CHAPTER - SIX

PROTEIN AND PROTEASES ACTIVITY DURING ADULT DEVELOPMENT OF ARMYWORM *MYTHIMNA SEPARATA* (WALKER)

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CHAPTER SIX

I. INTRODUCTION :

1. Development of Adult :

The proceses of escape of the adult insect from the cuticle of the pupa is known as eclosion. While in hemimetabolous insects the escape of the last larval instar is observed eclosion. The thorax of the enclosing cuticle splits along a line of weakness which in the pupa is T- shaped. To produce the split, the adult swallows air to increase its volume and then further increases its thoracic volume by pumping blood forwards from the abdomen. The mouth in Lepidoptera and Dipetra with an obtect pupa is sealed by a strongly sclerotised plate so that the adult insect cannot suck air directly into its gut. However, although some of the spiracles of the pharate adult connect with the pupal spiracles others do not, but open beneath the pupal cuticle. It is thus possible for the insect to pump air out of the tracheal system into the space between the adult and pupal cuticles and this air can be swallowed so as to increase the volume of the body (Chapman, 1969).

The pupa is escaped by primitive method in Diptera in which the pupa, aided sometimes by body spines and sharp cephalic processes or cocoon-cutter, makes its way to the surface of the pupation medium from which its body projects while the adult escapes. the adult emerges within the cocoon or cell and escapes by using its mandibles or, as in most fleas, by means of imaginal cephalic cocoon-cutters In the Siphonaptera, Coleoptera, male Strepsitera and Hymenoptera (Richards and Davies, 1977).

Number of insects emerge mainly at particular times of day, often at night or in the early morning. This may have some adaptive significance in giving the insect some degree of protection against predators while it is vulnerable in the period before it is able to fly (Chapman, 1969).

The emergence of species is timed so that the life history is synchronized with suitable environmental conditions and so that the meeting of the two sexes is facilitated. The temperature is particularly important in this, since to a large extent it governs the rate of development and the activity of the insect. It is common for male insect to emerge as adult a little before the female although the difference is not great.

Adult Growth and Development :

The adult pterygote insect does not moult but it undergoes some further development after emergence. The colouration, thickening and hardening of the cuticle take a little time to complete and in the majority of insects there is a period of some days during which the gonads and accessory reproductive glands attain their full size and become functional (Richards and Davies, 1977).

The somatic growth consists mainly of internal changes associated with the flight muscles, cuticle and fat body. Broadly, these changes are concerned with ensuring reproductive success. Thus, the flight muscles develop their full potential in terms of internal structure, stores of fuel and full complement of enzymes in preparation of migratory or mating flight, and the fat body builds up stores of fat and/or carbohydrate to support such activity and the development of gonads and accessory sex glands (Mordue *et al.*, 1980). In Corixidae the immature flight muscles of the newly emerged adult increase in size in the flying morphs but not in the non-flying forms (Scudder, 1971). In a few insects sexual maturity is accompanied by more striking changes (Richards and Davies,1977). The protein content of *Glossina* flight muscles increases two to three fold in the young adult (Bursell, 1973).

Ageing in Adults :

The sum total of those time-dependent reproducible changes both in structure and/or function for a given organism, species or strain during its total life span is known as process of ageing. As for senescence, the definition of ageing can be modified more specifically to include the sum total of those changes in structure, and function which, by virtue of their deleterious and degradative nature, result ultimately in the failure of the individual to survive and therefore, result in its death (from "old age") (Rockstein and Miquel, 1973).

Although, in general, males of many species of animals studied have been shown to manifest a shorter life span than the females, which suggests that duration of life is a secondary sex character, many exceptions, especially in insects, have been demonstrated even in the case of XY males versus XX females. In insects, diet may modify the rate of development, oviposition, fertility, and organ size, as well as life span (Rockstein and Miquel, 1973).

Corelation of and sequential changes in the ageing phenomena which correlate with biochemical changes suggest that ageing is not simple due to random error but is programmed. The ageing may be influenced by the activity of the corpora allata; in locust when these glands are removed the locusts live longer and show a number of features, including improved flight performance, which suggest that ageing has been slowed considerably. The situation is complex, however, since removal of the ovaries from locusts results in premature ageing

(Though this can be reversed if the corpora allata are removed also) (Mordue *et al.*, 1980).

2. <u>Metabolism during Adult Development</u> :

Lipid, carbohydrates and in some insects, certain amino acids are used as respiratory fuels to supply the energy for flight (Sacktor, 1970). Flight muscles contains enough fuel to initiate flight but the endogenous reserves are too small to sustain prolonged flight for which respiratory fuels must be supplied to the muscles from exogenous sources. The flight muscles derive their exogenous supply of respiratory fuels from the haemolymph, which itself contains an important reserve of organic materials. However, the major energy storage site in the insect is the fat body which is often considered the equivalent of the mammalian liver and adipose tissue combined (Kilby, 1965).

Protein Metabolism :

The dominant phenomenon in the life of adult insects in addition to maintenance is reproduction. In the female there is a continuous deposit of yolk at the time of egg production, whereas in the male probably different proteins are synthesized in connection with spermatogenesis and secretion of the accessory glands (Chen, 1966). In addition to hormones, the quality of the diet may have also a definite influence on ovarian development. A variety of amino acids included in the synthetic diet are found to be indispensable for egg formation. It is further known that in many insects egg maturation does not occur when the adult females are kept solely with sugar and water but without proteins (House, 1962).

Cytochemical finding indicate that yolk proteins can be synthesized within the ovary. Proteins provided by the follicular cells and the trophocytes are transferred to the oocyte where the final elaboration of the yolk spheres takes place. On the other hand, several investigations have demonstrated beyond doubt that egg proteins may be synthesized outside the ovary and then transferred without change to the oocyte. It indicates that yolk proteins are synthesized both within the ovary and in tissues outside it. In the ovary protein synthesis is initiated in the trophocytes and the follicle cells. Outside the ovary proteins are synthesized in the fat body, released into the haemolymph and taken up by the female gonads (Chen, 1966).

3. <u>Review of Literature on Adult Proteins and Proteases</u>:

The metamorphic events culminate into the formation of an imago. Adult pterygote insect doesnot moult but it generally undergoes some further development after emergence. most of the organs, particularly the reproductive ones, attain their full functional status. Along with this, certain age related physiological and biochemical changes also occur. In the female there is a continuous deposit of yolk at the time of egg production, whereas in the male probably different proteins are synthesized in connection with spermatogenesis and secretion of the accessory glands. It therefore seems reasonable to some differences in the protein metabolism between the two sexes.

Proteins in relation to prothoracic hormonal activity have been studied by Williams (1951). According to them the hormonal activity increase with simultaneous decrease in protein concentration as the adult develops concentration of haemolymph protein decreases during adult life. Hayes *et al.*(1970) worked on electrophoretic patterns of proteins in haemolymph from the adult medeira cockroach, *Leucophaea maderae* (F) during a period of 24 hrs. Parker (1971) compared the haemolymph proteins in two species of Leptinotarsa beetles. Proteins were separated from the haemolymph by use of PAGE. The electrophoretic patterns of haemolymph from *L. decemlineata* and *L. Juncta* adults demonstrated qualitative characteristics similar to those of their respective larvae. Effect of aging on amino acid turnover and rate of protein synthesis in the blowfly, *Phormia regia* was studied by Levenbook and Krishna (1971). According to them the rate of protein synthesis in young blowflies is higher than in aged individuals. Synthesis of blood and cuticular proteins in late pharate adults of the cercopia silkmoth investigated by Ruh and Willis (1974). Elliot and Gillot (1979) worked out organ specific study in the migratory grasshopper, *Melanoplus sanguinipes*. Phillips and Laughton (1965) studied the culticle proteins in adult *Locusta migratoria*. Carlisle *et al.*(1987) noted that in Locust feeding causes an appearance of a factor in haemolymph that stimulates the protein synthesis. Joplin and Denlinger (1990) studied the developmental and tissue specific control of the heat shock induced 70 KD related proteins in the flesh fly, *Sarcophaa crassipalpis*. Ichimori and Ohtomo (1990) studied the specific protein related to adult diapause in the leaf beetle, *Gastrophysa atrocyanea*.

Gooding and Huang (1969) studied trypsin and Chymotrpsin from the beetle, *Pterostichus melanrius*. These proteinases from the digestive tract have an optimal activity near pH 8 and temperature 47^0c where denatured haemoglobin is used as a substrate. The two proteinases occur in about equal concentrations in both males and females. Muraleedharan and Prabhu (1978) worked on food intake and midgut protease activity in the red cotton bug, *Dysdercus cingulatus fabr*. Martin *et al.*(1981) studied the digestive enzymes of detritus feeding stonefly nymphs (Plecoptera-ptronarcyidae). Partial purification and characterization of cysteine proteinase from metamorphosing tadpole tails of *Rana catesbeiana* was studied by Fujita *et al.*(1989). Partial purification and characterization of the major midgut proteases of grassbug larvae *Costelytra zelandica* (Coleoptera- Scarabaidae) was studied by Christeller *et al.* (1989). Pan *et al.* (1991) worked on the changes in composition and proteolytic enzyme activities of *Artemia* during early development. Ferreira *et al.*(1994) worked on the properties of the digestive enzymes and the permiability of the peritrophic membrane of *Spodoptera frugiperda* (Lepidoptera) larvae. Protease mediated prophenoloxide activation in the haemolymph of American cockroach *Periplaneta amaricana* was studied by Thangaraj *et al.*(1995) Regulation of digestive enzyme levels in insects was studied by Lehane *et al.*(1995). Moffatt (1995) studied the synthesis and secretion of trypsin in the midgut of *Stomoxys calcitrans.* Blackmore *et al.*(1995) studied the protein stimulation of trypsin secretion from the opaque zone midgut cells of *Stomoxys calcitrans.* Rosenfield and Vanderberg (1998) identified proteases from midgut and haemolymph of adult *Anopheles stephenis* mosquitoes. The peak midgut protease activity is shown by the fifth day old insect, which also consumes the maximum amount of food. It appears that proteins in the food stimulate midgut protease activity in this insect.

II. MATERIAL AND METHODS :

1. Material :

The culture of *M. separata* and chemicals used were same as in Chapter II (Material and methods).

A. Developmental Stages of Adult for Study :

The developmental period of the adult *M. separata* is of 9 days. The pupae which are freshly fromed were collected and kept in the small jar lined with blotting paper and containing folded pieces of dry maize leaf whorls. The pupae became dark brown, such male and female pupae were collected and kept separately in the big jars for emergence of moths. Generally the moth emerges early in the morning and soon after the emergence it was inactive and sluggish for some time. Newly emerged male and female moths were confined in the big jar in ratio 2:1 containing leaf whorls and pieces of folded paper for oviposition. The colour of full grown moth is pale brown in colour with beautifully designed patchy forewings which have a wavy border pattern on the fringe. The hind wings is practically white. Both sexes looked alike in antennal and wing pattern. The thorax and abdomen were full of scales. Cotton wad dipped in 10 percent honey solution was provided as food for the moths. The food was changed twice a day. The moths are found to be very active during night and remain hidden during day time. The adult developmental period is of 9 days in fed moth. The total developmental period of adult stages from 1^{st} -day to 9^{th} -day adults ($A_1 - A_9$) were taken for the study of protein and proteases activity.

2. <u>Methods</u> :

Estimation of Proteins and Proteases:

The estimation of proteins and enzyme assay of proteases were same as in chapter II (Material and Methods)

III RESULTS :

1. <u>Adult Developmental Period</u> :

The adult developmental period of *M separata* is of 9-days.

2. <u>Adult Proteins</u> :

The changes in proteins during adult development of fed moths of *M. separata* are represented in Table No. 4 and illustrated graphically in fig. No. 16.

In case of male moth, gradual increase in amount of proteins was observed on 1st to 5th -day, where as gradual decrease in the amount of protein was observed 6th to 9th -day of development of adults. In case of female moth gradual increase in amount of proteins was observed from 1st to 4th -day, where as amount of protein was found constant from 4th to 6th day. The decline in protien was observed from 8th to 9th day of development of female adults. The amount of proteins in female was more as compared to male.

The food (10% honey solution) contains high amount of carbohydrates which acts as instant source of energy for normal activity of moth and to initiate flight after a halt for feeding.

Table No. 4

Period of Adult Development (days)	Amount of Proteins (mg/gm of body wt.)	
	Male	Female
1	56	58
2	58	60
3	59	61
4	60	62
5	61	61.5
6	55	62
7	48	62.7
8	25	27
9	19	21

3. <u>Adult Proteases</u> :

A. Male adult proteases :

a. Acidic Proteases :

Partial characterization of acidic proteases :

The partial characterization of acidic proteases during male adult development revealed the maximum activity at pH 3.1 (Cathepsin D like enzyme) and pH 4.3 (Cathepsin B like enzyme), temperature 37^0C, 30 min. incubation time, 1% enzyme concentration and 2% substrate concentration.

Acidic proteases activity :

i. Cathepsin D like enzyme :

The changes in cathepsin D like enzyme activity during male adult development of *M. Separata* are illustrated graphcally in Fig 17.

Maximum enzyme activity was observed on 1^{st} -day of development of male adults. The gradual decrease in enzyme activity was observed during 1^{st} to 9^{th} -day of development of male adults. Minimum activity of enzyme was observed on 9^{th} -day of development of male adults.

ii) Cathepsin B like enzyme :

The changes in cathepsin B like enzyme activity during male adult development of *M. Separata* are illustrated graphcally in Fig 17.

Maximum enzyme activity was observed on 1^{st} -day of development of male adults. The gradual decrease in enzyme activity was observed during 1^{st} to 9^{th} -day of development of male adults. Minimum activity was observed on 9^{th} -day adults of development of male adults.

b. Neutral proteases :

Partial characterization of neutral proteases :

The partial characterization of neutral proteases during male adult development revealed the maximum activity at pH 7, temperature 37^0C, 30 min. incubation time, 1% enzyme concentration and 5% substrate concentration.

Neutral protease activity :

The changes in netural protease activity during male adult development of *M. Separata* are illustrated graphcally in Fig 18.

The maximum enzyme activity was observed on 1^{st} -day of development of male adults. The gradual decrease in enzyme activity was observed from 1^{st} to 9^{th} -day of development of male adults. Minimum activity of enzyme was observed on 9^{th} -day of development of male adults.

c. Alkaline proteases :

Partial characterization of alkaline proteases :

The partial characterization of neutral proteases during male adult development revealed the maximum activity at pH 7.6 (Chymotryps in like enzyme) and pH 8.3 (Trypsin like enzyme), temperature 37^0C, 30 min. incubation time, 1% enzyme concentration and 2% substrate concentration.

Alkaline proteases activity :

i. Chymotrypsin like enzyme :

The changes in chymotrypsin like enzyme activity during male adult development of *M. separata* are illustrated graphcally in Fig 19.

The maximum enzyme activity was observed on 1st -day of development of male adults. The gradual decrease in enzyme activity was observed from 1st to 9th -day of development of male adults. Minimum enzyme activity was observed on 9th –day of development of male adults.

ii. Trypsin like enzyme :

The changes in trypsin like enzyme activity during male adult development of *M. separata* are illustrated graphcally in Fig 19.

The maximum enzyme activity was observed on 1st -day of development of male adults. The gradual decrease in enzyme activity was observed from 1st to 9th –day of development of male adults. Minimum enzyme activity was observed on 9th -day of development of male adults.

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B. Female Adult proteases :

a. Acidic proteases :

Partial characterization of acidic proteases :

The partial characterization of acidic proteases during female adult development revealed the maximum activity at pH 3.3 (Cathepsin D like enzyme) and pH 4.4 (Cathepsin B like enzyme), temperature 37^{0}C, 30 min. incubation time, 1% enzyme concentration and 2% substrate concentration.

Acidic proteases activity :

i. Cathepsin D like enzyme :

The changes in cathepsin D like enzyme activity during Female adult development of *M. separata* are illustrated graphcally in Fig 20.

Maximum enzyme activity was observed on 1st -day of development of female adults. The gradual decrease in enzyme activity was observed from 1st to 9th -day of development of female adults.

Minimum enzyme activity was observed on 9^{th} -day of development of female adults.

ii. Cathepsin B like enzyme :

The changes in cathepsin B like enzyme activity during Female adult development of *M. separata* are illustrated graphcally in Fig 20.

Maximum enzyme activity was observed on 1^{st} -day of development of female adults. The gradual decrease in enzyme activity was observed from 1^{st} to 9^{th} -day of development of female adults. Minimum enzyme activity was observed on 9^{th} –day of development of female adults.

b. Neutral proteases :

Partial characterization of neutral protease :

The partial characterization of neutral proteases during female adult development revealed the maximum activity at pH 7, temperature 37^0C, 30 min. incubation time, 1% enzyme concentration and 5% substrate concentration.

Neutral protease activity :

The changes in natural protease activity during female adult development of *M. separata* are illustrated graphcally in Fig 21.

Maximum enzyme activity was observed on 1^{st} –day of development of female adults. The gradual decrease in enzyme activity was observed from 1^{st} to 9^{th} -day of development of female adults. Minimum enzyme activity was observed on 9^{th} –day of development of female adults.

c. Alkaline proteases :

Partial characterization of alkaline proteases :

The partial characterization of alkaline proteases during female adult development revealed the maximum activity at pH 7.8 (Chymotrypsin like enzyme) and pH 8.5 (Trypsin like enzyme), temperature 37^0C, 30 min. incubation time, 1% enzyme concentration and 2% substrate concentration.

Alkaline proteases activity :

i. Chymotrypsin like enzyme :

The changes in chymotrypsin like enzyme activity during female adult development of *M. separata* are illustrated graphcally in Fig 22.

Maximum enzyme activity was observed on 1^{st} -day of development of female adults. The gradual decrease in enzyme activity was observed from 1^{st} to 9^{th} -day of development of female adults. Minimum enzyme activity was observed in 9^{th} -day of development of female adults.

ii. Trypsin like enzyme :

The changes in trypsin like enzyme activity during female adult development of *M. separata* are illustrated graphcally in Fig 22.

Maximum enzyme activity was observed on 1^{st} -day of development of female adults. The gradual decrease in enzyme activity was observed from 1^{st} to 9^{th} -day of development of female adults. Minimum enzyme activity was observed on 9^{th} -day of development of female adults.

IV. DISCUSSION :

1. Adult Proteins :

Proteins provide the chief structural elements of muscles, glands and other tissues (Wigglesworth, 1972). In the female there is a continuous deposit of yolk at the time of egg production, yolk proteins are synthesized both within the ovary and in tissues outside it. Outside the ovary proteins are synthesized in the fat body, released into the haemolymph and taken up by the female gonads. In male different proteins are synthesized in connection with spermatogenesis and secretion of accessory glands. For example, a characteristic composition of free and bound amino acids has been found in the semen of honeybee drones (Novak *et al.*, 1960). It therefore, seems reasonable to expect some differences in the protein metabolism between the two sexes (Chen, 1966). A certain amount of protein may be converted into carbohydrate or fat and used for energy production (Wigglesworth, 1972).

In the present work in case of fed male moth gradual increase in the amount of proteins from 1 to 3rd -day of development of adults indicates the synthesis of proteins which are associated with reproductive functions like spermatogenesis and secretion of accessory glands. Decline in proteins from 3 to 6th -day of development of male adults indicates utilization of proteins during mating. Decrease in proteins from 6 to 9th – day of development male adults may be due to inactive physiological state of male. In case of fed female moth gradual increase in proteins from 1 to 4-day adults indicates synthesis of yolk proteins (vitellogenin) in the fat body, which are afterward translocated to the developing oocytes. Decline in the protein from 4 to 7th –day of development female adults suggests gradual oviposition. Decrease in proteins from 7 to 9th -

day of development female adults may be due to inactive physiological state of female.

Our results are in good agreement with the findings of Novak *et al.*(1960), Chen (1966) and Wigglesworth (1972).

2. Adult Proteases:

A. Male adult proteases :

The accessory glands (Paragonia) in adult male insects play an essential role in reproduction. The accessory gland secretion in *Drosophila* contains various amino acids, peptides and proteins (Chen, 1971; 1978). Friedel and Gillott, (1976) proposed that the secretory proteins are synthesized in the fat body, released into the haemoymph and taken up by the accessory glands. According to Kaulenas *et al.* (1979) some proteins increase in amount considerably during adult maturation.

In the present investigation maximum activity of acidic, neutral and alkaline proteases was observed in 1-day adults of *M. separata.* This suggests degradation of stored proteins in the fat body and utilization of free amino acids for the synthesis of secretory proteins, which are released in the haemolymph and taken up by the accessory glands. Steady decrease in enzyme activity from 1^{st} to 9^{th} -day of development adults suggests gradual decrease in protein degradation in the fat body. Minimum activity in 9-day adults indicates less role of proteases during late period of adult development. Our results are in good agreement with Kaulenas (1979); Chen (1971, 1978) and Friedel and Gillott (1976).

B. Female Adult proteases :

The processes like destruction of certain larval tissue and rejuvenation and remoulding of various tissues into adult take place during the metamorphosis of an insect. Since proteins are the first biological factors making their manifestation during development (Schmidt and Schwakl, (1975), number of plasma proteins increase during successsive stages of development (Kanost *et al.*, 1990). In adult insects proteins are prerequisite for egg maturation in females and spermatogenesis in male. Proteases are responsible for degradation of proteins (Chen, 1978).

Muraleedharan and Prabhu (1978) studied the food intake and midgut protease activity in the red cotton bug, *Dysdercus cingulatus Farb.* (Heteroptera : Pyurhocoridae.)

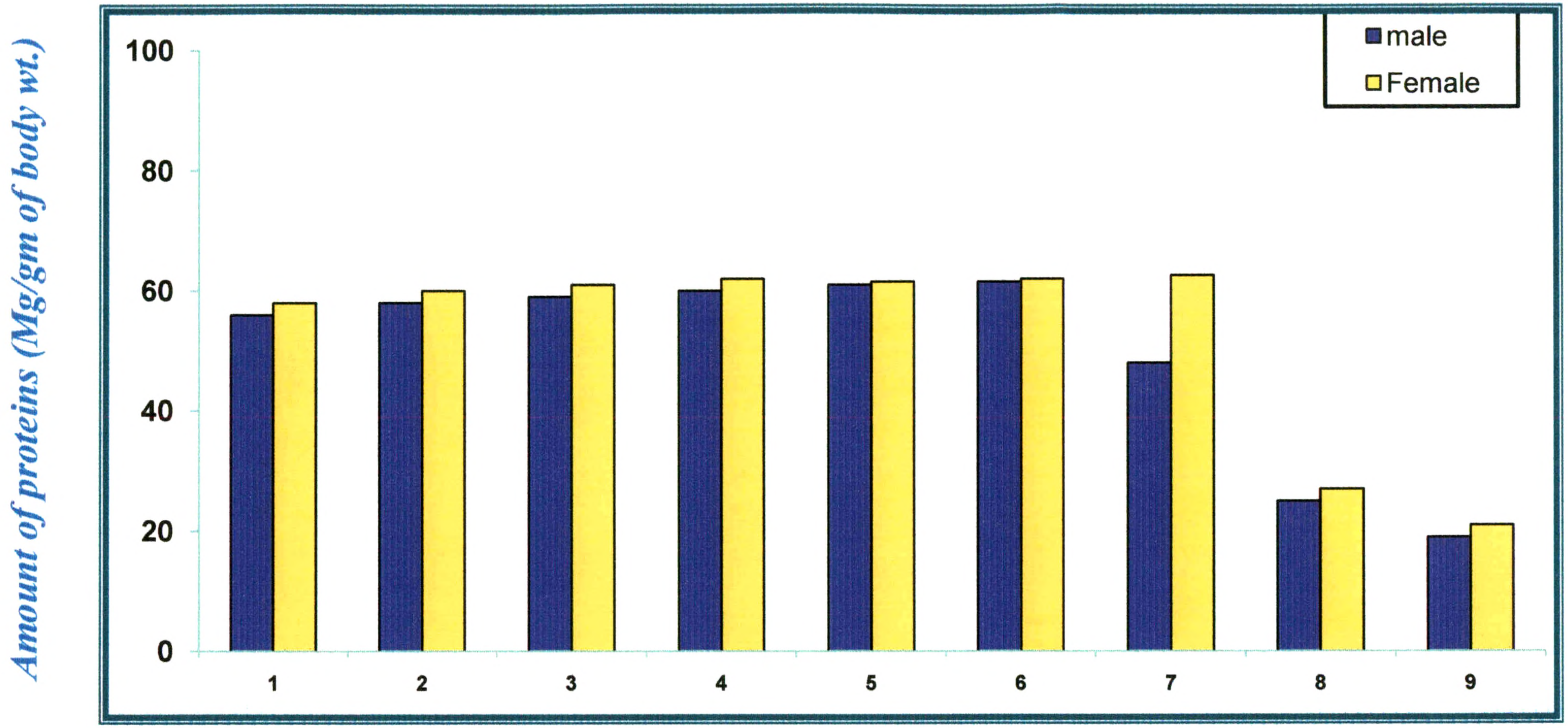
The probable regulatory mechanism involved in the secretion of digestive enzyme in insects have been reviewed by Dadd (1970) and Gooding (1975). Some degree of regulation of protease secretion in relation to food ingestion is proposed by many workers (Gooding, 1966; 1972 and Engelmann, 1969). In certain species of insects, proteins in the food stimulate midgut protease production (Shambaugh, 1954; Ishaaya *et al.,* 1971; Akov, 1972; Briegel, 1975). In this circumstance it was thought worth while to find out if food has any regulatory role in digestive enzyme secretion in the heteropteran insects.

In the newly emerged female *Dysdercus cingulatus*, midgut proteases are present at a low level. A residual level of midgut enzyme is noticed in many other species of insects like black file and mosquitoes (Yang and Davies, 1968; Shambaugh, 1954; Akov, 1972). In many other species of insects like *Tenebrio molitor* (Dadd, 1956) and *Rhodnius prolixus* (Persaud and Davey, 1971) a definite pattern of midgut protease activity is shown by the females of different age groups. The pattern of midgut protease activity in *Dysdercus cingulatus* is comparable to vitellogenic activity. The daily increase in the total protein content of the ovary of *Dysdercus cigulatus* during the first gonotrophic cycle has been demonstrate by Prabhu and Nayar (1971). Their observations show that a significant increase in the total protein content of the ovary is noticed in 3, 4, 5, and 6- day of old insects. This type of coordination between the gonotrophic cycle and the secretion of the midgut digestive enzymes is noted in many other insect species like *Aedes atropalpus*. Thus it becomes evident that there exists a close relationship between oogenesis and midgut protease activity in *Dysdercus cingulatus*. A high level of protease activity apparently leads to greater protein digestion and an increased availability of precursors in the haemolymph for the synthesis of yolk proteins during vitellogenesis. Vitellogenesis starts in the 3-day old insects and continues in the 4 and 5-day and completed in 6-day old insects (Jalaja and Prabhu, 1976) and this may be the reason why the 6-day insects show a steady decrease in the midgut protease activity. Detinova (1962) concluded that the completion of vitellogenesis slow down digestion in *Anopheles macculipnni*. According to Hudson (1970) *Aedes atripalpus* with mature or nearly mature eggs is incapable of synthesizing and releasing normal quantities of digestive enzymes. In the 7-day old *Dysdercus cingulatus* when the second batch of eggs start developing, a corresponding increase in the midgut protease level is also noted. Similar observations are also made in *Rhodnius prolixus* (Persaud and Davey, 1971) and *Dysdercus fasciatus* (Geering and Sacher, 1972)

The observation of Akov (1972) and Gooding (1974) on Glossina and Briegel and Lea (1975) on mosquitoes showed that different proteins stimulate different levels of proteolytic enzymes because different proteins would yield different amounts of amino acids during their digestion. Graf and Briegel (1985) isolate the trypsin isozymes from the mosquito, *Aedes aegypti* (L). Activity cycles and the control of four digestive proteinases in the posterior midgut of *Rhodnius prolixus* stal (Hemiptera: Reduviidae) was studied by Houseman and Downe (1983). Four digestive proteinases; Cathepsin B, Cathepsin D, lysosomal carboxypeptidase B and aminopeptidas have been characterized from the posterior midgut of *Rhodnius prolixus* stal (Houseman and Downe, 1980; 1981; 1982).

In the present investigations gradual decrease in enzyme activity of acidic, neutral and alkaline proteases from 1 to 9-day female adults of *M. separata* was observed. This suggests gradual degradation of stored proteins for the synthesis of yolk proteins in the fat body. Steady decrease in enzyme activity from 1 to 9 –day adult suggest gradual decrease in protein degradation in the fat body. Minimum activity in 9- day adult indicates less role of proteases after egg laying in the late period of adult development.

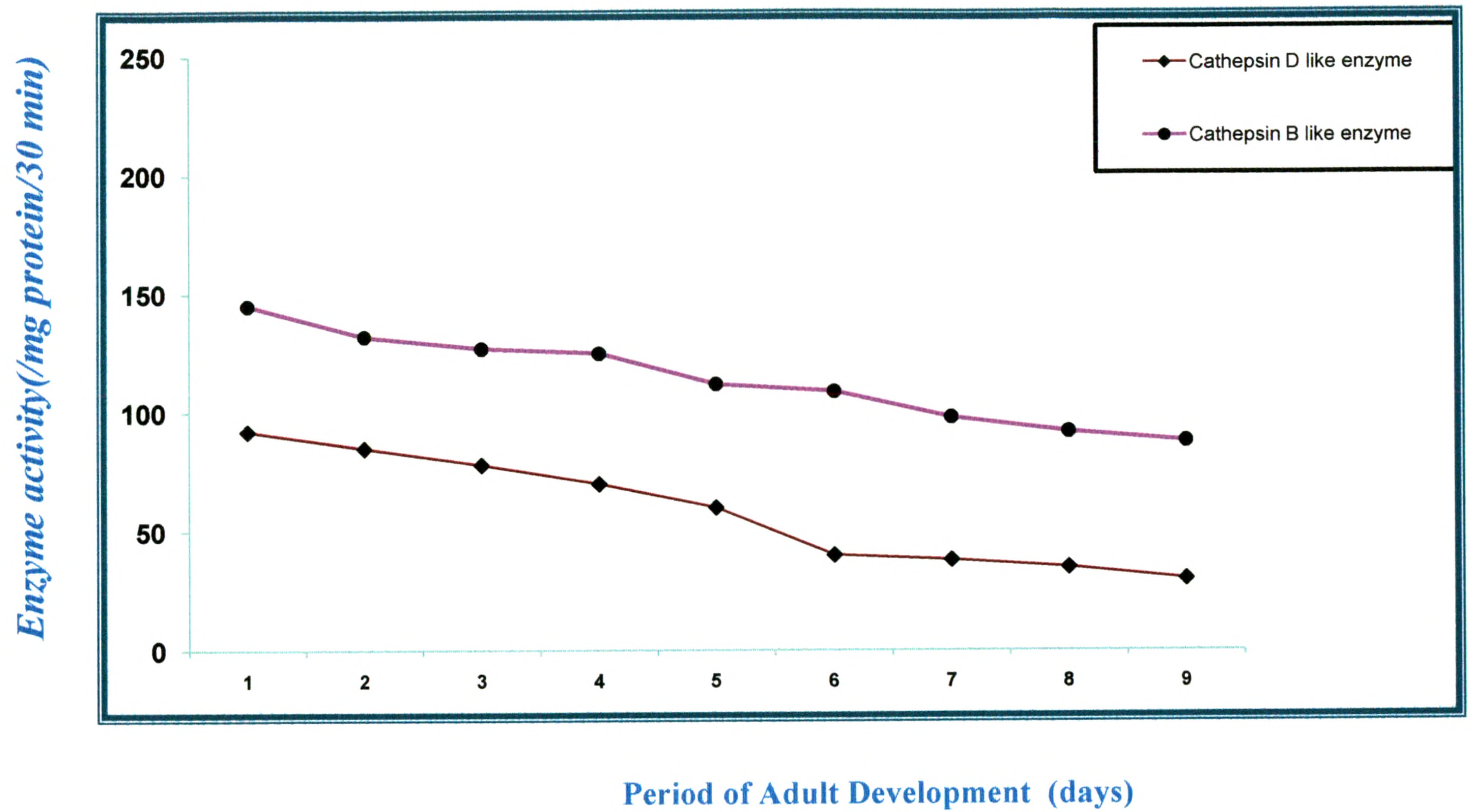
Our results are in good agreement with Jalaja and Prabhu (1971), Detinova (1961), Prabhu and Nayar (1971) and Chen (1978).



Period of Adult development (days)

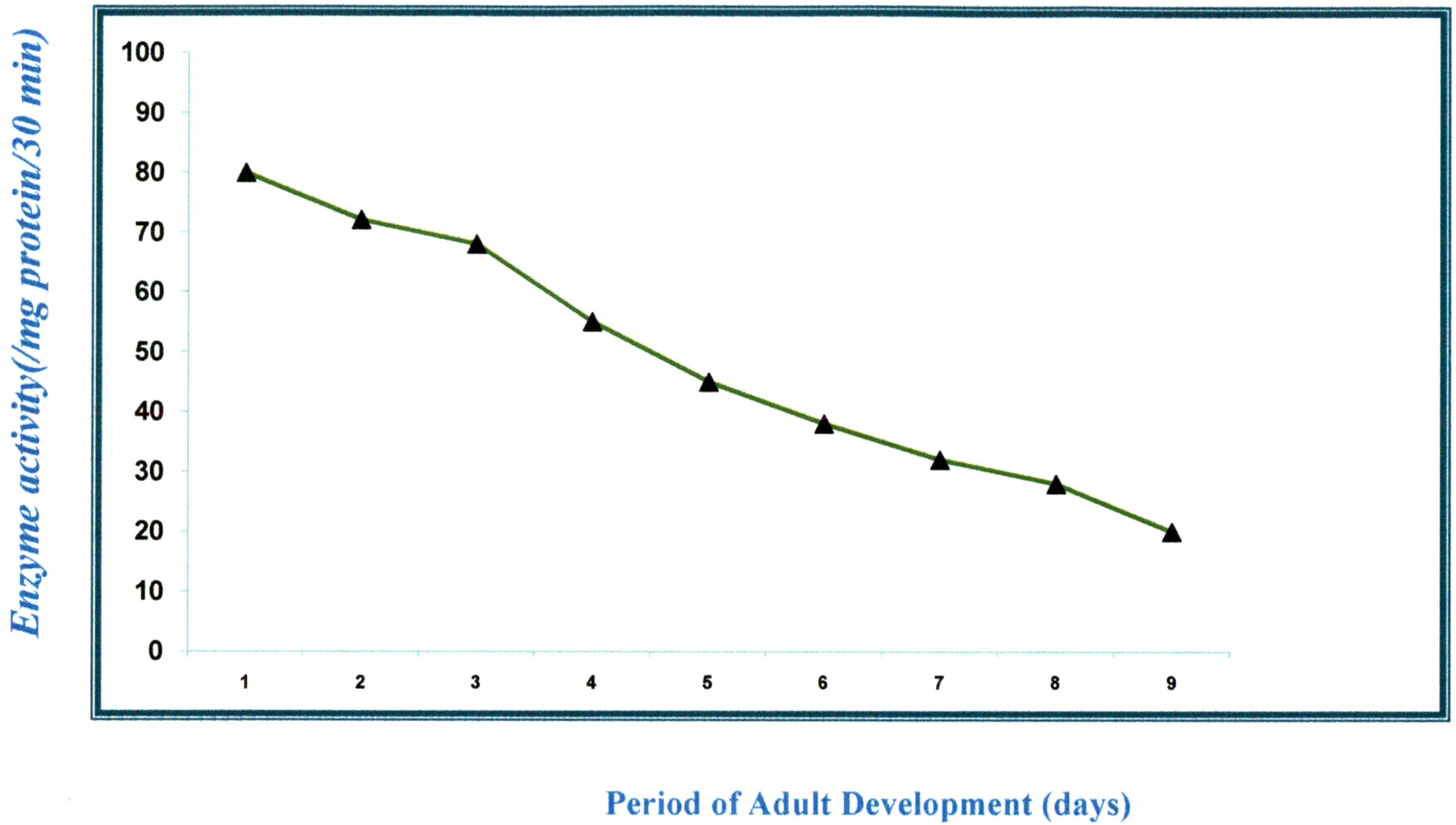
Changes in protein during adult development of *Mythimna separata*

Fig. No. 16



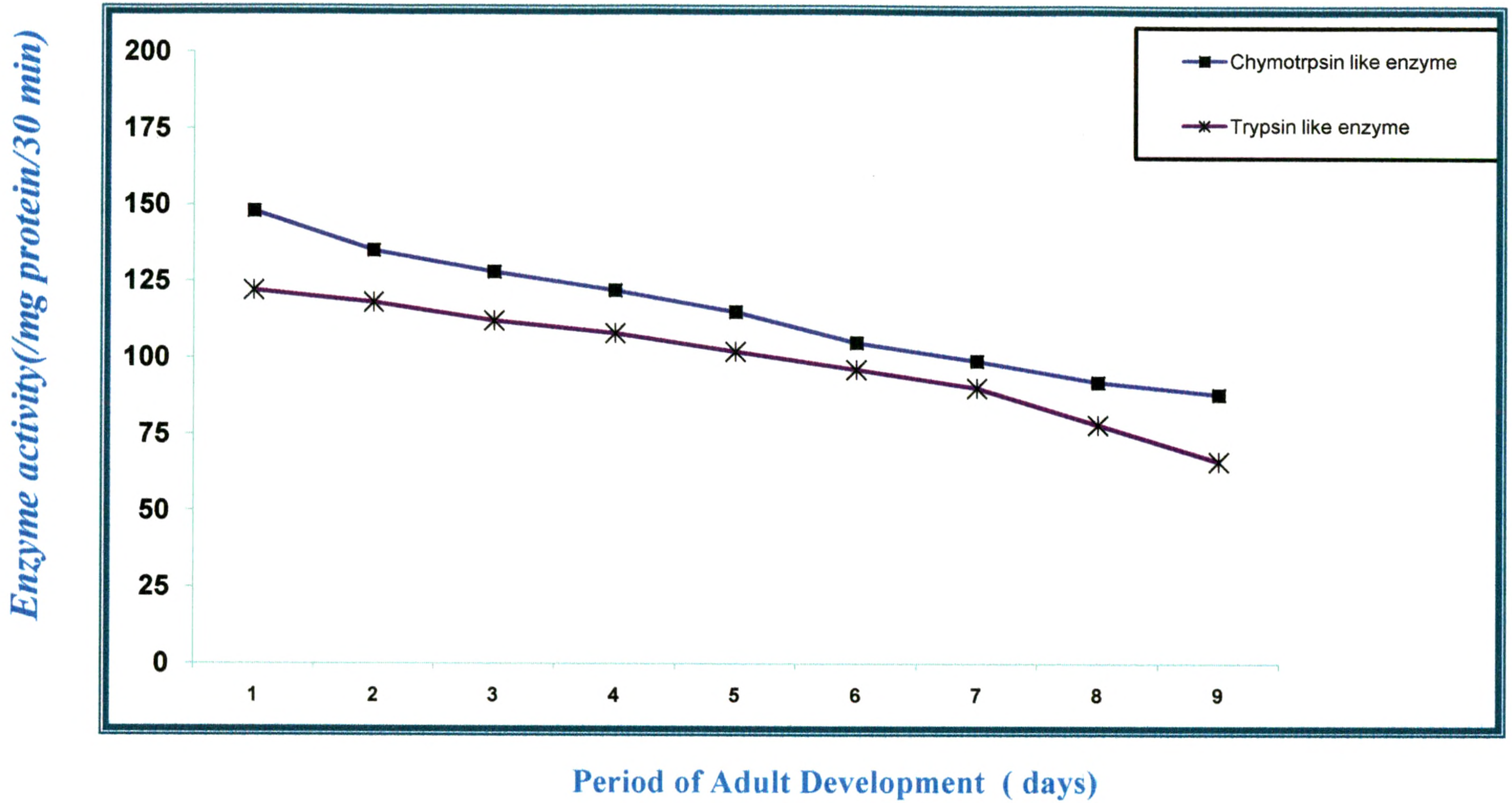
Acidic proteases activity during male adult development of *Mythimna separata*

Fig. No. 17



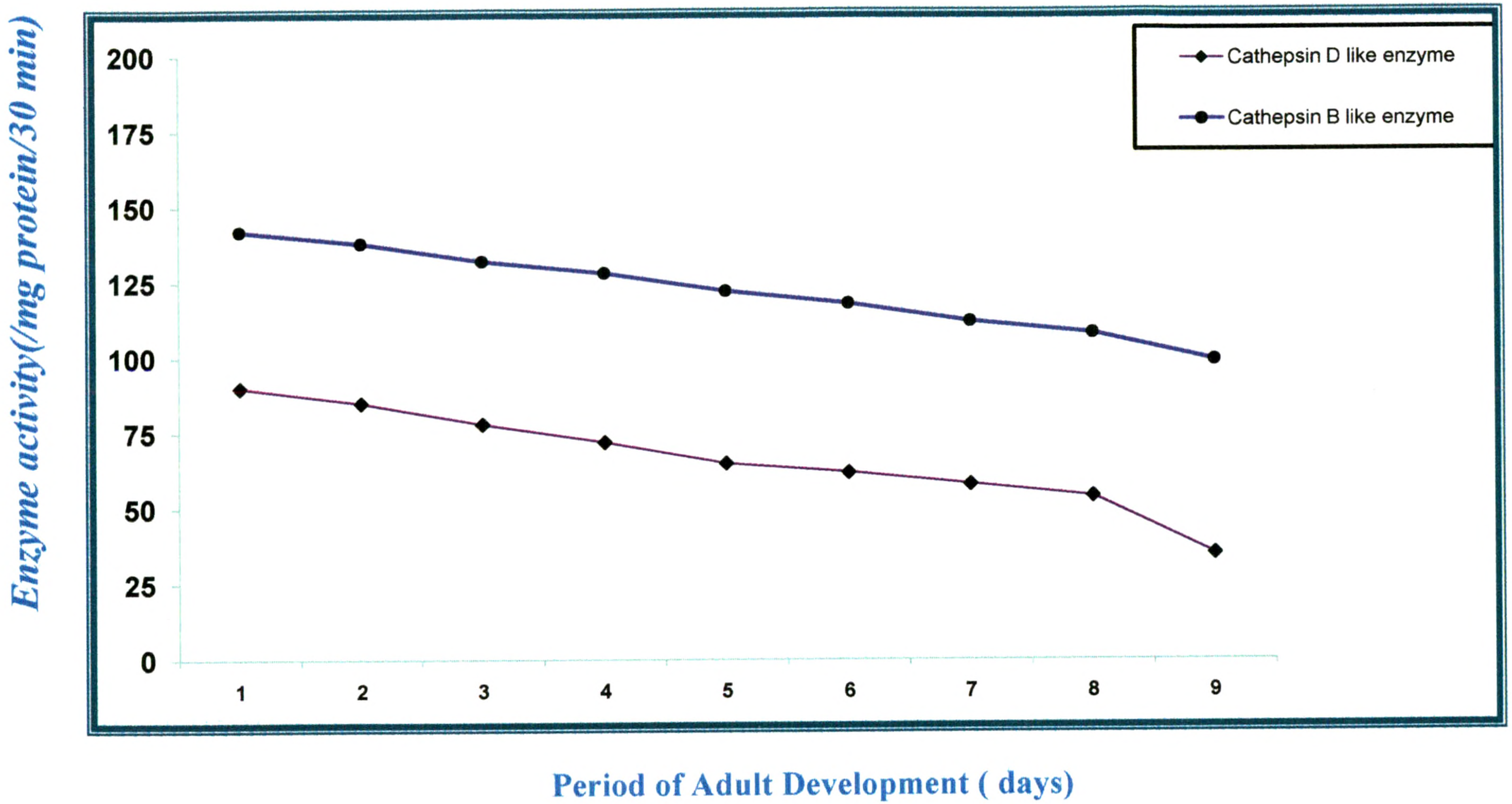
Neutral proteases activity during male adult development of *Mythimna separata*

Fig. No. 18



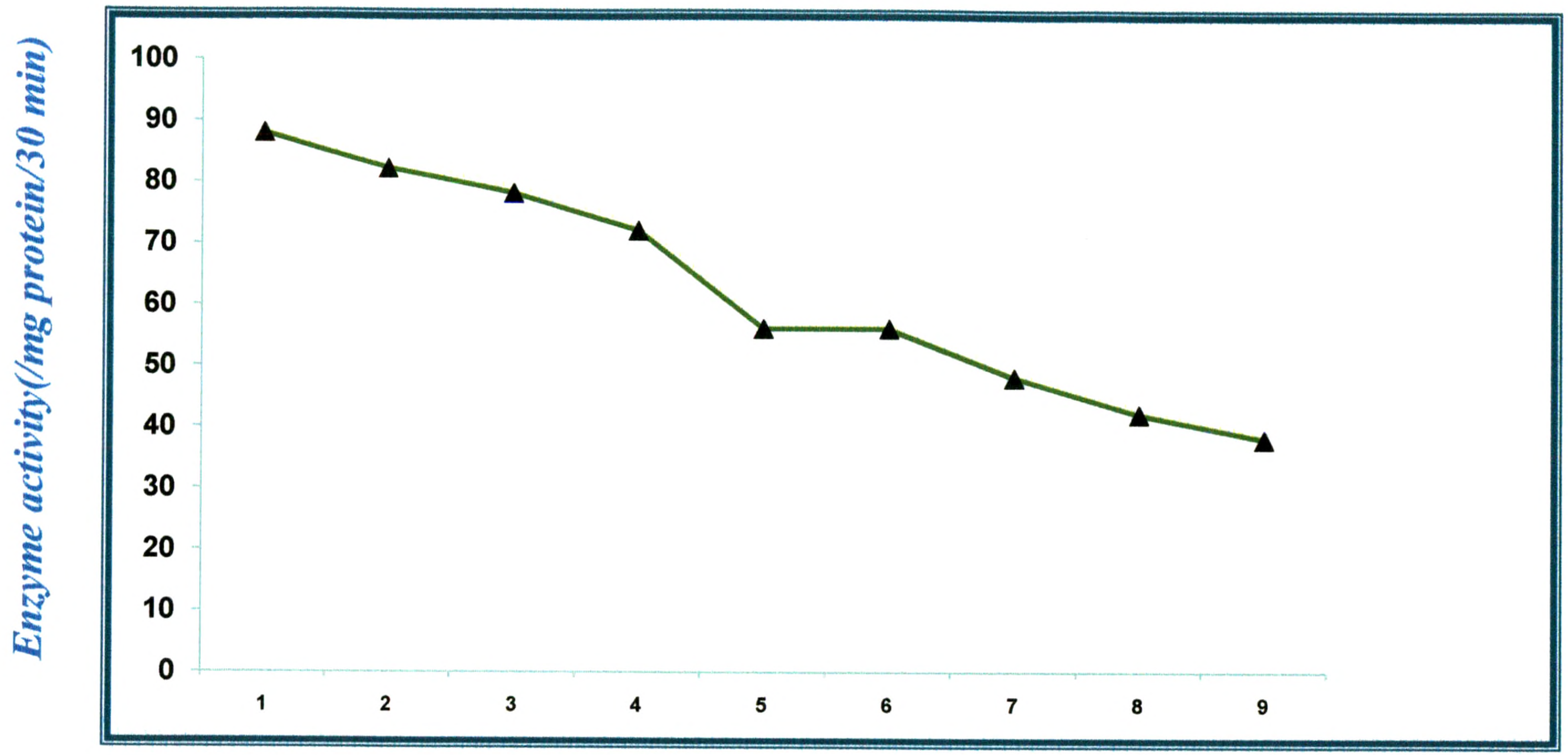
Alkaline proteases activity during male adult development of *Mythimna separata*

Fig. No. 19



Acidic proteases activity during female adult development of *Mythimna separata*

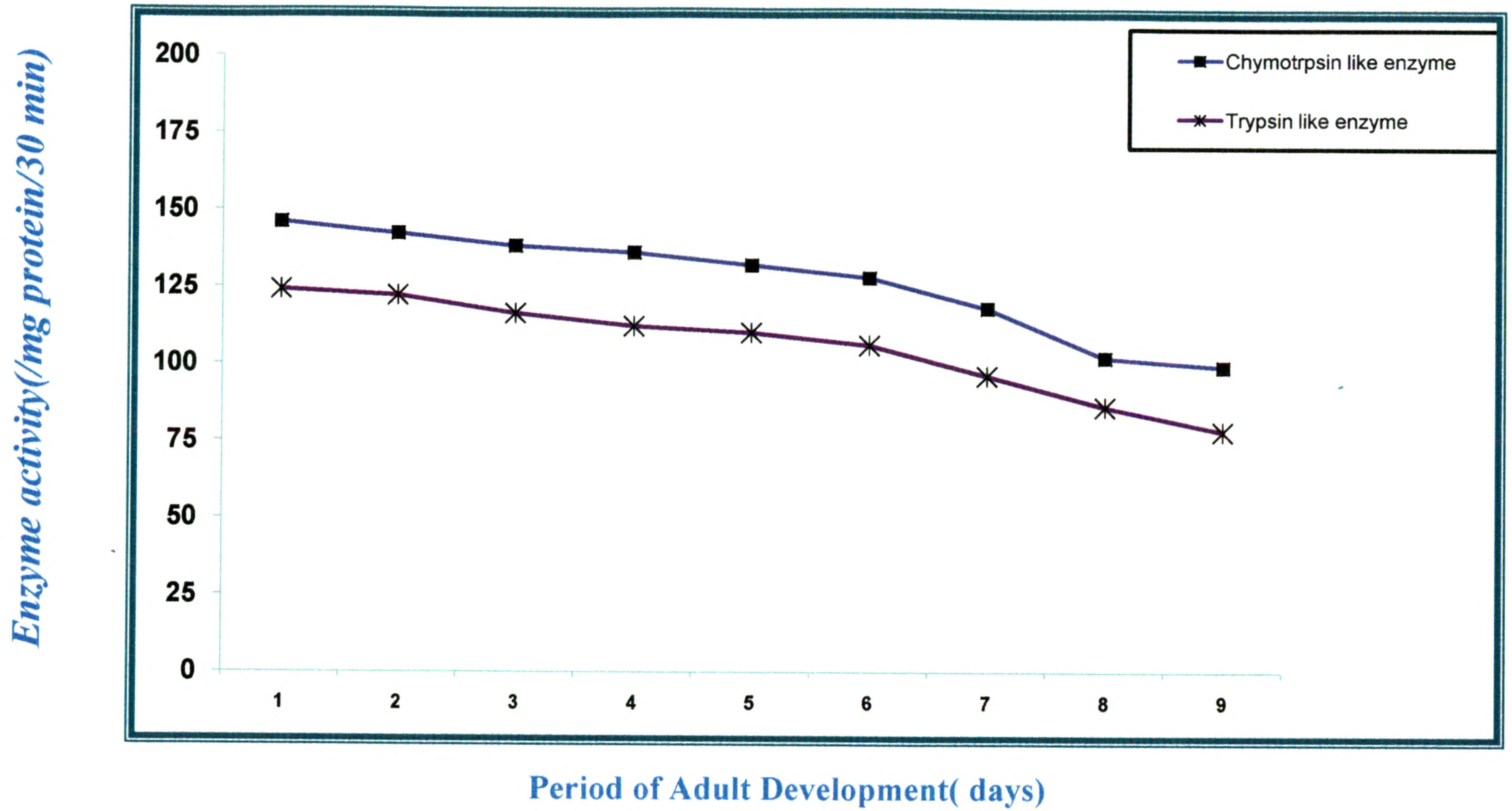
Fig. No. 20



Period of Adult Development(days)

Neutral proteases activity during female adult development of *Mythimna separata*

Fig. No. 21



Alkaline proteases activity during female adult development of *Mythimna separata*

Fig. No. 22

Fig. 1: Male and female moth

Fig.2: Mating

Fig 3: Female during oviposition in folds of paper

Plate No.7: Adult of *M.separata*