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 gonotrophic 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 (Koeppen and Wellmann, 1980). In *Oncopeitus*, 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 polyploidy 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 gonotrophic 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 keenigii*, 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 vitello-
genins 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 gonotrophic cycle (Elliott and Gillott, 1977). Although the fat bodies of 4 days old insects showed a lower amount of H³-leucine incorporation as compared to 2 and 3 days old insects, the amount of labelling 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
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,
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 gonotrophic 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 allatotectomised 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 gonotrophic 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
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 gonotrophic 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 indispensable 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
Jalaja, 1980). In *Dysdercus cingulatus*, the absence of protein uptake by the developing oocytes of allatostezotomized 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*. 