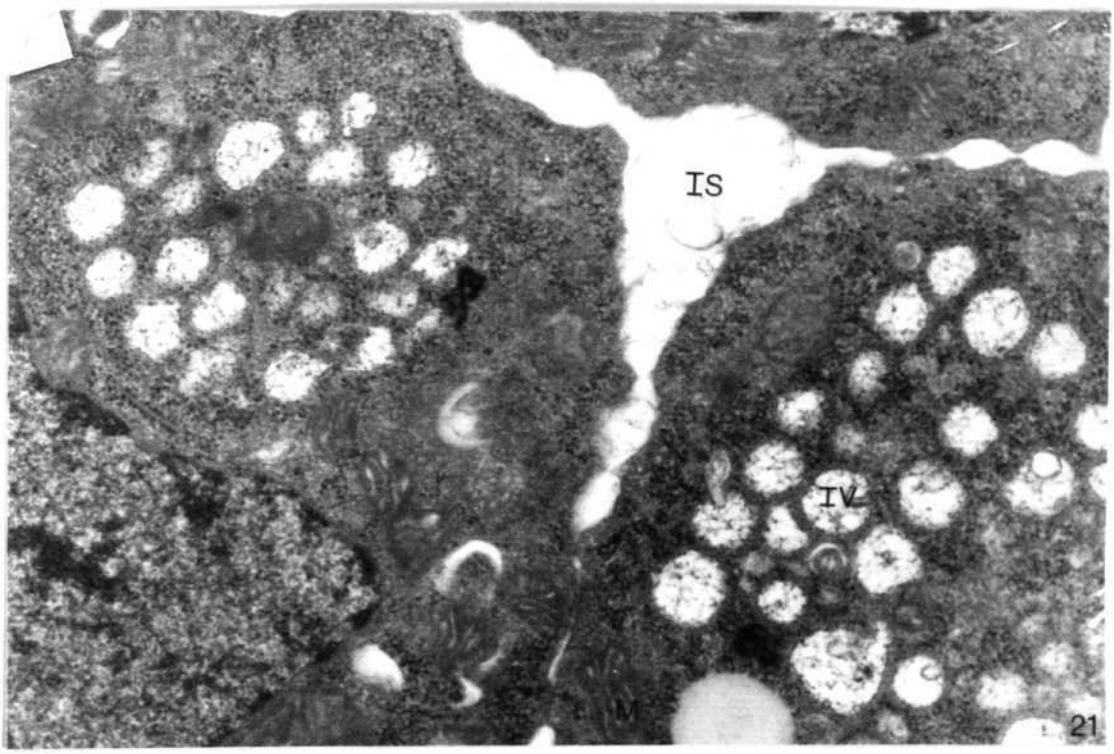

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TABLE 1

Effect of precocene on the body and ovary weight of *Dysdercus koenigii*

Treatment	Body wt. mg/Insect	Ovary wt. mg/Insect
Normal control 6 days old (7)	106 ± 9.84	45.15 ± 2.8
Acetone treated 6 days old (8)	105 ± 12.05	38.46 ± 2.61
Precocene treated (50 µg/insect) 6 days old (8)	60 ± 5.22*	3.53 ± 0.51*
Normal insect 3 days old (6)	62 ± 3.71	3.15 ± 0.44

The values represent the means ± S.D. of the number of determinations given in the parantheses.

* These values are significantly different ($P < 0.001$) from the corresponding control values.

TABLE 2

Effect of precocene on the fat body weight and RNA content of the fat body.

Age	Acetone treated			Precocene treated		
	Fat body wt. mg/Insect	Fat body RNA (µg) /Insect	/mg tissue	Fat body wt. mg/ Insect	Fat body RNA (µg) /Insect	/mg tissue
1 day (6)	3.74 ± 0.50	18.41 ± 6.99	4.99 ± 1.96	3.27 ± 0.70	9.08 ± 1.40	3.56 ± 0.50
2 days(7)	4.24 ± 0.90	18.74 ± 4.72	5.05 ± 2.75	4.08 ± 0.70	17.22 ± 3.40	4.20 ± 0.50
3 days(5)	3.98 ± 0.50	32.16 ± 5.80	6.65 ± 0.19	4.03 ± 0.40	30.46 ± 4.20	6.00 ± 0.90
4 days(14)	9.17* ± 2.40	85.16 ± 11.10	9.22 ± 1.60	4.37 ± 0.90	24.63 ± 4.50	5.52 ± 1.00
5 days(14)	8.81 ± 2.00	68.63 ± 11.50	8.21 ± 1.96	5.18 ± 1.10	29.35 ± 6.10	5.15 ± 0.50
6 days(10)	8.84 ± 1.20	75.20 ± 11.00	8.73 ± 1.50	5.37 ± 1.10	28.35 ± 5.10	4.84 ± 1.73

The values represent the means ± S.D. of the number od determinations given in parentheses.

* This value is significantly different ($P<0.001$) from acetone treated 3 days old insect as well as corresponding control value.

TABLE 3

Effect of precocene on H^3-uridine incorporation into fat body RNA.

Age	Acetone treated		Precocene treated	
	CPM/mg RNA	CPM/Insect fat body	CPM/mg RNA	CPM/Insect fat body
1 day (6)	2,59,340 ± 49,650	4037 ± 116	2,34,090 ± 39,570	2850 ± 667
2 days (7)	2,94,780 ± 65,000	5907 ± 893	2,77,090 ± 10,350	5705 ± 237
3 days (5)	1,80,830 ± 24,000	5086 ± 136	1,56,460 ± 30,130	4999 ± 431
4 days (13)	92,950 ± 24,420	6310 ± 1267	1,32,420 ± 26,700	* 3921 ± 1172
5 days (13)	84,610 ± 9,660	6625 ± 1165	1,16,990 ± 26,700	2939 ± 693
6 days (10)	71,580 ± 18,000	3820 ± 1625	86,433 ± 11,340	2785 ± 865

The values represent the means ± S.D. of the number of determinations given in parentheses.

* This value is significantly different ($P<0.001$) from the corresponding control value.

TABLE 4

Changes in the protein content of the fat body after precocene treatment.

Age	Acetone Treated		Precocene treated	
	Protein (µg)		Protein (µg)	
	/Insect fat body	/mg tissue	/Insect fat body	/mg tissue
1 day	134.04 ± 20.20	39.10 ± 0.70	86.96 ± 7.00	30.11 ± 0.80
2 days	204.32 ± 32.50	27.13 ± 3.80	148.70 ± 20.00	30.28 ± 3.70
3 days	152.27 ± 16.60	27.03 ± 2.20	106.04 ± 20.90	22.90 ± 1.60
4 days	393.38 ± 31.10	36.31 ± 3.60	227.59 ± 44.90	40.61 ± 2.20
5 days	378.02 ± 28.40	42.91 ± 3.40	284.97 ± 24.90	49.85 ± 3.00
6 days	399.78 ± 10.04	52.72 ± 4.00	264.57 ± 17.40	47.90 ± 1.50

The values are means ± S.D. of 3-5 determinations from 5-7 insects per group.

TABLE 5

Effect of precocene on the protein content of the haemolymph.

Age	Acetone treated	Precocene treated
	Protein (µg)/µl haemolymph	Protein (µg)/µl haemolymph
1 day	26.42 ± 4.65	30.94 ± 1.05
2 days	18.19 ± 3.35	18.30 ± 5.37
3 days	17.07 ± 1.91	10.45 ± 2.00
4 days	45.16 ± 7.60	14.89 ± 2.63
5 days	39.22 ± 4.06	16.50 ± 1.88
6 days	22.34 ± 0.42	15.75 ± 2.86

Results are mean ± S.D. of 3-5 determinations from 5-7 insects per group.

TABLE 6

The effect of precocene on the volume of corpus allatum.

Acetone treated		Precocene treated	
Age	Volume ($\mu m^3 x 10^5$)	Age	Volume ($\mu m^3 x 10^5$)
1 day (8)	15.27 ± 5.50	1 day (8)	11.14 ± 2.79
2 days (8)	17.95 ± 2.34	2 days (8)	7.90 ± 1.04
3 days (10)	22.34 ± 7.91	3 days (15)	13.09 ± 4.03
4 days (9)	28.04 ± 7.64	4 days (15)	11.29 ± 3.44
5 days (8)	21.46 ± 3.43	5 days (10)	8.06 ± 2.50
6 days (6)	10.81 ± 3.08	6 days (17)	9.67 ± 2.64

Results are means ± SD of the number of determinations given in parentheses.

TABLE 7

Effect of JH-III in precocene treated adult females.

Treatment	Ovary wt. (mg)/Insect	Protein (µg) /Insect ovary	Protein (µg) /mg tissue
Precocene treated	3.95 ± 0.32	198.47 ± 40.35	41.38 ± 6.26
5 µg JH on 4th day	12.04 ± 1.40	802.50 ± 27.18	67.29 ± 6.02
10 µg JH on 4th day	8.67 ± 0.35	437.32 ± 95.47	63.85 ± 7.11
Acetone treated	50.75 ± 4.50	4827.00 ± 385.50	121.46 ± 22.24

These values represent means ± S.D. of 4-5 determinations. For each determination, 5-7 insects were used.

TABLE 8

Effect of JH-III on fat body RNA content in 2 days old insects.

Treatment	Wt. of fat body	RNA(µg)/ Insect	RNA(µg)/ mg tissue.
Control-Acetone treated	5.234 ± 0.649	59.22 ± 8.17	15.25 ± 1.70
JH-5µg/Insect	7.501 ± 0.646	97.88 ± 20.52	25.31 ± 3.51
JH-10µg/Insect	5.612 ± 0.990	64.97 ± 10.92	19.18 ± 2.77
JH-15µg/Insect	5.438 ± 0.872	73.72 ± 15.41	19.59 ± 4.52

The values represent means ± S.D. of the 5-7 determinations, for each determination 4-5 insects were used.

CHAPTER VI

General discussion

In Dysdercus koenigii, a large number of oocytes in different ovarioles mature synchronously. This species lays eggs in a cyclical manner and the first gonotropic cycle is of about 7 days duration. The rate of oocyte growth in volume is not uniform in its progression. Previtellogenesis and the early phase of vitellogenesis are periods of relatively slow growth. Thereafter, the tempo of growth picks up dramatically until chorion formation (Goltzene, 1977; Ferenze, 1978). Rapid oocyte growth and vitellogenesis, as consequences of increased output of juvenile hormone from corpus allatum, has been convincingly demonstrated in several groups of insects (Engelmann, 1970; Rankin and Riddiford, 1978). Maturation of oocytes goes on hand in hand with the morphodynamic and functional changes in the follicle cells which have to keep pace with the steadily increasing volume of the oocytes (Anderson, 1964). The DNA content of the ovary was shown here to increase rapidly during egg maturation. Although the present study cannot offer any plausible explanation for this increase there might be two possibilities: increase in DNA material could be either due to the follicle cell proliferation or due to polyploid growth of the follicle cell nuclei (Koeppe and Wellmann, 1980). In Oncopeltus, polyploidy of follicle cells appears to

be dependent on JH (Koeppe and Wellmann, 1980). Also in Leucophaea, the follicle cells of vitellogenic oocytes synthesise large quantities of DNA which is regulated by JH (Koeppe and Wellmann, 1980; Koeppe et al., 1980a, 1981; Koeppe, 1981) and the rate of DNA synthesis in a terminal follicle increases 20-100 fold in comparison to a non-vitellogenic follicle. Polyploidization, as a rule, accompanies cell differentiation and enables the follicle cells to carry out their programmed functions (Brodsky and Uryvaeva, 1977; Nagl, 1978). This has been demonstrated convincingly in the follicle cells of Carausius morosus, in which there is a direct relationship between the degree of polypolidy and the developmental processes in the growing oocyte (Pijnacker and Godeke, 1984). Telfer (1979), suggested that polyploidy - a mechanism for gene amplification - is necessary during oocyte maturation for the increased synthesis of specific proteins required for maintaining the interfollicular spaces or for the production of proteins which may help in the incorporation of vitellogenin through the intercellular channels in the follicle epithelium. The binucleate condition of the follicle cells observed here may also serve the purpose of increasing the DNA material in the follicle epithelium.

As in many other Hemiptera, the present species also possesses telotrophic ovarioles (Mays, 1972; Schreiner, 1977a,b). During the previtellogenic growth of oocytes, large amounts of RNA are known to accumulate in the ooplasm and this RNA may be used up during the subsequent embryogenesis. The trophocytes of telotrophic ovarioles are known to be most actively involved in synthesising RNA in their large polyploid nuclei and exporting it to the oocyte through the trophic cords (Zinmeister and Davenport, 1971; Mays, 1972; Buning, 1972; Ullmann, 1973; Matuszaki, 1975; Ray, 1979; Ray and Ramamurty, 1979; Dittmann et al., 1981). The steady increase of RNA content of the ovary throughout the first gonotropic cycle in the present species indicates that it may be used firstly for yolk synthesis and later for embryogenesis.

During the first reproductive cycle in Dysdercus koenigii, the protein content of the fat body as well as haemolymph showed a significant quantitative change and this is directly related to the process of yolk deposition. During vitellogenesis, in a short time, remarkable amounts of proteins as well as other substances are deposited as yolk in the developing oocytes.

Electrophoretic studies have revealed that the vitellogenins produced by the fat body appear in the haemolymph of the adult female at about two days after adult emergence. The haemolymph protein concentration rose rapidly during early vitellogenic period and it remained high during active vitellogenic period but declined in 6 days old insects. These vitellogenins collectively form a large proportion of the haemolymph protein, so that their synthesis and uptake by the developing oocytes could account for the fluctuations in haemolymph protein titre during the gonotropic cycle (Elliott and Gillott, 1977). Although the fat bodies of 4 days old insects showed a lower amount of H^3-leucine incorporation as compared to 2 and 3 days old insects, the amount of label appearing in the haemolymph was 3 times more in the former than in the latter. This suggests that a greater proportion of the newly synthesised protein was being released into the haemolymph during active vitellogenic period and therefore it does not accumulate in the fat body (Brooks, 1969; Engelmann et al., 1971; Wyss-Huber and Luscher, 1972). The rate of protein synthesis as well as its release by the fat body declined drastically towards the end of the vitellogenic period and this corresponds closely with the depleted

protein content of the haemolymph in 6 days old insects (Slama, 1964).

The cytologically detectable cyclical activity of the fat body is consistent with its known biochemical rhythms of vitellogenin production (Wuest, 1978). In the present insect the well defined activity cycle begins with a short inactive phase (basal metabolism) during which non-specific protein synthesis occurs. From day 3 onwards, the fat body enters the phase of active vitellogenin production and its release, as reflected in the appearance of new protein bands in electropherogram (see Pl.1). This phase coincides with the short period of rapid yolk deposition. Thereafter the fat body returns to an inactive state, with low rate of protein synthesis during the post vitellogenic stage of oocyte development. The reason for this may be the presence of mature eggs in the ovariole which may cause partial feed-back inhibition of CA (Hagedorn and Kunkel, 1979). This, in its turn may be responsible for the lowered rate of protein synthesis by the fat body and the concomitant depletion of the haemolymph protein content. The haemolymph protein level is reflected in the observed changes both in the rate of synthesis in

the fat body and the rate of uptake by the developing oocytes (Bakker-Grunewald and Applebaum, 1977). These proteins enter the oocyte from the haemolymph through intercellular spaces in the follicle epithelium which have been demonstrated in several insects by vital staining, autoradiographic, electron microscopic as well as fluorescence-labelled antibody methods (Bier and Ramamurty, 1963; Ramamurty, 1964, Telfer, 1961).

The present studies on Dysdercus with tritiated amino acid (H^3-leucine) have yielded autoradiographic patterns that are largely in agreement with those described by several previous authors (Bier, 1962; Ramamurty, 1964; King and Agarwal, 1965; Melius and Telfer, 1969; Engels, 1972; Giorgi and Jacob, 1977b; Ramamurty and Engels, 1977a; Ray et al., 1981). The incorporation of label into the yolk spheres at the oocyte cortex is phase-specific, being confined only to vitellogenic stages and is conspicuously absent in the preceding as well as the subsequent stages. There are two female specific proteins (vitellogenins) in Dysdercus, recognisable as two distinct bands in polyacrylamide gel electrophoresis of haemolymph of mature reproductive females, and these are absent in the haemolymph of

males and immature females (see pl. I). These 2 bands showed similarity in their electrophoretic mobility with the two ovarian proteins. Both of them are probably glycolipoproteins as their chemical nature was convincingly shown in a variety of insect species (Yamasaki, 1974; Gellisen et al., 1976; Chen et al., 1976; McGregor and Loughton, 1977; Wyatt and Pan, 1978; Engelmann, 1979; Jensen et al., 1981). Vitellogenin(s) synthesised and released by the insect fat body are selectively taken up by the developing oocytes (Telfer, 1960; Hagedorn, 1974; Ferenz, 1978; Harry et al., 1979).

The CA produces JH in insects (Dahm et al., 1976). In the imaginal stage of most insects, JH is the principal gonadotropin that stimulates oogenesis (Engelmann, 1970; De Wilde and De Loof, 1973b). There is a large body of experimental evidence in several species of insects to show that JH controls the synthesis of protein yolk precursor, vitellogenin (Engelmann and Penney, 1966; Engelmann, 1969; Bell, 1969, 1970; Bell and Barth, 1970; De Loof and De Wilde, 1970b; Pan and Wyatt, 1971; Lanzrein; 1974), including the Hemiptera (Mundall and Engelmann, 1977; Kelly and Telfer,

1977). Surgical extirpation of CA before the natural induction of vitellogenin synthesis by the fat body blocks the oocyte maturation.

In the present species the weight of the fat body increased significantly during the first gonotropic cycle and this increase is most probably due to hypertrophy of the cells and not occasioned by cell proliferation. No cell proliferation has been found to take place in fat body during vitellogenesis in Locusta (Reid and Chen, 1981). The total RNA content of the fat body increased rapidly from 3 to 4 days (Hagedorn et al., 1973; Behan and Hagedorn, 1978; Chen et al., 1979). This RNA presumably supports the increased rate of protein synthesis during the active vitellogenic period. The fat body in the vitellogenic females of the cockroach Leucophaea maderae contain a prominent population of large polysomes each made up of approximately 30-40 ribosomes, whereas fat bodies of non-vitellogenic females and males of all ages do not exhibit such large number of polysomes (Engelmann, 1977). Reid and Chen (1981) demonstrated the accumulation of ribosomes in the fat body of female locusts after adult ecdysis and this is soon followed by the

appearance of vitellogenin polysomes. JH analogues stimulated RNA synthesis in the allatectomised locust and a major portion of this is shown to be rRNA (Chen et al., 1979), as reflected in the massive accumulation of stable ribosomes in the fat body (Lauverjat, 1977; Couble et al., 1979; Reid and Chen, 1981). Therefore, it appears that the primary action of JH on the fat body involves selective stimulation of transcription of vitellogenin genes, accompanied by synthesis of rRNA and build-up of protein synthesizing apparatus (Reid and Chen; 1981).

In the present study, injection of actinomycin-D inhibited the total RNA synthesis. Also it has almost completely blocked the subsequent peak of RNA build-up (normally found in the active vitellogenic females) when actinomycin-D was administered on the 4th day. When the insects were given this drug at progressively later time points on day 5 and 6, less and less inhibition of RNA production was observed. Our results suggest that, by day 4, a major quantity of RNA necessary for vitellogenin synthesis has already been synthesised and accumulated. In Aedes aegypti, actinomycin-D inhibited RNA synthesis in vivo and prevented

normal increase in the rate of yolk protein synthesis, but allowed synthesis to proceed at the rate expected for the tissue assayed at the time of injection (Hagedorn et al., 1973). On this evidence the authors suggested that the synthesis and accumulation of long lived mRNA for yolk protein takes place. They also suggested that the synthesis of vitellogenin is controlled in this insect at the transcriptional level. In the same insect species, injection of either α-amanitin or cordycepin together with ecdysterone did not inhibit the vitellogenic protein synthesis, whereas actinomycin-D was found to be inhibitory (Fong and Fuchs, 1976). It is well known that actinomycin-D inhibits synthesis of all RNA-species while α-amanitin and cordycepin, in general, seem to be more selective. Therefore, it is possible that ecdysone does induce transcription of not mRNA, but of rRNA and/or tRNA, either or both of which may be needed for subsequent translation (Fong and Fuchs, 1976). However, in Leucophaea maderae, α-amanitin inhibited the incorporation of uridine into rapidly labelled RNA and protein synthesis (Engelmann, 1974).

In Dysdercus koenigii, Precocene-I inhibited egg maturation in a dose dependent manner (Ramalakshmi, 1983). However, in the present study, a single topical application of 50 µg of P-II resulted in total inhibition of vitellogenesis. After its topical application precocene readily appears in the haemolymph of insects (Bergot et al., 1980; Feyereisen et al., 1981). In Heteroptera, since vitellogenesis is under the influence of JH (Kelly and Davenport, 1976; Rankin and Riddiford, 1978; Davey, 1981), it seems reasonable to suggest that precocene depresses the haemolymph JH titre in the present insect also, although JH-assay was not carried out after precocene treatment.

In Dysdercus koenigii the incorporation of H^3-uridine into the fat body total RNA increased steadily during the period of egg-maturation to reach the maximum, accompanied by a small rise in tissue RNA content in 4 days old insects and it remained high in 5 days old insects. On the other hand, in precocene-II treated insects, H^3-uridine incorporation into the fat body RNA diminished gradually. This may be attributed to the deficiency of JH output occasioned by the degeneration of CA cells. The haemolymph protein concentration in

precocene treated animals did not show the usual increase with the advancement of age and remained fairly low throughout, as compared to the acetone treated controls. The observed increase in the fat body protein titre after precocene treatment, might be due to the accumulation of other (non-vitellogenic) proteins (Coles, 1964; Elliott and Gillott, 1979).

In acetone treated control females the volume of the CA increases and reaches a peak value on day 4 after the imaginal moult, midway during the first gonotropic cycle. This observation is in accord with the findings of previous authors (Jalaja and Prabhu, 1977; Judson et al., 1979; Tiwari and Shrivastava, 1979). It indicates that the egg maturation is positively correlated with allatal activity in the present species of Dysdercus. Such a direct correlation of allatal volume with oocyte growth is consistent with the well known gonadotropic role of the CA in the imaginal life of many insect species because allatectomy in most insects results in failure of egg maturation. A parallelism between the rate of JH synthesis and CA volume during the ovarian development is reported for Nauphoeta cinerea (Lanzrein et al., 1978) and Leptinotarsa

decemlineata (Schooneveld et al., 1977; Kramer, 1978). The CA volume could increase either by multiplication of the cell numbers (Johansson, 1958; Barth and Sroka, 1975) or merely by increase in the cytoplasmic volume (Schooneveld, 1970; De Laurence and Charpin, 1978) or by both the methods (Szibbo and Tobe, 1981). In adult females of Dysdercus koenigii, it is seen that P-II inhibits the increase of the volume of CA, which occurs normally during the first gonotropic cycle (Bowers and Martinez-Pardo, 1977; Judson et al., 1979).

Ultrastructural studies of the CA clearly indicate that P-II not only inhibits the increase of CA volume but also produces abnormal morphology of CA cells which is associated with massive autophagy and degeneration of various cell organelles (Unnithan et al., 1977; Liechty and Sedlak, 1978; Schooneveld, 1979a,b; Feyereisen et al., 1981).

In Dysdercus koenigii, apart from the disorganization of the basement membrane, mitochondria, cisternae of the rER and polysomal configuration of the ribosomes in P-II treated insects, the main difference, as compared to the acetone controls, is the accumulation of several

intracellular vacuoles and aggregations of numerous vesicles, the latter being filled with diffuse electron dense materials. These vesicles probably represent autophagic vacuoles resulting from lysosomal activation. In Oncopeltus fasciatus, Leichty and Sedlak (1978) and in Diploptera punctata, Feyereisen et al.,(1981) have described extensive damage to the cytoarchitecture of the allatal cells at the dosages of Precocene II used by them. However, in our species of Dysdercus koenigii at the dosage employed, such severe damage is not in evidence. Conceivably the susceptibility of the CA cells to P-II may vary in different species of insects and also with the dosage.

P-II induced effect is possibly due to a reactive precocene metabolite, produced by CA cells (Brooks et al., 1979,Muller et al., 1979, Feyereisen et al., 1981). The CA from adult female of Locusta migratoria rapidly metabolises precocene to dihydrodiols in the presence of high levels of epoxidases and this causes selective cell death in CA (Pratt et al., 1980, 1982). However, the effect of precocene is spontaneously reversible with passage of time in other insect species such as Drosophila melanogaster (Landers and Happ, 1980, Wilson et al., 1983),

and in aphids like Acrythosiphon (Mackauer et al., 1979) and Myzus (Hale and Mittler, 1981). Such reversibility is not in evidence in the present species, since the ultrastructure of the CA was not investigated after JH treatment.

While the vitellogenesis is controlled by the CA in a large number of insects, its role in the regulation of previtellogenesis is controversial. According to Masner (1968) the CA is required for the differentiation of the follicular cells. However, Joly (1968) pointed out that ovarian reaction to ablation of CA varies in different species. Some need the CA only from the stage of vitellus deposition whereas others require them for previtellogenesis as well (Girardie, 1962; Pluot, 1973). The CA is indispensible during the previtellogenic growth period of oocytes in Panstrongylus megistus (Furtado, 1979). Previtellogenesis in Dysdercus is most likely regulated by BH, because previtellogenic growth occurs even after precocene treatment.

The consistently large amounts of PF-positive materials accumulated in the neurosecretory cells of the brain, observed in precocene treated females of

Dysdercus is most likely due to inhibition of the release of neurosecretory colloid from the cells. The results shown here are at variance with those reported for Oncopeltus fasciatus by Unnithan et al., (1978), because these authors believe that precocene inhibits synthesis of the neurosecretory A-cells and this could be restored by JH-III application.

Extirpation and reimplantation techniques have revealed that both the neurosecretory cells of the brain and CA are essential for vitellogenesis in the females of Dysdercus cingulatus (Jalaja and Prabhu, 1976, 1977). Only the CA plays a direct gonadotropic role in females and the neurosecretory cells serve to activate the CA (Jalaja and Prabhu, 1977). However, these authors have not paid any attention to the fat body protein synthesis and its hormonal regulation.

In most insects studied, the CA have been shown to stimulate the de novo synthesis of the female-specific protein in the fat body (Doane, 1973; Engelmann, 1974; Highnam and Hill, 1977; Hagedorn and Kunkel, 1979) including some Hemipterans (Coles, 1965; Kelly and Telfer, 1977; Mundall and Engelmann, 1977; Rankin and

Jackle, 1980). In Dysdercus cingulatus, the absence of protein uptake by the developing oocytes of allatectomized females have been attributed to the failure of the follicle cells to differentiate (Jalaja and Prabhu, 1976). However, the present study clearly indicates that CA (JH) not only controls the vitellogenin uptake by the developing oocyte (Slama, 1964; Jalaja and Prabhu, 1976, 1977) but also regulates the protein synthesis by the fat body in Dysdercus koeningii.

General Summary

Chapter III

In the red cotton bug, Dysdercus koenigii, the first reproductive cycle is of 7 days duration. The ovary of each side consists of 7 telotrophic ovarioles. About 9-11 oocytes undergo maturation in each ovariole during the first reproductive cycle. The ovarian growth is divisible into 3 different phases each of which is characterised by morphological and biochemical processes.

a) Pre-vitellogenic phase lasting the first 2 days after emergence.

b) Vitellogenic phase lasting through the next 3 days.

c) Post-vitellogenic phase lasting the next 2 days.

The oocyte undergoes euplasmic growth during the pre-vitellogenic phase mainly by deriving macromolecules, especially RNA, from the nurse cells via the tropic cords. It also synthesises its own proteins to a limited extent. During this phase, there is a small increase in the ovarian weight which is reflected

in a two fold increase in the total protein content. A high rate of H^3-leucine incorporation seen in scintillation counting studies, also suggests an endogenous protein synthesis.

The ovarian weight increases significantly during the next vitellogenic phase. This increase is accomplished by the oocytes through the sequestration of large quantities of exogenous proteins mainly vitellogenins of glycolipoproteinaceous nature, which are synthesised mostly in the fat body and transported via the haemolymph to the developing oocytes. This is clearly demonstrable by autoradiographic studies using H^3-leucine.

During the last post-vitellogenic phase a further increase in the ovarian weight is seen. The main feature of this phase is the occurrence of chorion deposition which is also evident from autoradiographs.

The weight of the fat body increases gradually till the early vitellogenic phase. There is a sharp increase in the fat body weight from the pre-vitellogenic to the active vitellogenic phase, and this weight

decreases again during the post-vitellogenic phase. A similar pattern is seen in the changes occurring in the DNA, RNA and protein contents in the whole fat body. The increase in DNA and RNA contents seen during this stage may sustain the increased synthesis of the yolk protein precursor (vitellogenin). The total protein content thus remains low upto early vitellogenic period, increases significantly during the rest of the vitellogenic period but declines thereafter. Scintillation counting studies using H^3-leucine clearly indicate a high rate of protein synthesis by the fat body and its simultaneous release into the haemolymph which was the highest in 4 days old insects.

The various aspects of studies undertaken clearly demonstrate the synthesis of the female specific yolk-protein precursor by the fat body, its release into the haemolymph and then its sequestration into the oocytes wherein it constitutes the major yolk protein. This conclusion is further supported by the evidence from electrophoretic studies of the fat body and ovarian homogenates as well as haemolymph of both males and females.

Chapter IV:

Effect of actinomycin-D:

The effect of actinomycin-D, a well known inhibitor of RNA synthesis, was investigated on fat body RNA content. Females of different age groups, namely, 2 days, 3 days, 4 days and 5 days old insects, were injected with various dosages of 0.1, 0.25, 1.0 and 2.5 µg of actinomycin-D/insect. The total RNA and protein contents were estimated after 24 h. 2 and 5 days old insects injected with actinomycin-D showed no effect at all. The maximum effect i.e., maximum inhibition of RNA synthesis, was seen in 3 days old insects injected with actinomycin-D. These results suggest that 2 days old insects are in the pre-vitellogenic phase and hence there is no significant RNA synthesis in the fat body due to which actinomycin-D cannot bring about its inhibitory effect. In the 3 day old insects which enter the active vitellogenic phase a large amount of RNA synthesis occurs to meet the required increase in yolk protein synthesis. At this time actinomycin-D exerts its highest inhibitory effect. In 4 days old insects, since most of the RNA,

required for the yolk protein synthesis is already accomplished and only limited RNA synthesis continues to occur, actinomycin-D causes only partial inhibition. When the insects become 5 days old, since almost all the RNA synthesis is completed and they enter into the post-vitellogenic phase, actinomycin-D does not show any effect on the total RNA content of the fat body. These conclusions are based upon the total RNA content and are confirmed also by estimations of the total fat body protein in the insects of the same age groups, injected with actinomycin-D.

Chapter V

Effect of an anti-juvenoid Precocene-II:

Precocene-II, dissolved in acetone, was topically applied at a dosage of 50 µg/female within 10-15 minutes after emergence. This dose of precocene was found to be optimum because there is negligible effect on the mortality rate and at the same time a complete inhibition of vitellogenesis is achieved. The body weight drops to one half in experimental insects. The ovaries of insects treated with precocene remained

considerably small and contained only undifferentiated eggs, whereas in acetone treated insects the ovarioles showed the presence of a series of well differentiated mature oocytes. In 6 days old normal and acetone treated insects, the ovarian weight was 45 mg/insect and 38 mg/insect respectively, while it was drastically low at 3.5 mg/insect in precocene treated ones. This corresponds with the weight of the previtellogenic ovary during normal growth phase, thereby indicating a clear-cut inhibition of vitellogenesis. Histologial preparations showed that in the ovaries of experimental insects, vitellogenesis was inhibited and the oocytes were devoid of yolk platelets. This pattern was confirmed by tracer studies using H^3-leucine as a protein precursor.

The effect of precocene II was also studied on the weight, RNA and protein content of the fat body. In control insects, the fat body weight increased significantly (nearly 3 fold) from 3 to 4 days, whereas 4 days old experimental insects retained more or less the same fat body weight as that of 3 days old experimental insects. This may be attributed to the deficiency of juvenile hormone which has an important

role in the control of growth and synthetic activity of the fat body. Precocene also blocks the increase in total RNA content as well as the total H^3-uridine incorporation, which occurs normally in 3-4 days old insects, as evidenced by scintillation counting studies. In precocene treated insects, the total RNA synthesised was only 50% of the control value. This suggests that the increased RNA synthesis of the fat body at this stage may be regulated by a high JH titre. As this is very low in precocene treated insects, it results in a very low amount of RNA synthrsis. The increase in the haemolymph protein content that normally occurs from 3-4 days was also blocked, thereby indicating that the increased RNA synthesis at this stage most probably supports the synthesis of female-specific proteins (vitellogenin) which are selectively incorporated into the developing oocytes.

Exogenous JH-III application to precocene treated insects counteracts the deleterious effect of this substance only to a very small extent when compared to the acetone controls. This is shown by the slight increase in the protein content of the ovaries. In this insect, the pre-vitellogenic growth seems to be

controlled by brain hormone. But a certain titre of JH may also be required during this phase to make the fat body competent for its growth as well as vitellogenin synthesis. In precocene-primed insects even after treatment with JH, the fat body is not able to actively synthesise vitellogenin. This might be due to the inadequate titre of JH in the previtellogenic phase.

Ultrastructural studies revealed a number of degenerative changes in the allatal cells of precocene treated insects, such as the disorganisation of the basement membrane, cisternae of the rER and polysomal configuration of the ribosomes as well as clustering of mitochondria. Several intracellular vocuoles and numerous vesicles filled with an electron dense material also appear. The latter may be the autophagic vacuoles resulting from lysosomal activation. The resultant chemical destruction of CA is irreversible with time in the present insect because even when the precocene treated insects were left for 30-40 days, they did not lay any eggs.

The cyclical changes in CA volume which were seen in acetone treated insects were not in evidence in precocene treated insects. The increase in the CA volume which occurs in acetone treated insects from the pre-vitellogenic to the active vitellogenic phase, most probably reflects the increased JH synthesis because a high JH titre is required for sustaining the high rate of protein synthesis during this phase.

PF staining of neurosecretory cells revealed intensely stained cells in precocene treated insects and very lightly stained cells in acetone treated insects. The intense staining seen in the experimental insects may be attributed to the feedback inhibition of the release of neurosecretory material, resulting in its accumulation in these cells.

Lastly topical application of 5 µg JH, to 2 days old normal insects which were still in the previtellogenic phase, resulted in a 40% increase in fat body weight and a 60% increase in the total RNA content of the fat body. These values are close to the values of RNA content observed in 4 days old insects. This shows that initially a low titre of JH is present in haemolymph

and in the vitellogenic phase when the JH titre increases the RNA synthesis also undergoes a spurt. One may infer that the fat body growth and its RNA synthesis is under the control of JH. It may be concluded from the data presented here that JH not only controls the vitellogenin uptake by the developing oocytes but it also regulates the yolk protein synthesis by the fat body in Dysdercus koenigii.

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