SUMMARY

In the present investigation the changes in the haemolymph metabolites like proteins and lipids during the growth, metamorphosis and reproductive periods have been studied in two hemimetabolous and two holometabolous insect species. The proteins and lipids play an important role in the formation of cuticle during metamorphosis and are also used for the growth and formation of organs.

Protein fractionation with polyacrylamide gel disc electrophoresis has been done in the different developmental stages and during the adult life. In Dysdercus similis, six protein fractions have been observed in both the sexes. In Sphaerodema rusticum, seven protein bands in males and eight in females; in Danais chrysippus, nine protein bands in males and eleven in females; and in Sarcophaga lineatocollis eleven protein fractions in males and twelve in females, have been observed.

Female sex specific protein has been found to be absent in Dysdercus similis, while in the other three insect species, female sex specific protein/proteins have been observed. The female sex specific protein is synthesized "de novo" at the time of vitellogenesis in Danais chrysippus and Sarcophaga lineatocollis, while in Sphaerodema rusticum it is present from the very beginning.
From the results on protein fractionation in different developmental stages, it is clear that in *Dysdercus similis*, the stage specific protein/proteins are absent. The stage specificity is shown only by the fluctuations in the concentration of different protein fractions. In *Sarcophaga*, it has been shown by the total disappearance of haemolymph protein fractions. Fraction 5 in the late pupal stage and fraction 1 of the newly emerged males and females disappeared from the haemolymph. In *Danaus chrysippus*, the stage specificity is shown by the disappearance and appearance of the new protein fractions. In the third stage larvae, 9 protein fractions could be detected, while in the larvae one day before moulting to pupae, a new protein fraction i.e. fraction 1 appeared for the first time. In newly formed pupae, 11 protein fractions have been observed and a new protein fraction — fraction 12 appeared during this stage, while in the six-days-old pupae with darkened pupal case, protein fractions 1 and 9 have been found to be missing while another protein fraction i.e. protein fraction 8 appeared for the first time.

In the newly hatched adults, three protein fractions have been found missing from the haemolymph of both the males and females; they are fraction 1(Rm 3-4), fraction 9(Rm 64-66) and fraction 12(Rm 85-87). These three protein fractions could not be seen during the adult life of males and females.
On the second day, in female adults two new proteins have been observed; they are protein fraction 5 ($R_m$ 23.6-26.0) and 11 ($R_m$ 89-91). These proteins could not be seen during the larva and pupal stages, and appear only in the adult females.

Apart from this, a number of protein fractions showed oscillations in their concentration during the growth and development of immature stages and also at the time of moulting. The protein fractions are used during these stages for the incorporation in the newly synthesized cuticle. They are probably used also for the darkening of the pupal case. Some of the protein fractions are also used probably for incorporation into the developing and differentiating tissues.

It has also been observed that the development of both the sexes involved different protein fractions in *Dysdercus similis*, *Sarconhaga lineatocollis* and *Danais chrysippus*. The differences in the fluctuations in the concentration of protein fractions of both the sexes can be related to the physiological differences and the need of proteins, specific to that sex.

Protein fraction 3, in *Dysdercus similis*, *Sphaerodema rusticum* and *Sarconhaga lineatocollis* and fraction 1 in adults and fraction 2 in the immature stages in *Danais chrysippus* are the proteins of higher concentration with
low molecular weight, and are considered to be the "Common Insect Proteins".

As regards the presence of "Major Blood Proteins", protein fractions 1 to 4 in the males and females of *Dysdercus similis*, fractions 2, 3, 4, 5 and 6 in females, and protein fractions 1 to 5 in males of *Sarcophaga lineatocollis*, protein bands 2, 3, 4, 5 and 9 in females and 2, 3, 4 and 6 in males of *Danais chrysinus* and protein fraction 3 in *Sphaerodema rusticum* are the "Major Blood Proteins".

In *Dysdercus similis*, all protein fractions appeared to be the vitellogenic female proteins, although the oscillations are more pronounced in the fractions 3 and 5. In *Sphaerodema rusticum* fractions 3, 4, 5, 7 and 8; in *Sarcophaga lineatocollis* protein fractions 2, 3, 4, 9 and 12, and in *Danais chrysinus* fractions 2, 4, 5, 9 and 11 are vitellogenic female proteins.

Protein fractions 2 and 4 in *Dysdercus similis*, fractions 5, 6 and 10 in *Sarcophaga lineatocollis*, and protein fractions 7, 8 and 10 in *Danais chrysinus* showed oscillations during the reproductive cycle in males, and hence are male specific/limited proteins.

Human serum albumin like protein is found to be absent in all the four insect species.
The concentration of vitellogenic female proteins is found to be low in unmated females of *Dysdercus, Sarcophaga* and *Danais* when compared with the concentrations found in the normal mated females. The concentration fluctuations of the vitellogenic female proteins in unmated females followed the same pattern as has been noted for the normal females. The sex specific protein/proteins appeared in the haemolymph samples irrespective of whether the females were mated or unmated. During the second reproductive cycle of unmated females in *Dysdercus similis*, the concentration in different vitellogenic female proteins has been found to be still lower.

As regards the total protein concentration in the haemolymph, a gradual rise during the nymphal instars in *Dysdercus similis*, and during the larval growth in *Sarcophaga* and *Danais* can be attributed to the continuous digestion process and also to the increased blood volume. A slight drop in the concentration in the fifth instar male *Dysdercus* is probably due to the morphogenesis and histogenesis processes specific to the male physiology.

The decline in the protein concentration in the pupal stages and during adult development is obviously due to the incorporation of proteins in the newly laid cuticle. The haemolymph proteins as such, or after degradation, help probably in the darkening of the pupal case. The proteins are also incorporated into the other developing and differentiating tissues. It is also possible that the
haemolymph proteins are taken up by the fat body during the pupal stage, just for storage.

During adult life, in Dysdercus and Sarcophaga, after an initial drop, the concentration rose to the maximum level before the start of vitellogenesis. Similar results have been observed for Danais and Sphaerodema also. The haemolymph protein concentration declined in all the four insect species at the time of egg maturation and vitellogenesis. The concentration fluctuations followed the same pattern during the second reproductive cycle in Dysdercus i.e. a rise in the concentration during the preoviposition period and then a decline during the oviposition period.

Although no violent fluctuations could be seen for the male haemolymph protein concentration, the pattern appears to be the same as has been observed for the females.

As regards the total haemolymph lipid concentration, after a gradual rise from third to fourth instar in Dysdercus, and during the larval growth and early pupal stage in Sarcophaga and Danais, it declines in the fifth instar nymphs of Dysdercus and during the late pupal stage in Sarcophaga and Danais. This can be attributed to the histogenesis, laying of new cuticle and also to their utilization in energy production at the time of moulting. In newly emerged males of Sarcophaga and Danais, the haemolymph lipid concentration has been found to be more
than the concentration values observed for females.

From newly emerged stage, the concentration started rising again both in males and females, reaching to a maximum level before the vitellogenesis and egg maturation processes started in all the four insect species. The lipid concentration then declined, probably due to the utilization of haemolymph lipids by the maturing oocyte in females and spermatozoa in males. The same cycle is repeated again during the second reproductive cycle in *Dryadecus* although the concentration has been found to be low as compared to the first reproductive cycle. The fluctuations related to the reproductive cycle in females have also been noted in *Sphaerodema*.

The total protein and lipid concentrations in the haemolymph of the unmated females have been found to be lower than the concentration found for the normal females in *Dryadecus*, *Sarcophaga* and *Danais*, although it followed the same type of oscillations in the concentration as have been observed for normal females.

The differences in the concentration of haemolymph metabolites between the two sexes has been probably due to the differential activity of the fat body and other metabolite synthesizing tissues in the two sexes.

The length of the last oocyte increased gradually both in mated and unmated females but the increase in length
has been more pronounced in mated females. The length of the last oocyte increased with the decrease in the concentration of both proteins and lipids in the haemolymph. The concentration in vitellogenic female protein fractions also increased during the early periods of adult life, decreasing at the time of oocyte maturation. The concentration of haemolymph metabolites like proteins and lipids reflects the balance between the protein synthesis and lipid concentration and their drainage into the growing oocytes. Although the growth in the last oocyte occurred throughout the adult life up to the maturation, the same could not be reflected in the protein fractionation studies and in the haemolymph protein and lipid concentration. During this period the protein synthesis and lipid turnover rate appear to be higher than their drainage into the maturing oocytes, hence a notably high concentration of haemolymph metabolites has been observed.

The number of eggs laid has been found to be less in unmated females in Drosophila and Danaids. Likewise in Sarcophaga also the number of first instar larvae laid was less during the instamments of larviposition. The laying during the first reproductive cycle in unmated females of Drosophila has been slow, and only a few eggs have been laid.

Mating in Drosophila increases egg maturation and production, while in Sarcophaga and Danaids mating does not
influence maturation but laying. It is assumed that the modeling in some way alters the availability of haemolymph metabolites by way of influencing the protein synthesis and lipid mobilization. It is also probable that a certain level of metabolites is necessary to ensure the oocyte maturation, which could not be attained in the case of unmated females, thus affecting the growth of oocyte and also the laying.