DISCUSSION

The haemolymph or blood of insects possesses properties that have attracted to it a major share of attention of insect physiologists (Reviews by Wyatt, 1961, 1968; Florkin and Jeuniaux, 1964; Buck, 1963; Chen, 1966).

The separation and characterization of one of the most important haemolymph reserves, i.e. proteins, have been done in a number of instances, for example Telfer (1954, 1960); Laufer (1960, 1961, 1963); Whittaker and West (1962); Coles (1964, 1965 a,b); Terendo and Peir (1965, 1966, 1967 a,b); Adiyodi (1967); Adiyodi and Nayar (1966, 1968); Prabhu and Nayar (1972); Kulkarni and Mebrotra (1970); Engelmann and Penney (1966); Wilkens (1969, 1969); de Toof and de Wilde (1970); Perassi (1972, 1973) and others.

As far as the author is aware only a few workers have studied the protein fractions in all the developmental stages and during the adult life of a single insect. The work of Chen and Levenbook (1966), Hudson (1966), Loughton and West (1965), Lensky (1971) is of particular importance when the fractionation studies in the developmental stages and during the adult life are taken into consideration.

As regards the total protein concentration in the haemolymph during different stages in the life cycle of an insect and during the adult stages, many results have
accumulated in the past few years. The work of Hill (1962), Hill et al. (1968), Orr (1964), Nowosieński and Patton (1965), Minks (1967), Mills et al. (1969), Slama (1964) and few others deserve special mention.

The total lipid concentration in the haemolymph has been reported for only a few insect species, for example, Sridhara and Bhat (1965), Ludwig and Wismeister (1953), Martin (1969) Wlodawer and Visniewska (1965) and others.

For the purpose of the present study two insect species with hemimetabolous and two insects with holometabolous type of development were selected in order to review and understand the biochemical and physiological complexities of growth, moulting and reproduction in these two groups of insects.

The protein pattern of the haemolymph has been followed during the immature stages of Dysdercus similis, Sarcophaga lineatocollis and Danais chrysippus to study the differences relating to growth and metamorphosis. At metamorphosis, it is generally accepted that haemolymph proteins act as a store of protein (Wyatt, 1961).

The polyacrylamide gel disc electrophoresis revealed six protein fractions with no evidence of stage specific fractions in Dysdercus similis. The concentration in all the fractions fluctuates independently during the nymphal stages.
and adult development. Protein fractions 1, 2, 5 and 6 show oscillations in the concentration during the development and transformation of third, fourth and fifth instar male nymphs. During the nymphal development in females the concentration in all the protein fractions except for fraction 2, fluctuates with the growth and molting. While the transformation from fifth instar to adult female involved three protein fractions i.e. protein bands 4, 5 and 6, only one protein band, namely fraction 6, appears to be involved in the transformation to adult males from fifth stage male nymphs. Laufer (1960) in many saturniid moths, Schmidt (1965) in Formica polyctena and Hiroshi (1968) in Bombyx mori have also discussed the development and sexual differences of haemolymph proteins. As has been noted for Dysdercus similis, a stage specific protein has not been reported for other insects like Oncopeltus fasciatus (Terando and Feir, 1967), Rhodnius prolixus (Coles, 1965), seven species belonging to four genera of Triatomidae (Van Sande and Karchar, 1960), and others.

A maximum of twelve protein bands in females and eleven in males could be observed in the haemolymph samples of Sarcopha lineatocollis. A reduction in the concentration of protein fractions 5, 9 and 11 has been observed from early to late larvae. During the transformation from larvae to pupae seven protein fractions are involved. The drop in the concentration of protein fractions 2, 3, 5 and 11 has
not been so pronounced, but it has been very significant in protein fractions 1, 4 and 6. An almost similar situation has been observed in the studies in Rothschildia orizaba and Malacosoma americanum (Toughton and West, 1965), Phormia regina (Chen and Levenbook, 1966), Samia cynthia ricini (Masako, 1968), Bombyx mori (Kobara and Kawai, 1969).

As the pupa darkened, a further drop in the concentration of protein fractions 1, 2, 3, 4, 6 and 11 is noted. Protein fraction 5 disappeared from the haemolymph in the late pupal stage.

During the transformation from late pupal stage to adult female, the concentration in protein fractions 4 and 6 dropped further, along with a decline in the concentration of two other protein fractions, i.e., 8 and 10. Protein fraction 5 which has not been observed in the haemolymph from late pupal stage appeared again in the haemolymph of newly emerged insects. Protein fraction 1 has been found to be missing in newly emerged males and females. In newly emerged males protein fractions 6, 7, 8, 9 and 10 have got less concentration. Similarly in just emerged females, concentration in fractions 6, 8 and 10 have been observed to be low. In females, protein fraction 7 does not show any oscillations throughout the larval pupal moulting and also during adult emergence.

In Danais chrysippus, characteristic protein bands in the larval, pupal and adult stages have been observed.
In early third stage larvae, a maximum of 9 protein bands could be seen. A reduction in the concentration of protein fractions 4, 5, 7 and 10 has been observed from early third stage larvae to late larvae (one day before moulting to pupae). An increase has been observed in the concentration levels of fractions 2, 3, 6 and 9, while no oscillation could be seen in the concentration of fraction 11. A new band appears in the late larvae (one day before moulting to pupae).

The transition from larvae to pupae involved protein fractions 2 and 11. While an increase in the concentration has been noted in the fractions 1, 3, 4, 5, 7, 9 and 10, the concentration in fraction 6 does not fluctuate. A new protein fraction has been observed, i.e., fraction 12 in the newly hatched pupae. As the pupae darkened, a drop in the concentration of protein fractions 6 and 12 has been noted. A rise has been observed in the concentration levels of protein fractions 2, 3, 4, 5, 10 and 11. Fractions 1 and 9 have been observed to be missing and, instead of these two, a new band could be seen, i.e., fraction 8. No change could be seen in the concentration level of fraction 7.

The transformation from pupae to adults, both in males and females, involved different protein fractions. During the transformation from pupal to adult females, the concentration declined in five protein fractions, namely, fractions 4, 5, 7, 8 and 11, while transformation from pupae to adult males involved six protein fractions 2, 4, 5, 6, 8 and 10.
During the development from larvae to adult, it can be seen that protein fraction 1 (Rm 3-4) and fraction 9 (Rm 64-66) are found only in larva and pupa. Fraction 12 (Rm 85-87) has been noted only in pupae.

From these observations, it is clear that in *Dysdercus similis*, the stage specificity in each instar and during the adult development is shown by the fluctuations in the concentration of different protein fractions, while in *Sarcophaga linnaeiocollis* this has been shown by the total disappearance of haemolymph protein fraction 5 in the late pupal stage and fraction 1 in the newly emerged males and females.

In *Danais chrysippus* this has been shown by the appearance and disappearance of the protein fractions during larvae, pupae and adult stages. Protein fraction 1 (Rm 3-4) in the late larvae (one day before moulting to pupae), fraction 12 (Rm 85-87) in the newly formed pupae, and fraction 8 in the late pupae (with darkened pupal case) appeared for the first time. In the late pupae, fractions 1 and 9 have been found missing, while in the newly hatched adults, fractions 1, 9 and 12 are found missing from the haemolymph.

Wyatt (1968) suggested that the differences not found in the protein picture of a bug can be correlated to the hemimetabolous nature of the development. This phenomenon appears to be true for *Dysdercus*, although it does not hold
true for all the insects having hemimetabolous type of
development; for example, in *Haemopota cinerea* (Adiyodi,
americana* (Steinhaur and Stephen, 1959; Siakotos, 1960 a,b),
stage specific proteins have been observed.

Characteristic protein bands in larvae, pupae, and
adult stages have been observed in a number of holometabolous
insects like *Phormia regina* (Chen and Levenbook, 1966),
*Tenebrio molitor* (Po-Chedly, 1959; Butler and Leona, 1966;
Laverdure, 1972), *Drosophila melanogaster* (Chen, 1959; Duke
and Fantelouris, 1963), *Ostrinia nubilalis* (Chippendale and
Beck, 1966), *Protonotera quinquimaculata* (Hudson, 1966),
*Calliphora* (Munn and Greville, 1966) and others.

But there are still other insect species with the
holometabolous type of development, where stage specificity
in the protein picture has not been observed, a situation
found and expected for the hemimetabolous insects. Masako
(1968) in *Samia cynthia ricini*, and Reno (1971) in the two
species of *Lentinotarsa*, did not find any stage specific
proteins during development.

As far as the author is aware, only three hemipteran
species have been investigated for the qualitative protein
fractionation of the haemolymp. Although from these studies,
it is very difficult to draw any general pattern regarding
the presence and the absence of the stage specific protein or
proteins, it is suggested that in this insect order i.e. Hemiptera, no stage specific protein is present.

The oscillations in the concentration of various fractions at specific stages, can be correlated to the utilization of different fractions at the particular stage in the morphogenesis and in the laying of new cuticle. Support for this suggestion comes from the observation of Radomir (1971) in Dizippus morosus in which the sharp decline in the two glycoprotein fractions occurring during the moult may obviously reflect the formation of new cuticle. Willis (1970) in cecropia silk worm, and using immunodiffusion technique in Periplaneta americana, Fox et al. (1972) observed that one or more proteins in the cuticle are identical with the specific haemolymph proteins suggested that haemolymph proteins traverse the epidermal cells to be incorporated into the newly formed cuticle. In a very recent study using radio tracer and electrophoretic technique in Manduca sexta (Koenne and Gilbert, 1973), it has been proved that the haemolymph carrier proteins are indeed incorporated into the cuticle.

According to Coles (1965) and Tobe and Loughton (1967), the haemolymph proteins once in the epidermal cells could not be secreted unchanged as the part of the newly deposited cuticle. It is proposed that these proteins, after entering the epidermal cells are probably synthesized and modified in such a manner that they are identical with the haemolymph
proteins and then are incorporated into the cuticle. It is also possible that epidermal cells possess protein synthesizing machinery, secreting the same proteins into both, cuticle and haemolymph, supporting the view of Locke and Krishnan (1971) and Koenne and Gilbert (1973).


In *Sarcophaga lineatocollis* five protein fractions 1, 2, 3, 4 and 6 are accumulated in the haemolymph in the late larval instar. A drop in the concentration of these fractions along with fractions 5 and 11 has been observed at the time of moulting from late larval stage to pupal stage. These proteins are probably taken up by the cuticle and fat body, and some of them are used up in differentiating tissues. The accumulation of proteins by the fat body has also been observed in *Pieris brassicae* (Chippendale and Kilby, 1969) and *Malacosoma americanum* (Loughton and West, 1965).
A further drop in the concentration of protein fractions 1, 2, 3, 4, 6 and 11 in the late pupal stage can be explained by assuming that these proteins are taken up by developing and differentiating tissues like gonads etc. Fraction 5 does not appear in the fractionation of haemolymph from late pupal stage, and is probably utilized in the darkening of the cuticle. The probable utilization of this fraction in the formation of adult cuticle cannot be ruled out. The disappearance of fraction 5 can also be explained by keeping in mind the suggestion of Boyd and Mitchell (1966) that the fractions undergoing rapid turnover after puparium formation, are being degraded to provide substrates for the synthesis of other haemolymph proteins.

Similarly, in Danais chrysippus also a decline in the late larval stage, one day before moulting to pupae, has been observed in the protein fractions 4, 5, 7 and 10. These proteins are probably used by the differentiating tissues. A new protein fraction i.e., fraction 1 appeared during this stage. In newly hatched pupae, concentration declined in the protein fractions 2 and 11. These fractions are taken up by the newly laid pupal cuticle. Fraction 12 appeared for the first time in this stage.

In the late pupal stage with darkened pupal case, two protein fractions have been observed to be missing, fractions 1 and 9. The concentration decreased in two other
fractions, fractions 6 and 12. These proteins are probably used up in the differentiating adult cuticle and other tissues. Protein fraction 8 has been observed for the first time in this stage.

In newly emerged females the concentration has been found to have declined in the protein fractions 4, 5, 7, 8 and 11 while in newly emerged males the concentration has declined in fractions 2, 4, 5, 6, 8 and 10. These fractions are probably utilized for the incorporation into the adult structures.

Considering the differences in protein picture with reference to the concentration of proteins, of the male and female nymphs and adults of *Dradeecus similis* and in the newly emerged males and females of *Sarcophaga lineatocollis* and *Danais chrysamus* it can be inferred that different protein fractions are involved during the moulting of both the sexes. Each stage of the life cycle is characterized by definite and specific protein bands, and the blood of immature stages can be used to distinguish the sex of the particular insect. This type of protein differences in the immature stages has been reported by Laufer (1960) in *Cecropia* and *Cynthia* silk worm, Hiroshi (1962) in *Bombyx mori* and *Formica polyctena* by Schmidt (1965).

As regards the presence of "Common Insect Protein" of Whittaker and West (1962), fraction 3 in *Dradeecus similis*,


Sphaerodema rusticum and Sarcophaga lineatocollis, and fraction 1 in Danaus chrysippus appear to be the "Common Insect Proteins". In all the four insect species, the general staining density and relative mobility pattern suggest that these fractions are probably homologous. According to Whittaker and West (1962) the members of class insects may have a common protein in the haemolymph although this homology is not an established fact. These proteins may be constituting a category of proteins, similar in function, if not in origin. They suggested that these proteins might be contributing to the colloidal osmotic pressure of the insect haemolymph provided that the dye staining density of these bands represents a large concentration of protein molecules of low molecular weight. The presence of such a common insect protein with a low molecular weight has been reported in Periplaneta americana (Stephens, 1956; Adiyod and Nayar, 1968); Musca domestica (Bodnaryk and Morrison, 1966); Galleria mellonella (Denuce, 1958); Phalophora cactoria and Samia cynthia (Laufer, 1960); Phormia regina, Smerinthus carisi, Placotoma minor, Blatta orientalis, Oncopeltus fasciatus (Stephens, 1956) and Locusta migratoria migratorioides (Tobe and Loughton, 1967, 1970). In many other insect species, workers have not mentioned the presence/absence of this type of protein in their studies.

As it is already known, the insect haemolymph acts as a store of protein, and various fractions accumulate in the
haemolymph at specific stages to provide raw material for
the growth and development. Hence a high concentration in
different fractions is expected at different periods of the
life cycle. The haemolymph protein fractions 1 to 4 in males
and females of *Dysdercus similis*, fractions 2 to 6 in females
and 1 to 5 in males of *Sarcophaga luteocollis*, fractions
2, 3, 4, 5 and 9 in females and 2, 3, 4, and 6 in males of
*Denalis chrysinae*, and fractions 3, 4 and 5 in females and
protein fraction 3 in males of *Sphaerodema rusticum* are
"Major Blood Proteins", as these proteins are deeply stained
with protein dye. The concentration in these protein fractions
is moderately high, and they show maximum activity i.e., they
fluctuate with the development and other physiological activi-
ties. The high concentration in different fractions at each
developmental stage probably helps in bringing up the osmotic
pressure of the body fluid to a reasonable level which is
needed for the normal functioning of the insect body.

In all the four insect species, one significant
observation is that a protein fraction with a mobility
comparable to that of a mammalian serum albumin could not
be observed. The absence of such a protein is consistent
with the earlier observations on *Drosophila melanogaster*
(Wunderly and Gloor, 1953; Chen, 1958); *Culex* (Chen, 1959);
*Onconelthys fasciatus* (Terando and Feir, 1967); *Periplaneta
americana* (Siakotos, 1960 a,b); *Melolontha* sps., *Neoclinion
gertiff* and *Lymantria dispar* (Fries, 1956). In *Phormia regina*
Chen and Levenbook (1966) also could not detect, by amidoschwartz staining, a protein with \( R_m \) value comparable to human serum albumin, but an albumin band was found present when a mixture of serum and haemolymph was run on a single gel.

In some other insects like *Bombyx mori* (Duccechi, 1902; Drilhon, 1954); adult *Hydrometopa piceus* (Florkin and Duchateau, 1943); larval honey bee (Bishop et al., 1925); three Lepidoptera species (Clark and Ball, 1956); *Musca domestica* (Bodnaryk and Morrison, 1966); *Sobistocerca gregaria* (Kulkarni and Mehrotra, 1970); seven species of Lepidoptera and one Hymenoptera species (Whittaker and West, 1962), a protein comparable to that of human or mammalian serum albumin has been found to be present.

At the present stage of our knowledge, it does not appear justifiable to characterize the haemolymph protein bands as either albumin or globulins. The classification of insect haemolymph proteins will be possible only when more accurate physiological and biochemical data become available, using more adequate purification procedures (Whittaker and West, 1962; Chen and Levenbook, 1960; Kulkarni and Mehrotra, 1970).

No differences between immature stages and adult haemolymph of both sexes in *Dyaderrus similis* could be seen when investigated for the presence of female sex
specific protein. In both the sexes six protein fractions are present in *Dysdercus similis*. The sex specific differences in the qualitative protein picture were also not found in other insects like *Rhodnius prolixus* (Coles, 1965), *Onconemus fasciatus* (Terando and Feir, 1967); *Tenebrio molitor* (Pemerick and Butz, 1970); *Anisolabis littorea* (Leader and Bedford, 1972). A distinctly sex specific protein was also not observed in *Protonarca quinquimaculata* (Hudson, 1966).

A protein associated with sexual dimorphism has been observed in the haemolymph of *Hyalophora cecropia* (Telfer and Williams, 1953; Telfer, 1954, 1960), many saturniid moths (Lauffer, 1960), *Naunboeta cinerea* (Adiyodi, 1967); *Rothschildia oryzaba* (Loughton and West, 1965). This protein i.e. female sex specific protein, although present in the male haemolymph, occurred in much higher concentration in that of the female. According to Telfer (1954), this particular antigen band which has higher concentration in the blood of females and also found in the egg yolk might be acting as a means of transport for the lipid material. It seems very likely that there is a quantitative adjustment of protein in the two sexes of such insects, as has been suggested by Stephen and Steinhaur (1957).

In *Sphaerodema rustigianum* protein fraction 4 (Rm 14-16) is an extra protein fraction found only in females; hence
it is female sex specific protein, a feature comparable to that of *Triatoma infestans* (Perassi, 1973). In *Sarcophaga lineatocollis*, no differences, between the sexes, were noted in the protein fractionation study, in immature stages, newly emerged and in one day old adults. The first appearance of female sex specific protein was noted from the second day of the adult life. This is in agreement with the findings of Wilkens (1968, 1969), Engelmans et al. (1971) on *Sarcophaga*, where the haemolymph was fractionated immunologically. In *Danais chrysinnus*, two extra proteins i.e. fraction 5 (Rm 23-26) and fraction 11 (Rm 88-91) have been observed in the haemolymph from two-days-old adult females. These two female sex specific proteins are found to be missing from the haemolymph of newly emerged females and from the haemolymph of males of any age. This result is comparable to the observations in *Schistocerca gregaria*, where Kulkarni and Mehrotra (1970) have observed two female proteins, in *Triatoma infestans*, Perassi (1973) observed two female antigenic components by immuno-chemical assays, and in *Lentinotarsa dejeani* de Loof and de Wilde (1970) have observed three sex specific proteins.

The observation that in *Sarcophaga lineatocollis* and *Danais chrysinnus* the female sex specific protein or proteins are detected late in the haemolymph is consistent with the observations in other insect species like
Leucophaea maderae (Engelmann and Penney, 1966; Engelmann, 1965; Wyss Ruber and Luscher, 1972), Schistocerca gregaria (Engelmann et al., 1971), Aedes aegypti (Roth and Porter, 1964; Nagedorn and Judson, 1972), Triatoma infestans (Perassi, 1972). The sex specific proteins in these insects could be demonstrated only during the period of oocyte maturation.

The presence of sex specific female protein/proteins could be observed irrespective of vitellogenesis in the insects like Locusta migratoria migratorioides (Tobe and Loughton, 1967), Formica polyctena (Schmidt, 1965); Masonia vitripennis (King et al., 1972); Gryllus domesticus (Kunz and Petzelt, 1970); Musca domestica (Bodnaryk and Morrison, 1968), Periplaneta americana (Adiody and Nayar, 1966; Thomas and Nation, 1966; Bell, 1969; Prabhu and Rama, 1970; Prabhu and Nayar, 1970), Aulacophora foveicollis (Shukla and Gupta, 1974).

The concentration in various protein fractions showed oscillations during the adult life of the insects with respect to their physiological needs. The rise in the concentration of various fractions during the early days of adult life in Dysdercus similis may be attributed to the continuous feeding behaviour of this species, and to the release of proteins from the protein synthesizing and accumulating tissues like fat body etc. Likewise, in Sarconbaza lineatocollis and Danaus chrysippus also, the concentration rose in different fractions during the early period while a drop in the concentration of
different protein fractions has been observed during the later period. These proteins are probably utilized in the histogenesis and also in the various physiological activities related to the reproduction. Some of these proteins are probably decomposed to peptide and amino acid level, and as such utilized in the various physiological processes of the body. They do not disappear completely at any time during adult life, i.e. they remain in traces. Their total decomposition and complete mobilization is probably not needed.

In addition to maintenance, the dominant phenomenon in the life of adult insect is reproduction. In the female, there is a continuous deposit of yolk at the time of egg production, whereas in the male, no such demand is made for haemolymph reserves like protein etc. at any time. It seems reasonable, therefore, to expect some differences in the protein metabolism between the two sexes.

Very little work has been done so far to find the different protein fractions which are essential for the physiological processes in connection with the male reproductive cycle. Bodnaryk and Morrison (1966) working on *Musca domestica*, showed that the three protein fractions accumulated in the haemolymph of milk-fed male and suggested that they are perhaps more essential to the overall protein metabolism of the males. Linský and Kalinsky (1971) reported
the presence of three haemolymph proteins in the reproductive organs - seminal vesicle, mucous gland and penis of honey bee drones. They also established that the haemolymph proteins of low molecular weight are selectively taken up by these organs. Shukla and Gupta (1974) have reported the presence of male sex specific protein in Aulaconhora foecicola.

In the present study some protein fractions have shown oscillations in their concentration with respect to the physiology of reproduction in males. In Dystiscus similis fractions 2 and 4, in Sarcophaga lineatocollis fractions 5, 6 and 10 and in Danais Chrysonus protein bands 7, 8 and 9 fluctuated in relation to the reproductive cycle in males. These proteins are thus named "Male Sex Linked Proteins".

There is considerable difference of opinion concerning the mode of yolk formation in insects. It has been found through cytochemical findings that yolk proteins can be synthesized within the ovary (King et al., 1972). The possibility of incorporation of foreign body or protein outside the ovary was reported for the first time by Wigglesworth (1943). The proposed incorporation of blood proteins as suggested by Wigglesworth has been found in many insects, for example - Pyrrhocoris apterus (Slama, 1964); Protoparce quinquimacula (Hudson, 1966); Rothschildidia orizaba and Malacosoma americanum (Loughton and West, 1965);
Phormia regina (Chen and Levenbook, 1966); Schistocerca gregaria (Hill, 1962; Vukarni and Verhotra, 1970).

In some insect species, the sex specific/limited female protein appears late at the time of vitellogenesis. The "de novo" synthesis of female protein/protiens has been reported for Lernophaea maderae (Engelmann and Penney, 1966); Locusta migratoria migratoroides (Tohe and Loughton, 1967); Musca domestica (Bodnaryk and Morrison, 1968); Sarcophaga bullata (Wilkens, 1960; Engelmann et al., 1971). In the present study, both in Sarcophaga lineatocollis and Danais chrysippus, the sex specific protein fractions are synthesized "de novo", at the time of vitellogenesis.

In the present study, certain protein fractions clearly show oscillations with respect to the physiology of reproduction in females. These sex limited vitellogenic female proteins accumulated in the haemolymph before the yolk deposition process started in the maturing oocytes, and hence are named "Vitellogenic female proteins".

According to de Loof and de Wilde (1970), it is difficult to give a satisfactory definition for the term "Vitellogenic Protein". Every protein which is demonstrable in the egg yolk is vitellogenic. In most female insects of which the haemolymph has been investigated, only one sex specific protein has been found, but in the Colorado beetle Lentinotarsa decemlineata females at least three sex specific
proteins are present, and only one of these is vitellogenic in the sense of the definition given by de Loof and de Wilde (1970). A sex specific protein thus is not necessarily "Vitellogenic". In the present work, blood proteins showing oscillations in their concentration with the stages in the maturing oocytes are considered to be the "Vitellogenic Female Protein".

In *Dysdercus similis*, all fractions to some extent acted as vitellogenic female proteins but oscillations have been more pronounced in the protein fractions 3 and 5. In *Sphaerodema rusticum* protein bands 3, 4, 5, 7 and 8 fluctuated with the demand of the ovary. A similar condition has been reported for *Rhodnius prolixus* (Coles, 1965), *Periplaneta americana* (Mills et al., 1966; Menon, 1966; Prabh and Nayar, 1970, 1972); *Triatoma infestans* (Perassi, 1973).

In *Sarcophaga lineatocollis* the concentration in protein fractions 2, 3, 4, 9 and 12, and in *Danais chrysippe* the concentration in protein bands 2, 4, 5, 9 and 11 fluctuated with the reproductive cycle. The other insects in which the vitellogenic female proteins have been observed are *Fyalophora* (Telfer, 1954, 1960, 1961, 1965); *Tenebrio molitor* (Pennick and Butz, 1970); *Birisia brassicae* (Jamy, 1967); *Ostrinia nubilalis* (Chippendale and Kilby, 1967).

The total protein concentration was estimated during the different developmental stages and in the adult life.
In *Dysdercus similis*, a gradual rise in the haemolymph protein concentration has been observed during the three nymphal instars studied i.e., third, fourth and fifth. Similar results have been reported in other hemimetabolous insects like *Rhodnius* (Coles, 1964, 1965); *Oncomelius fasciatus* (Terando and Feir, 1967; Bassi and Feir, 1971); *Locustamigratoria migratorioides* (Winks, 1967; Hill and Goldsworthy, 1970). In males, the concentration is found to be slightly higher during the third and fourth instar, dropping slightly in the fifth instar males. The gradual rise during the nymphal stages is due to the continuous feeding habits of the insects. The drop in the protein concentration in the fifth instar male nymphs is attributed to the probable utilization of proteins in the morphogenesis and histogenesis process, specific to the male physiology.

In newly emerged males, the protein concentration in the haemolymph is slightly higher than in the females. The concentration dropped both in one-day-old males and females. The observations on the haemolymph protein concentration of male and female adults, agree with the findings of Prabhu and Nayar (1971) and Zalaja and Prabhu (1971) on *Dysdercus cingulatus*. The slight differences noticeable are probably due to the differences in rearing methods, nutrition and other factors. The protein concentration rose up to three days of age both in males
and females, although the concentration in the male remained less than that of the protein concentration level of the female, while in the newly emerged male it was higher than the concentration in the female.

The concentration observed in three-days-old females is almost double in the value observed in three-days-old males. The drop in the haemolymph protein concentration is more steep in females on the fourth day, as compared to the males of the same age. This drop in the concentration can be correlated to the vitellogenesis and to the possibility that all the excess proteins were apparently utilized by the growing oocytes, confirming the observations of Prabhu and Nayar (1971), Zalaja and Prabhu (1971), Gupta (1971).

As regards the protein concentration in males, it is almost parallel to that of females. The rise and subsequent fall in the blood protein concentration in the males could be due to their being used for the reproductive physiology of males, like spermatogenesis, accessory gland secretion etc. The differences in the concentration of haemolymph proteins of the sexes can be explained by the fact that the testicular tissue growth requires a far smaller consumption of reserve material when compared with the corresponding requirements of the growing oocytes (Tlama, 1964).

These oscillations in the haemolymph protein concentrations are repeated again during the second
reproductive cycle, which is extended from the seventh day of adult life up to the thirteenth day of age. The concentration gradually increased to a maximum level for the second time in ten-days-old adult female, and then declined with the active growth of oocytes i.e., during vitellogenesis up to the thirteenth day. Prabhu and Mayar (1971) and Zalaja and Prabhu (1971) have reported the protein concentration in haemolymph of Dysdercus sanguinolentus only for the first 7 days of adult life.

Similarly a high protein concentration was observed in the young females of Sphaerodema rusticum. This concentration dropped to a low level during the oocyte maturation period, as the proteins are incorporated into the developing oocytes as yolk proteins. After the yolk deposition is completed, a rise in the haemolymph protein concentration was observed.

In Sarcophaga lineatocollis, the total protein concentration increased from the early to late maggot stage, probably because of the continuous digestion process and also due to the increase in the blood volume (Coles, 1965). During the pupal stages, the noticeable drop in the haemolymph protein concentration is due to the utilization of proteins during the metamorphosis and in the laying of new cuticle. The haemolymph protein concentration dropped further at the time of the adult emergence. The fall in the haemolymph protein concentration during the
pupal stage can be explained also by keeping in mind the suggestion of Chippendale and Kilby (1962), Loughton and West (1965), that the released haemolymph proteins again start accumulating in the fat body for storage during this period, to make them available for further metabolic processes during subsequent days.

The haemolymph protein concentration, in *Danais chrysippus* during larval life, increased from early to late caterpillar as has been observed for *Sarcophaga lineatocollis*. The concentration dropped slightly in early stage pupae with a further drop in the late pupal stage. At emergence, the concentration was found to be still lower.

The feature of rise in the protein concentration in growing larvae, decrease in the pupae and during adult development and a further drop in the concentration at the time of emergence, is also shown in *Dielaphila euphorbia* (Heller and Moklowska, 1930); *Evalophora ceratonia* (Chefurka, 1953); *Papillia japonica* (Juddi s, 1954); *Bombus mori* (Wyatt et al., 1954); *Phormia regina* (Chen and Levenbök, 1966); *Calliphora* (Mann and Greville, 1969); *Dixippus morosus* (Radomir, 1971); *Pieris rapae* (Kim, Lee and Kim, 1969); *Leptinotarsa dec.* (Reno, 1971).

Wyatt (1961) suggested that the haemolymph proteins are an important source of amino acid for the synthesis of protein of the adult tissues. Here also the oscillations
in the protein concentration during growth and molting indicate that the proteins are utilized during metamorphosis in the histogenesis of the body organs.

The haemolymph protein concentration during adult life in relation to reproductive cycle of the fly *Sarcophaga bullata* has been reported previously by Wilkens, (1968, 1969). The results here, on the related species *Sarcophaga lineatocollis*, confirm his observations. The protein concentration in the haemolymph at emergence is low, dropping further up to the second day of the adult life. The haemolymph proteins are probably used up in various metabolic processes during this period. As the demand exceeded the protein synthesis, the haemolymph protein concentration was found to be low. According to Orr (1964), at least two days are required by the fat body to process the dietary proteins to make them available for the haemolymph in *Phormia regina*. Probably, the same mechanism operates in *Sarcophaga* also. The concentration then started rising again, and a maximum protein concentration was observed on the third day of the adult life. The concentration then declined from three-day onwards up to 5 days of age. The haemolymph proteins are incorporated in the oocytes for the formation of yolk in the developing ova, during this period. From 5 days to 7 days of age, there is an increase in the concentration of haemolymph proteins.
In males, although no marked oscillations could be observed in the haemolymph protein concentration, still a maximum concentration has been noted on the third day of adult life, dropping slightly on the fourth day. As very little quantity of metabolites is required for the formation and maturation of spermatozoa, oscillations in the protein concentration are also not expected.

In Denaia chrysirinus, the concentration rose to 5.87±0.26 g/100 ml in two-days-old imagos. This rise can be attributed to the synthesis and release of proteins at a higher rate, to build up the protein concentration to such a level as to provide sufficient raw material for the yolk formation. A drop in the protein concentration has been noted on the fourth day i.e., at the time of vitellogenesis.

As noted in the other insect species, during the present investigation, although a cyclic pattern does exist, no very marked oscillations in the protein concentration could be observed in the male adults at the time of spermatozoa formation.

Similar oscillations in the protein concentration related to the female and male reproductive cycle have been observed in Locusta (Tobe and Loughton, 1967); Schistocerca gregaria (Hill, 1962; Hill et al., 1968; Pinamonti et al., 1966; Kulkarni and Vehrotra, 1970); Pyrrhocoris apterus
(Slama, 1964); *Leuconhaea maderae* (Engelmann and Penney, 1966); *Dermestes frischii* (Küthe, 1972); *Periplaneta americana* (Menon, 1964; Mill, et al., 1966; Bell, 1963).

Very little is known about the concentration and composition of haemolymph lipids in insects. Most of the available information on the composition of lipid is from the studies carried out on whole insects (Gilbert, 1967). Reviews by Wyatt (1961) and Florkin and Jeuniaux (1964) also could not give full attention to this aspect of insect biochemistry. Lipid metabolism has been studied without much reference to haemolymph lipid, by Gilmour (1965), Fast (1964), Gilby (1965) and Gilbert (1967).

The lipid concentration in the haemolymph was reported for *Bombyx mori* larva (Sridhara and Bhat, 1965); *Acheta domesticus* (Nowosielski and Patton, 1965; Weng and Patton, 1969); *Galleria mellonella* larva (Wlodaver and Wisniewska, 1965); Queen *Macrotermes natalensis* and *Macrotermes solitans* (Omılık, 1969); *Pyrhocoris apterus* (Martin, 1969); *Popillia japonica* (Ludwig and Wiegmeister, 1953; Bennett and Shotwell, 1972) with some details.

From the available literature on the total lipid concentration in the haemolymph, it is clear that no efforts have been made so far to get a clear picture of fluctuations in the lipid concentration in the insect haemolymph throughout the developmental period and during the adult life.
In the present study, the total lipid concentration has been estimated in the different developmental stages and during the adult life of *Dysdercus similis*, *Sarcophaga lineatocollis* and *Danais*, while the lipid concentration has been observed in relation to the reproductive cycle in *Sphaerodema*.

In *Dysdercus similis*, the total lipid concentration increased from third to fourth instar, and dropped during the fifth instar stage, both in males and females. No very marked sexual dimorphism could be observed in the lipid concentration during nymphal stages. In this it has behaved like protein concentration. The drop in the lipid concentration in fifth stage nymphs suggests that it has been preferentially utilized for the energy production and also in the morphogenesis of the adult structure.

From newly emerged stage, the concentration starts rising again both in males and females and reached a maximum value of $2.24 \pm 0.12$ gm/100 ml in females and $3.16 \pm 0.01$ gm/100 ml in males on the third day. From the fourth day onward up to the sixth day, the concentration decreased, but this drop in the concentration has been more pronounced in the normal female. These results clearly show that the oscillations found in the lipid concentration follow the haemolymph protein concentration pattern, and behave in the same manner. The concentration started rising again, both in the males and females, after the seventh day of
adult life. The concentration reached a maximum on the tenth day and declined thereafter, i.e., followed the same pattern during the second reproductive cycle. During the second reproductive cycle the lipid concentration was found to be low as compared to the first reproductive cycle. The decrease in the lipid concentration from the fourth day to the sixth day can be related to the reproductive cycle. The haemolymph reserves during this stage, are taken up for the formation of yolk. The same story has been repeated in the second reproductive cycle. In their studies on the total and neutral lipids of the whole body of *Dysdercus koenigii* (Agrawal and Rao, 1969) could not detect any regular increase in the lipid content with the moulting cycle and age.

In *Enhaeredema rustigum* the total lipid content has been found to be higher than what has been observed for the other three insect species, during the present investigation. The concentration dropped in females during oocyte maturation. In the female, after oviposition a slight increase in the lipid concentration has been observed. The observed fall in the lipid concentration can be explained by their drainage into the maturing oocytes.

In *Sarconophaga lineatocollis* a gradual increase in the lipid concentration of the haemolymph was noted during the maggot stages and in the early pupal stage. During the late pupal stage, the lipid concentration decreased in the haemolymph. The haemolymph lipids, during this stage, have
probably been used for energy production, laying of the new cuticle and in the histogenesis of the adult structures.

In newly emerged males, the haemolymph lipid concentration has been found to be more than that of the females. The reason might be that the females utilized far more of their stored lipid during the transformation from the larval to adult structures.

Lipid concentration was found to be maximum in three-days-old adults, and then it decreased. The decrease was more pronounced in females than in males. The haemolymph lipids during this stage are probably utilized for egg maturation. The most energy-consuming processes in the insects are movements like flight, oogenesis, embryogenesis and moulting, particularly at metamorphosis.

In Danais chrysippus, the haemolymph lipid concentration gradually increased during the caterpillar stages, followed by a gradual decrease during the pupal stages. These results are similar to those observed for Sarcoptes lineatocollis. The lipids, during the immature stages, have been utilized both as an energy source and also for the synthesis of the new cuticle during metamorphosis.

The sexual dimorphism in the lipid content was reported in the studies of whole larvae, pupae and adult, and the condition seems peculiar to Lepidoptera (Gilbert, 1967). In the present investigation, the sexual dimorphism in the
haemolymph lipid concentration has been noted in the newly emerged adults where male haemolymph contained more lipid than the female haemolymph. The females appeared to have used more of their stored lipids during metamorphosis than did the males. This dimorphism in the lipid content of the haemolymph was evident throughout the adult life, the male haemolymph containing more lipid than the females, as has been observed by Gilbert (1967).

The fluctuations in the lipid concentration during the adult life can be related to the reproductive cycle of the insects. During adult development, the females utilize far more lipid than the males. Females convert a large percentage of their endogenous substrate into egg (yolk, cytoplasm, chorion, etc.).

The monarch butterfly is a continental migrant and also utilizes lipid during flight. Prior to migration this species lay down a store of fat as do migratory birds (Beall, 1948). Since its food is only nectar which does not contain lipid, it evidently converts carbohydrates to lipid during the period of repose and then uses it during flight. During the present study, the butterflies were kept in cages and were fed on sugar solution and fresh nectar. They were prevented from flying large distances. The high lipid concentration during the adult life in males can be explained by the fact that the prevention of flight results in the conservation of lipid (Dowroese and Gilbert, 1964).
The growth of the oocytes has been taken as a criterion to measure the reproductive physiological growth of the female, by observing the length of the last oocytes. In normal mated females, a gradual increase in the size of the last oocyte has been noted in all the four insect species studied. The growth rates were found to have increased during active yolk deposition period. The length of mature terminal oocyte was found to be 1.2 mm in Dysdercus similis, 2.1 mm in Sphaerodema rusticum, 1.84 mm in Sarcophaga lineatocollis and 1.52 mm in Danais chrysippus. The increase in the size of the last oocytes has been noted from the very first day after emergence. During early days of adult life, the same could not be reflected in the protein fractionation studies and in the haemolymph protein and lipid concentrations. During this period, the protein synthesis and lipid turnover rate appeared to be higher than their drainage into the maturing oocytes; hence a noticeable high concentration of haemolymph metabolites was found in spite of the steady growth of the oocytes. Later on with the increase in the length of the oocytes the concentration of lipid and protein in the haemolymph dropped. The concentration of the vitellogenic female proteins fluctuated as the oocyte matured. The results clearly indicate that the haemolymph protein and lipid concentration and the growth of the oocytes are correlated with each other. The concentration of the nutrients (Protein and lipid) reflects the balance between protein synthesis and lipid concentration in the haemolymph
and their drainage into the maturing oocytes. Firstly, the concentration of both haemolymph proteins and lipids increased and then, with the increase in the size of oocyte length, the concentration of haemolymph reserves declined. These results are in agreement with findings on Sarcophaga bullata (Wilkens, 1968, 1969); Musca domestica (Bodnaryk and Morrison, 1966, 1968); Locusta migratoria (Wigmans et al., 1968), and others.

It has been reported for a number of insect species that the mating not only influences egg maturation but also often stimulates oviposition (Engelmann, 1970). Mating was found to be absolutely essential for full maturation of the oocytes in thysanuran Thermobia domestica (Watson, 1964). More eggs are laid by the mated females in Melanoplus bilituratus (Riegert, 1965) and Cimex lectularius (Davis 1964).

Here in Dysdercus similis, the protein fractionation studies of the haemolymph from virgin females of three to thirteen days of age, showed that the protein fractions have oscillated independently during the period of vitellogenesis. Although egg laying occurred, the virgin females have laid eggs "reluctantly" and the number of eggs laid are also less. All protein fractions, particularly fractions 3 and 5, which have shown clear oscillations in the concentration in the normal females, behaved in the same manner in the virgin females. The concentration in all the protein fractions has been observed to be low when compared to the concentration
values of the protein fractions for normal females.

The total haemolymph protein and lipid concentration also have followed the pattern, similar to what has been observed for the normal females, but the concentrations have been found to be low when compared to the concentration values of the normal females.

During the second reproductive cycle, the concentration of these haemolymph metabolites was found to have been much affected. No egg laying occurred during the second cycle in the virgin females. Although the length of the last oocyte had increased, it was half the length found for the normal females. The condition seen here is comparable to that found for *Rhodnius prolixus* (Buxton, 1930; Coles, 1965; Davey, 1965; Khalifa, 1950; Pratt and Davey, 1972), where the first wave of oogenesis proceeds at almost the same rate in mated and virgin females, although only two thirds of the eggs had been laid, whereas the second wave is inhibited in the virgin.

Other documented evidences on the effect of mating come from some more hemimetabolous insects species, where mating accelerates egg maturation and thus increases the total number of eggs laid during the lifetime, for example, *Periplaneta americana* (Roth and Willis, 1965); *Diploptera* (Stay and Roth, 1958; Engelmann, 1959; Roth and Stay, 1961). *Leucophaga* (Engelmann, 1960; Roth and Stay, 1962);
Nauphoeta cinerea (Roth, 1964); Schistocerca gregaria (Morris, 1954; Hamilton, 1955; Hignam and Haskell, 1964); Locusta migratoria manitensis (Mo, 1950).

In virgin females of Sarcoptes lineatocollis, the protein fractionation studies have shown that the oscillations in the concentration of the vitellogenic female proteins were similar to those observed for the normal females, although fluctuations were not so pronounced. The total protein and lipid concentration in the haemolymph dropped at the time of oocytes maturation, as was seen in the normal females. The concentration of these metabolites in virgin females has been found to be less than that of the normal females, and hence the growth of the terminal oocyte was also found to have been affected, although the length of the last oocyte had increased with age both in the mated and virgin females. In virgin females, the terminal oocyte measured 1.36 mm, whereas in the normal females it was 1.82 mm in length. The virgin females laid a few first instar larvae, that too, "reluctantly", as compared to the normal females in the first and subsequent brood. The laying was delayed in the virgin females. It appears that a certain level of protein and lipid concentration is necessary to enhance the complete oocyte maturation and oviposition. In other words, mating in some way controls the synthesis and release of the haemolymph metabolites governing in turn the normal growth rate of reproduction in the females.
The results in the present investigation on this dipteran species correspond to the findings on other insect species belonging to the order Diptera, for example, *Drosophila annaeophila* (Guyenot, 1913); *D. melanogaster* (Chiang and Hudson, 1950); *Musca domestica* (Hampton, 1952); *Lucilia cuprina* (Mackerras, 1933); *Culex pipiens* (Deduit, 1957); *Aedes aegypti* (Lang, 1956) and *Anopheles punctulatus* (Robert and O'Sullivan, 1948) where mating does not influence oocyte maturation but does influence oviposition.

In the majority of Lepidopteran species also, mating as such has nothing to do with the oocyte maturation. The short-lived lepidoptera notably noctuid moths, often has a full complements of eggs on the day of emergence. Virgin females of this order have also been reported to have retained their mature eggs until shortly before death. In *Cynthia, Acronia, Luna* and *Promethea silkworms* (Rau and Rau, 1914); *Bombyx mori* (Nokia, 1941); *Mamestra brassicae* (Bonnemaison, 1961) and in many others, mating is followed by a burst in egg-laying which indicates that eggs had simply been stored.

During the present study, in virgin females of *danaus chrysippus*, a gradual increase was noted in the growth of the last oocyte till it attained a length of 1.48 mm, slightly smaller than that of normal females on the fifth day. Here in this insect species, probably oocyte maturation progressed irrespective of whether females were
mated or unmated. In virgin females the concentration in the individual protein fractions was found to be low as compared to that of normal females, although they behaved in the same manner. All vitellogenic female proteins have shown oscillations comparable to those of normal females, although the concentration was found to have been low and the oscillations were not so pronounced.

As regards the total protein concentration, except for the slightly lower concentration, no very marked differences between the mated and virgin females were noted. The oscillations in the concentration have followed the same pattern as have been observed for the normal females.

The lipid concentration was found to have been affected by mating in this insect species. The concentration showed the oscillations, observed for the normal females at the time of vitellogenesis, but it could not reach that level and was found to be much less.