STUDIES ON SOME ASPECTS OF DIAPAUSE PHYSIOLOGY OF THE TROPICAL TASAR SILKWORM, *ANTHERAEA MYLITTA* DRURY (LEPIDOPTERA : SATURNIIDAE)

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for the degree of Doctor of Philosophy

by

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DEDICATED TO

The memory of my departed parents

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28.8.97.

This is to certify that the thesis entitled "Studies on Some Aspects of Diapause Physiology of the Tropical Tasar Silkworm, ***Antheraea mylitta*** Drury (Lepidoptera : Saturniidae)" is a bonafide piece of research work carried out by Mr. Ashok Kumar Sinha during the last five years.

Mr. Sinha has fulfilled the requirements of the University of North Bengal for submission of his thesis for the Degree of Doctor of Philosophy (Ph.D.) in Zoology.

In habit and character Mr. Sinha is fit and proper person for Ph. D. degree.

I forward this thesis for submission to the University of North Bengal.

(D. C. Deb)

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Ashok Kumar Sinha

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I. INTRODUCTION

Silk from nature was known to the Pre-Aryan civilization in India as evinced from Hindu scriptures and epics. Over thousands of years, silk has embraced the Indian culture and tradition. It is known as 'Queen of textiles' for its lustre, elegance, softness, smoothness, comfortness and luxurious appearance. In India sericulture was established at the beginning of the Christian era between the Gangetic valley and Brahmaputra river (Watt, 1893). In ancient India kings and queens had virtually assumed the divine right for the use of silk. Thus, the use of silk was limited to a few wealthy hands and was beyond the reach of the common people. Temple priests were encouraged to wear silk on special occasions by the kings who bestowed presents of the fabric upon the former. The said tradition was continued by Muslim Nawabs like Tipu Sultan of the then Mysore state and Siraz-Ul-Doula of Murshidabad in the 18th century. It was perhaps in the 20th century only that the silk started coming within the reach of the better-off people at large. The British East India Company at that time exploited the silk industry and developed silk centres in many parts of the country and exported large quantities of raw silk produced in West Bengal to England. After the Second World War sericulture kept spreading its roots into the mass and milieu of rural India.

The native non-mulberry silk of India, also known as wild silk as because the silkworms survive under natural conditions on wild food plants, has been an integral part of Indian scenario since pre-historic days even before the utility of the mulberry silkworm was discovered by a Chinese princess. In fact, the mulberry silk originated in China about 2200 B.C. and thereafter spread to other parts of the world. According to historians mulberry silk was introduced in India from China through Kotan (Tibet) by about

140 B.C. (Rangaswami et al , 1976). By the fourth century A.D. sericulture was well established in India and Central Asia. Sericultural practices were mostly confined to the northern and eastern India prior to 19th century and during later period, it was spread to southern peninsula (Mukherjee, 1898; Nanavaty, 1965).

Non-mulberry silk manifested the tricolour of its ancient culture symbolising in turn the Yogic cream of tasar, the peaceful white of eri and the golden bliss of the muga. The unique feature is that India is the only country in the world producing all the four kinds of silk viz. mulberry, tasar, eri and muga. In addition, it is the only country in the world producing golden yellow muga silk in Assam.

Tasar silk has found mention in the legend, folklore and fable since the dawn of civilization itself. The word "tasar" is apparently derived from the sanskrit word "Trasara" (Shuttle) and there is evidence in sanskrit literature that certain wild silks were cultivated in India since time immemorial. The master creators of the world famous Indian tasar are the 'adivasi' (aboriginal) forest tribes known by various names such as Santhals, Kol, Ho, Baiga, Budha, Munda, Oraon etc. These poor simple people have been rearing tasar silkworm over the centuries in complete harmony with forest and nature. In fact, tasar is the most important variety of non-mulberry silks as 95% of their global production is presently contributed by tasar alone. Tasar silk moths are mainly distributed in temperate and tropical zones of India.

While production of temperate tasar in China dates back to Hau and Wei dynasties, the rearing of temperate tasar silkworm (*Antheraea yamamai*) has a very recent tradition in Japan. The emphasis to temperate tasar culture is quite recent practice in India and is now being practised in the sub-Himalayan belt of North and North-Eastern India such as in the states of Jammu & Kashmir, Himachal Pradesh, Manipur, Mizoram and Arunachal Pradesh exploiting the vast Oak flora (Quercus sp.) for the rearing of *Antheraea proylei* which has been obtained through successful hybridization of indigenous *Antheraea roylei* with Chinese *Antheraea pernyi.*

However, the Indian tropical tasar silk insect *Antheraea mylitta* Drury (Lepidoptera : Saturniidae) is confined mainly to its tropical belt comprising the states of Bihar, Orissa, Madhya Pradesh, Andhra Pradesh, Maharastra, West Bengal ande Karnataka . The dense humid tropical forests sprawling over the central and southern plateau are the habitat of tropical tasar species. In India natural forest occupies about 44.50 million hectares in the tropical tasar cultivating states. Out of this tasar food plants are available in about 11.16 million hectares. Approximately, 1.32 lakh tribal families are engaged in tasar silkworm rearing and an equal number is engaged in the processing of silk such as reeling, spinning, weaving, dyeing and manufacturing of finished silk commodities. India is the second largest producer of tasar silk in the world after China. Because of the unique appearance of tasar silk, it has a novel value and a ready market, earning a considerable amount of foreign money. *A. mylitta* is polyphagous in nature. This traditional tasar silk producing insect thrives on three major food plants viz. Arjun *(Terminalia arjuna)* , Asan (*Terminalia tomentosa*) and Sal (*Shorea robusta)*. Other primary food plants for this species are Sidha (*Lagerstroemia parviflora*), Jarul(*Lagerstroemia speciosa*), Saoni *(Lagerstroemia indica*), Ber (*Zizyphus mauritiana*) and Anjan (*Hardwickia binata*). The important secondary food plants of *A. militta* are Haritaki (*Terminalia chebula*), Bahera (*Terminalia belerica*), Jamun (*Syzygium cumini*), Mahua (*Madhuca indica)*, Kinjal (*Terminalia paniculata*) etc.

A. mylitta has nearly 34 eco-races out of which 'Daba' is one of the most widely distributed eco-races (Jolly et al., 1979) contributing a major share to the total Indian non-mulberry silk production. This eco-race is reared variably between June and January and is predominantly of two types, bivoltine and trivoltine. First crop (generation) is raised during July-August following the natural emergence of moths and egg laying during mid-June to mid-July. This is the seed crop for the next only one commercial crop (in case of bivoltinism) or two crops (in case of trivoltinism). In case of bivoltine breeds, the second crop reared during September-October is the commercial crop and the cocoons thus produced are either subjected to reeling or the pupae inside remain in diapause till about the next June to produce the seed crop of the next year. For trivoltine breeds, second crop reared during September-October is also considered as the seed crop while the third crop raised during November - January becomes the commercial

crop. The pupae obtained from the third crop also remain in diapause till the next June and are used as seed crop during July-August . Under natural condition bivoltine breed occurs in the regions having well defined hot and cold spells like Bihar plateau and hilly tracts of Uttar Pradesh, Orissa and Madhya Pradesh while the trivoltine breed occurs in West Bengal, some parts of Orissa, Andhra Pradesh, Maharashtra and Karnataka where temperature is more equitable.

The life cycle of *A.mylitta,* being a holometabolan insect, has four stages viz. egg, larva, pupa and adult (moth). The eggs are ellipsoidal and bilaterally symmetrical along the micropylar axis . On washing they are yellowish creamy in colour(Fig.1). The body of the larvae on hatching is brownish yellow (Fig.2) but turns green at late first instar. Besides it possesses prominent white lateral and brick red or white dorsal shiny spots and violet or white tubercles with setae. The larvae pass through 4 moults and five instars. A mature larva is green or yellow and rarely blue in colour (Fig.3). After completion of feeding it starts spinning and envelops itself in a dense ovoid cocoon which is suspended from a branch of the food plant with the help of a long stock . At the end of spinning the larva gradually turns to pupa inside the cocoon . Though the tasar pupae are light to deep brown in colour (Fig.4), the adults (moths) are highly heterochromatic . The males are usually brown and the females are yellow or grey. Rarely brown females and grey (Fig.5) yellow males are observed. Gynandromorphs are also rarely found in this species (Chaudhuri et.al., 1995; Chaudhuri and Sinha, 1997).

A. mylitta undergoes facultative pupal diapause in bivoltine and/or trivoltine breeds from the middle of November to the middle of June. In the bivoltine breed pupal diapause starts from the middle of November and extends upto the following June, while, in the trivoltine breed, diapause starts from the middle of December and also extends upto June. Thus, pupal period in *A. mylitta* varies from 6 to 7 months in the diapausing generations but the time varies from 20 to 25 days in the non-diapausing progeny. A very asynchronous (erratic) emergence of moths takes place after the diapause. Due to its over-wintering nature, a major portion of tasar seed cocoons becomes abortive due to asynchronous (erratic) emergence and

Fig. 1 : Phtograph of eggs of *A.mylitta*.

Fig.2 : Photograph of 0-day-old first instar larvae of *A.mylitta*.

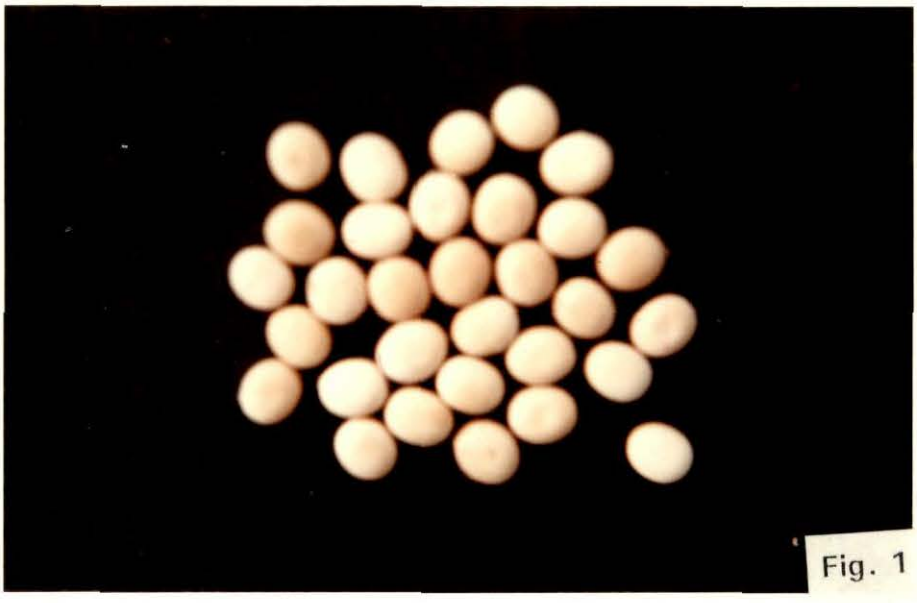


Fig. 1

Fig.2

Fig. 3 : Photograph of mature fifth instar larvae of *A.mylitta*.

Fig. 4 : Photograph of pupae of *A.mylitta* , a.male, b.female.

Fig.3

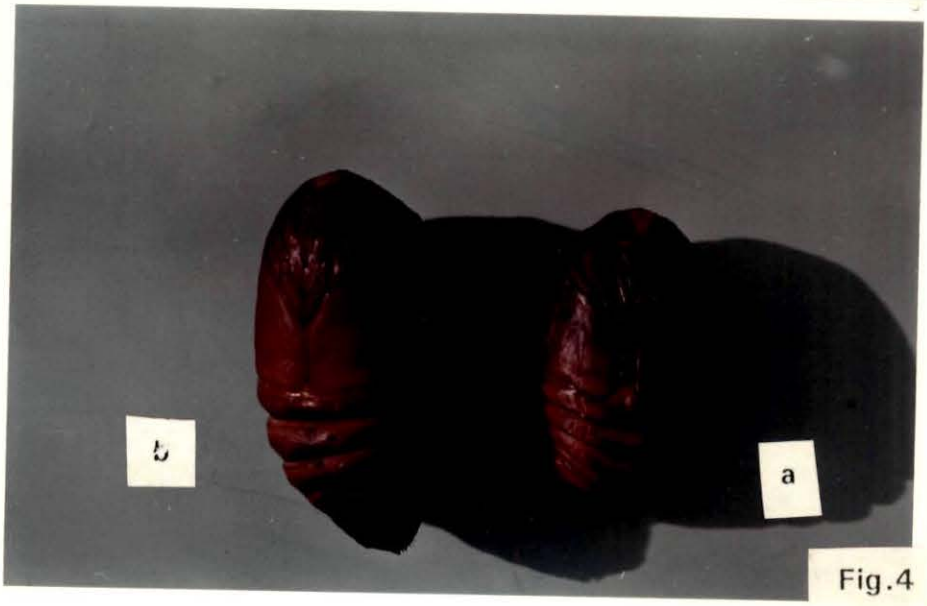


Fig.4

Fig. 5 : Photograph of moths of *A.mylitta,* a.male, b.female.

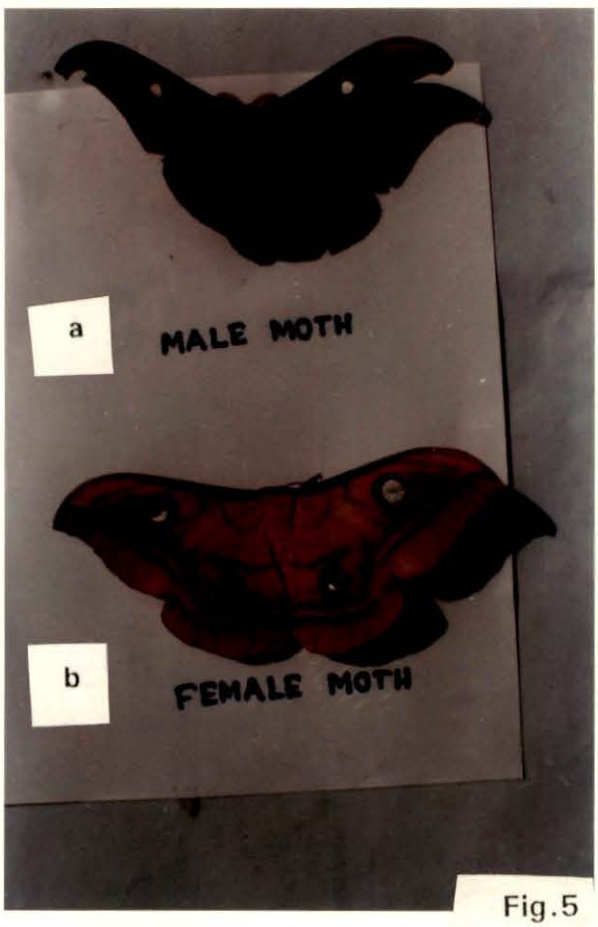


Fig.5

pupal mortality, causing practical problems to the tasar silk industry especially in seed production leading to poor fecundation, low hatchability etc. Further, during prolonged pupal stage a certain amount of energy is spent as maintenance cost. The possible cause of these impeding factors in this silkworm has been attributed to the fluctuating ambient environmental conditions during diapause (Kapila et al., 1992; Sinha and Chaudhuri, 1992). The availability of leaves of food plants varies at different geographic locations in India. In general, the leaves are available from June to December. This may provide a chance for one more crop. However, very little is known about the physiology of diapause of this species. Again, the plenty of this insect's food plants in India remains unutilized. So, the prospect of raising an additional crop is quite high, provided the technology for regulating/ terminating the diapause in *A. mylitta* can be evolved, the erratic emergence of months can be synchronised with congenial ambient conditions and food plant abundance and prolonged maintenance energy could be made use for egg or silk production.

Diapause in insect development is a result of the genetic adaptation to environment during the evolution of a given species. The expression of diapause in lepidopterous pupae is due to the partial failure of the endocrine system regulating growth and development (Williams, 1952; Wigglesworth, 1970; Meola and Adkisson, 1977). Kopec (1922) first provided evidence in the gypsy moth, *Lymantria dispar* that moulting and metamorphosis required the presence of a "brain hormone" which later on was named as prothoracicotropic hormone (PTTH). In response to endogenous and/or exogenous cues, PTTH is released from its neurohemal organ (Agui et al., 1980) into the haemolymph and transported to the prothoracic glands (PGLs). This hormone (PTTH) then activates the PGLs to synthesize and secrete ecdysone which is hydroxylated to 20-hydroxyecdysone, the ecdysteroid that elicits moulting in insects (Bollenbacher et al., 1984; Bollenbacher and Granger, 1985). In diapausing pupal brain PTTH synthesis occurs but its secretion is inhibited (Bollenbacher and Granger, 1985; Bowen et al.,, 1985; Gelman et al., 1992). Indeed it is the neuroendocrine system that serves the crucial role of transducing the environmental signals into many facets of the diapause programme. At some point during diapause the endocrine system regains its competence to function, and pupal development resumes when environmental conditions (photoperiod and temperature) become conducive (Gilbert et al., 1980; Denlinger, 1985).

PTTH has the homology with the vertebrate insulin and insulin-like growth factors (Nagasawa et al., 1984, 1986; Jhoti et al., 1987; Ishizaki and Suzuki, 1988; Kawakami et al., 1990). Further, insulin and insulin-like materials were active physiologically in insects (Hemmingsen, 1934; Dixit and Patel, 1964; Bhakthan and Gilbert, 1968; Seecof and Dewhurst, 1974; Ishay, 1975; Mosna and Barigozzi, 1976; Tager et al., 1976; Ishay et al., 1977; Davis and Shearn, 1977).

The efficacy of exogenous ecdysteroids in terminating pupal diapause has been well documented by many authorities (Williams, 1968; Zdarek and Denlinger, 1975;Waldbauer et al., 1978; Bradfield and Denlinger, 1980; Browning, 1981). The biochemical profiles of developmental stages and also of adults indicate to some extent the diapause physiology particularly with respect to its onset and termination. Several studies have been made on the biochemical aspects of diapause in several species of insects (Ichimasa, 1976; Brown, 1980; Gelman and Woods, 1983; Chippendale, 1988; Denlinger and Tanaka, 1989; Friedlander, 1989; Pullin and Bale, 1989a, 1989b; Joplin et al., 1990; Pullin et al., , 1991; Friedlander and Reynolds, 1992; Pullin 1992). However, biochemical profile during pupal diapause in A. mylitta is still unknown. This is essential for understanding the physiological adaptiveness through the mobility of stored energy during diapause and subsequent development pattern.

In India only a very little attempt has been made for understanding the influence of light and/or temperature on the pupal diapause of A. mylitta. The observation is that short days and long nights sustain diapause. On the other hand, a reverse situation terminates it (Jolly et al., 1971). Further, the influence of six phytohormones has been tested for pupal diapause termination. Topical application of atleast one tested hormone shows a good promise for diapause termination in *A. mylitta* (Jolly et al., 1973; Ahsan et al., 1976). However, these preliminary observations attract attention for further investigation with exogenous hormones.

Thus, a thorough information on larval growth and development and initiation and termination physiology of pupal diapause of this insect is eagerly looked into for an understanding how A. mylitta enters, maintains and terminates its diapause. The knowledge expected to help in solving the

practical problem for better seed production at the right time as well as to ensure the possibility for raising an additional crop in tasar industry. With this background the present investigation was aimed at the study of the following aspects in both non-diapause and diapause generations of the 'Daba' race of *A mylitta*:

1. Phenology with regard to larval pupal and adult characters under the ambient field conditions,

2. Timing of PTTH release for larval-pupal transformation through starvation and neck ligation experiments,

3. Biochemical status of cholesterol protein, DNA, and RNA in the haemolymph, fat body and gonads of pre-pupae, pupae and adults,

4. Effect of vertebrate insulin on the termination of pupal diapause , and

5. Effect of exogenous 20-hydroxyecdysone on the termination of pupal diapause.

2. REVIEW OF LITERATURE

The term "diapause" was first coined by Wheeler (1893) for the embryonic diapause in insect. It was extended by Henneguy (1904) to dormancy at any stage of development, including the arrest of reproduction by adults. Insect diapause represents a syndrome of behavioural and physiological characteristics all of which enhance the insect's survival during long periods of environmental adversity (Denlinger, 1985). The insect anticipates the period of adversity and is fully prepared when the adverse condition actually arrives. Thus, diapause is a kind of anticipatory strategy for survial that developed and programmed genetically by insects facing extreme environmental conditions (Lavenseau et al., 1986). This period is characterized by very low metabolic rates and, for most part, apparently by no morphological changes, organ development or tissue differentiation. Nevertheless, development is continued during diapause and can be demonstrated at least at the level of neurosecretory cells in the central nervous system (Beck, 1980). It is important to point out that the diapause is a transitory developmental stage and that there are many factors influencing the rate of diapause development. These include photoperiod, temperature, water, sensory stimuli and nutritive factors. Insect diapause results from the exposure of sensitive stages to distinct stimuli. This stage in the life history is usually fixed and is characteristic for each species (Behrens, 1985). Nothing is known about the signals indicating that the preparations have finished and diapause can start. However, the diapuase in insects may be either "obligatory" i.e. every individual of every generation undergoes a period of diapause as part of its life history regardless of the environmental conditions prevailing during its development; or "facultative" that is one that may or may not be manifested, depending on the environmental conditions prevailing during certain critical stages of insect's development. In addition, a few species have the capacity to diapause in two or more different developmental stages.

2.1 Phenology : Environmental cues and initiation maintenance and termination of pupal diapause .

The 'Daba' race of *A.mylitta* is though a bi- or trivoltine breed, it is subjected to pupal diapause during the winter months.

Pupal diapause is quite abundant among other lepidopterans (Beck, 1980; Saunders, 1982) and among higher dipterans (Denlinger, 1981b) although it can occasionally be found among other holometabolan insects. This is characterized by a strongly suppressed metabolic rate, cessation of adult differentiation and marked resistance to transpiratory water loss (Beck, 1980; Behrens, 1985). These diapause characteristics may occur variably in different species at different times during the pupal life span. Pupal diapause in *Papilio xuthus* is determined as a developmental commitment in response to photoperiods and temperature experienced during larval stages (Nakahama et al., 1986). Insects with facultative diapause mainly utilize the daylength as the environmental cue for induction and termination of diapause, the daily cycle of light and dark changes precisely with the seasons of the year. Indeed, many insects are known to have "biological clocks" that measure with utmost precission the duration of light and dark in each day (Saunders, 1976; Beck, 1980; Denlinger, 1985). Pupal diapause in the Chinese oak silkworm, *Antheraea pernyi* is determined by short-day photoperiods to which last two larval stages are exposed (Tanaka, 1950). Though the larval exposure to daylength is an essential requisite for pupal diapause, the nature of diapause response may even be influenced by the photoperiods experienced by the eggs and their parents. Pupal diapause in the horn fly, *Haematobia irritans* (Depner, 1962; Wright, 1970) and in the flesh fly, *Sarcophaga bullata* (Henrich and Denlinger, 1982; Rockey et al., 1989, 1991) has been claimed to be influenced by such maternal determinants. As a result of high sensitivities even the insects that live inside the fruits and the pupa inside the cocoon (*Antheraea*) are affected by photoperiod (Chapman, 1969). In *Antheraea pernyi* all larval instars are susceptible to markedly increasing sensitivity to photoperiod towards the later stagesand the effects are cumulative (Mansingh and Smallman, 1967). In *Philosamia cynthia* and *Telea polyphemus* the pupal diapause is inducible only by short photoperiods during the 4th and 5th larval instars (Behrens, 1985).

Diapausing insects are often divided into so-called long-day species and short-day species. Under natural conditions a long-day species enter diapause during the late summer of early autumn, in response to daylengths that are shorter than the population's critical daylength. Autumnal daylengths become progressively shorter until the winter solstice (Decembef 22), after which the daylengths progressively increase. It has been shown that the rate of diapause development in some species may be sensitive to daylengths through autumn (Beck, 1980). Although it is commonly the scotophase (nightlength) which is measured by insects, reports in the literature generally describe the photophase (daylength) in diapause studies (Beck, 1980).

Though it is widely documented that photoperiod plays a major role in diapause induction in insects (Andrewartha, 1952; Danilevski, 1961; Beck, 1980; Saunders, 1982), particularly in species of temperate regions, other environmental cues may also influence the response to daylength. Among these are temperature and thermoperiods (Saunders, 1973; Beck, 1983) and changing photoperiods (Tauber and Tauber, 1973).

The effect of photoperiod on animals is not only related to bioclimatic adaptations but also to the temporal organization of the internal processes that characterize the living system. The relationship between season and daylength varies with latitude (Behrens, 1985). Therefore, the critical photoperiod for diapause induction in a given species varies between the local populations of different latitudes. However, evidence indicates that local natural populations (as in flesh fly) exhibit a large amount of variability in response to diapause - inducing environmental factors (Henrich and Denlinger, 1983). The critical daylength or point of transition between very high and very low incidences of diapause has been quite sharply defined (Beck, 1980). The critical photoperiod appears to increase at higher latitudes (Danilevski, 1965; Bradshaw, 1976). Temperature can modify the critical photoperiod and provide a degree of flexibility in the photoperiodic response (Ohtaki and Takahashi, 1972). Short daylength induces and long daylength accelerates the termination of diapause. The noctuid moth, *Diparopsis castanea* from tropical Africa aestivates as a pupa when the host plants stop growing because of drought (Lees, 1955). In Europe, the southern population (at 43°N) of the cabbage moth *Mamestra brassicae* displays hibernation and aestivation diapause in pupa, but no aestivation can be found in the Northern population (at 48°N) (Gruner and Sauer, 1984). Thus, an increase in latitude is known to be associated with an increase in the critical daylength in diapause response.

Thus, the intensity of diapause can vary geographically (Danks, 1987) and possibly from year to year at the same location in response to environmental conditions during its initiation and termination phases. Few examples of geographical variation in the duration of post-diapause development however, have been reported (Tauber and Tauber, 1976; Danks, 1987).

Under natural conditions, insects are exposed to daily thermoperiods and photoperiods in which cryophase (night time) temperatures occur during the scotophase (night length) and thermophase (day time) temperatures coincide with

daylight hours (photophase). Experimental studies have shown that these natural phase relationships are of significance in the determination of diapause. Low scotophase temperatures tend to increase the incidence of diapause and high scotophase temperatures tend to suppress the diapause (Beck, 1983) Goryshin (1964) studied the combined effects of thermoperiods and photoperiods on the induction of pupal diapause in three lepidopterous species, the sorrel dagger moth, *Acronycta rumicis*, the satin moth, *Leucoma salicis* and the cabbage butterfly. *Pieris brassicae*. In all the three species thermoperiod has a definite influence on the incidence of diapause. It is also reported that in some insect species diapause induction is thought to be only temperature-dependant and indpendant of photoperiod under natural conditions (Matthee, 1978; Claret and Carton, 1980; Behrens, 1985).

Thus, the sequence of requirements must normally be fulfilled during the ontogeny of an insect for diapause expression. A genetic capacity for diapause is the first pre-requisite . The second requirement for diapause expression is a maternal history of non-diapause. Mothers that hve undergone pupal diapause cannot produce diapausing progeny (Henrich and Denlinger, 1982). The third requirement is exposure to short daylength during late embryonic development followed by reinforcement of short daylength during early larval life as in the case of pupal diapause of flesh flies (Denlinger, 1971; Vinogradova, 1976). Temperatures must remain cool during larval development to elicit a high diapause incidence (Denlinger, 1972; Saunders, 1971) and likewise, the temperature shortly after pupariation must be cool (Gibbs, 1975). Failure to meet any one of the requirements will either completely avert diapause or greatly reduce the diapause incidence. Thus, programming of diapause can be analysed as a sequence of developmental criteria, all of which must be fulfilled in order for diapause to be expressed (Denlingear, 1985).

The induction of pupal diapause in *Antheraea pernyi* and *Antheraea polyphemus* by short-day photoperiods was demonstrated by Tanaka (1951) and Mansingh and Smallman (1967). This photoperiodic control can be largely or completely nullified by slightly high temperatures. *A. pernyi* shows considerable stability under a range of temperatures; only at 32°C the diapause-inducing effects of short days are sharply reversed (Mansingh and Smallman, 1971). Indeed, the termination of diapause in this saturniid species is unique among known instances; regardless of whether the pupae are previously exposed to low temperature treatment or not, adult development is initiated under long-day photoperiod (Williams

and Adkisson, 1964). In other saturniids such as *Hyalophora cecropia* and *Antheraea polyphemus*, however, diapause can be terminated either by chilling or by exposure to long-day photoperiod (Williams and Adkisson, 1964); Mansingh and Smallman, 1967). Among diapausing pupae the duration of diapause is variable. If larvae receive many short days diapause duration is short, while exposure to only a few short days late in larval life produces a diapause of much longer duration (Denlinger and Bradfield, 1981). The insects whose diapause is determined by the photoperiodic conditions usually require a certain number of successive short or long-days for the onset of diapause. For example, larvae of *Sarcophaga argyrostoma* require 13-14 short-days to raise the incidence of pupal diapause to 50% (Saunders, 1971) and larvae of *Acronycta rumicis* need 11 short days (Goryshin and Tyshchenko, 1970). In certain insects the requirement of short days is known to be temperature compensated (Saunders, 1981).

Diapause inducing effect of short-days and the stages sensitive to photoperiod have also been demonstrated in many multivoltine insects (Danilevski, 1961; Saunders, 1976). It has been suggested that the insects are able to discriminate between short and long-day regimes by their time-measurement mechanisms and to store the photoperiodic information. This information is ultimately summated and the result is transmitted to the hormonal system which decides whether the insects enter diapause or not (Denlinger, 1985). Thus, the determination of pupal developmental fate by the photoperiodic information experienced during larval instars may be translated ultimately in terms of the neuro-endocrine mechanism.

Jolly and his collaborators undertook some preliminary investigations in India on the pupal diapause of *A.mylitta*. If the diapausing pupae are kept in continuous darkness or exposed to light upto only 12 hr/day at 20°C the diapause is sustained. On the other hand, exposure of one or more week old diapausing pupae to continuous light or to only 18 hr/day the diapause terminates. But a long day treatment of the insects (pre-pupae) beginning during the first week after spinning interferes with the diapause termination. A preliminary chilling followed by a long-day exposure of the diapausing pupae induce diapause termination only to a trace level. In summary, the authors' conclusion is that short days and long nights favour the persistence of diapause, reversively it is terminated (Jolly et al., 1971).

From the foregoing account of current status of knowledge on the environmental impact on pupal diapause in the lepidopterans in general it transpires that a great deal of diversity exists in diapause physiology. *A.mylitta* in particular, warrants priority for the study of diapause physiology since excepting the preliminary attempt by Jolly et al., 1971), there is no sound information and also because of a potentiality for furtherance of commercial exploitation of this insect based on the knowledge which may emerge out of such study.

2.2 Endocrine mechanism of pupal diapause :

The endocrinology of pupal diapause has been subjected to intensive investigation by several workers. In a classical study on *Hyalophora cecropia* pupae, Williams (1946, 1947, 1952) demonstrated that the brain has a key function in diapause regulation. Active brains from non-diapausing pupae transplanted into the diapausing ones caused the onset of post-diapause development by activating prothoracic glands. Furthermore, brains from chilled donors were able to function even in unchilled diapausing recipients. These experimental results indicated that the brain of the diapausing pupae was quite inactive for the furtherance of developmental process. Because the activation of the brain leading to a release of peptidic prothoracicotropic hormone (PTTH) is a normal pre-requisite for initiation of adult development. PTTH is a neurohormone (Ishizaki and Suzuki, 1980). In non-diapausing individuals the brain releases the PTTH which is required to initiate adult development a little prior to pupation. On the other hand, in diapausing pupae the adult development can be initiated only after the stimulation of prothoracic glands (PgIs) by the factors of brain. Thus, PTTH induces the PgIs for the release of ecdysteroids necessary for adult development and differentiation. It has already been established that inactivation of the PgIs leading to a deficiency of ecdysteroids is the main cause of pupal diapause of holometabolan insects (Bowen et al., 1984; Denlinger, 1985) and in general, the regulation of diapause in lepidopterous insects mainly depends on this endocrine basis (Raabe, 1982; Chippendale, 1983; Yamashita, 1983; de Wilde, 1983; Denlinger, 1985). In response to diapause programming signals received during larval stage, the pupal brain stops releasing PTTH, and hence the production of ecdysone from the PgIs, thereby interrupting the adult development which is manifested as pupal diapause (Bollenbacher et al., 1984; Denlinger, 1985; Bodnaryk, 1987). Just prior to pupal diapause the PgIs of both diapause and non-diapause destined individuals actively produce a surge of ecdysone that triggers pupariation (in flies) and pupation (Ohtaki and Takahashi, 1972; Calvez, 1976; Walker and Denlinger, 1980). In individuals not programmed for diapause a second surge of ecdysone is soon

released and leads to initiation of adult development. However, individuals programmed for diapause fail to release the second surge of ecdysone and ecdysteroid titre drops to levels that are undetectable with biossay techniques (Walker and Denlinger, 1980). Though the Pgls appear conspicuously inactive during pupal diapause, a low concentration of ecdysone is maintained in the haemolymph (Denlinger, 1985). Diapausing pupae of *Hyalophora cecropia* maintain a rather constant level of 5-6 pg ecdysteroid μl^{-1} haemolymph (Mc Daniel, 1979) and in *Heliothis virescens* ecdysteroid titer remains around 60 pg μl^{-1} haemolymph (Loeb, 1982). This was detected by radioimmunoassay, and is far below the amount required to initiate adult development. When the prothoracic glands again become active at the termination of diapause, ecdysone is released not as a brief pulse, but at a high level that may sustain for several days (Denlinger, 1985).

The significance of the first ecdysteroid peak during the final larval instar and its importance for pupal commitment have been described in several lepidopteran insects (Riddiford, 1985; Smith, 1985; Nagata et al., 1987). The second secretion of ecdysone, occurring after gut purge, is required for the induction of pupal cuticle formation (Riddiford, 1976; Truman and Riddiford, 1974; Truman et al., 1974). The response of the prothoracic glands to PTTH is varied, according to developmental stages and species. It appears that the external signal input in brain regulates the secretion of PTTH, the key factor for controlling the pupal diapause. Among the signal input systems, some aminergic neurons in the brain play a key role for transmitting the 'off' and 'on' signals to PTTH secretory cells in the brain (Evans, 1980; Orchard, 1982, 1984). Recently, it has been estbablished that biogenic amines are particularly implicated in the response to photoperiod variation and also in the regulation of development especially in diapause induction and termination (Puiroux et al., 1990 ; Fields and Woodring, 1991). Furthermore biogenic amines control energy metabolism in insects and as releasing factors, regulate the secretion of other hormones (Rauschenbach et al., 1993).

Cyclic nucleotides may regulate dipause, acting possibly as a second measenger of PTTH or other neurohormones (Bodnaryak, 1983; Denlinger, 1985). In both *Hyalophora cecropia* and *Antheraea pernyi*, one of the earliest biochemical signs of adult development is a pulse of cAMP activity in the brain (Rasenick et al., 1976, 1978; Berry, 1981; Resenick and Berry, 1981). The response of the Bertha armyworm, *Mamestra configurata* to cyclic mucleotides differs markedly from the response of silkmoths (Bodnaryk, 1975, 1978, 1981). Recent study suggests

a dual control of pupal diapause in *M. configuarata* by cyclic neucleotides, cAMP to maintain diapause and cGMP to terminate it (Bodnaryk, 1975, 1987). On the other hand, in *H. cecropia*, cAMP in median neurosecretory cells of the brain acts to transduce photoperiodic signals to terminate diapause (Rasenick et al., 1976, 1978).

The possible role of ecdysteroids and the juvenile hormones in the induction of larval and/or pupal diapause in holometabolan insects has been investigated indirectly (Denlinger, 1985; Chippendale, 1983) and in a number of instances the titres of these hormones have been determined during the inductive periods (Ohtaki and Takahashi, 1972; Calvez, 1976; Claret et al., 1978; Ismail et al., 1979; Yin and Chippendale, 1979; Walker and Denlinger, 1980; Bean et al., 1982; Loeb, 1982; Bean and Beck, 1983; Sedlak et al., 1983 Gelman and Woods, 1983; Gharib et al., 1984; Briers et al., 1982). The observations suggest that the changes in the haemolymph levels of ecdysteroids are not consistent during the pre-diapause development of either larval or pupal diapause -destined animals. It appears that photoperiod-induced pupal diapause programme in *Manduca sexta* is stored by the brain-retrocerebral complex during larval-pupal development, without affecting any significant change in ecdysteroid and juvenile hormone endocrinology during this period. This elevated brain centred programme is then expressed during the pupal period as a curtailment of PTTH release (Bowen et al., 1985).

PTTH : In larval and pupal diapause PTTH is the primary endorcine element affected by the environmental cues such as photoperiod, temperature and moisture. Although its presence in the insect brain and its role in stimulating the moulting process was postulated more than six decades ago (Kopec, 1922), it is only recently that progress in the isolation and characterization of PTTH has been made (Agui et al., 1980; Ishizaki and Suzuki, 1980, 1988; Gilbert et al., 1981; Bollenbacher Nagasawa et al., 1984, 1986 ; Bollenbacher and Granger, 1985; Jhoti et al., 1987; Kawakami et al., 1990; Gelman et al., 1992; Thyagaraja et al., 1992). In pupal diapause growth, moulting and metamorphosis of larvae reared under diapause inducing conditions are not overtly different from those in case of non-diapausing larvae, yet the resulting pupae diapause. During this time, however, specific covert endocrine signals such as elevated juvenile hormone titres, could be programming the brain in the short-day larva to prevent the release of PTTH in the pupa. It is the release of PTTH which is apparently curtailed at discrete times

during the larval or pupal period that results in diapause (Bowen et al., 1985). Analysis of the titers of PTTH in brains of diapausing *Antheraea* (williams, 1967) suggested that the arrest of development in this species probably occurred at the level of release, since the levels of PTTH in short-day brains remained high during diapause (Bollenbacher and Granger, 1985). Recent investigation affirms that diapause is not due to a lack of synthesis of PTTH molecule, but rather to an inhibition of its release (Gelman et al., 1992).

The control of PTTH release and the precise timing of this event during development is governed by three factors : (1) the body weight of the developing insect, which at a given stage of development reaches a threshold level necessary for the neurohormone's release (Truman, 1972; Nijhout and Williams, 1974; Goodman et al., 1985); (2) a change (decline) in the haemolymph titre of juvenile hormone that is permissive for PTTH release (Nijhout and williams, 1974; Fain and Riddiford, 1976; Rountree and Bollenbacher, 1986); and (3) a circadian gating mechanism within the brain (Truman, 1972) that specifies the time of PTTH release during a 24 hr cycle (Bollenbacher et al., 1987). The sequence of the neuro endocrine events and the clock mechanism controlling the events reside within the brain-corpus cardiacum-allatum complex (Tomioka and Bollenbacher, 1989). The release of PTTH is confined to a narrow window around the time of the head critical period (HCP) and this release time is differnet in male and female larvae. The HCP can be defined as the time during which the presence of the brain becomes unnecessary for a subsequent moult; it can be defined more precisely as the time during which PTTH release from the brain occurs.The duration of the HCP in a population represents the range of time over which PTTH release ceases in that population (Knobloch and Steel, 1989). Thus, the most rapidly developing insects would cease PTTH release at the beginning of the head critical period while the most slowly developing ones would cease the release at its end.Hence, the most rapidly developing animals must commence to release PTTH prior to the beginning of the head critical period (Knobloch and Steel, 1989). This interpretation of the relationship between the head critical period and PTTH release is entirely consistent with earlier literatures (Truman and Riddiford, 1974; Bollenbacher et al., 1987).

The time of PTTH release is a pre-requisite to proper interpretation of events controlling metamorphosis. In *Manduca sexta* it appeared that the release of PTTH during the last larval instar is triggered by attainment of a critical weight of 5 g. (Nijhout and Williams, 1974). Evidence indicates that the interactioin among

the three metamorphic hormones in Lepidoptera is more complex than in the early classical scheme (Nijhout and Williams, 1974; Safranek et al., 1980). Perhaps the best criteria for insuring physiological synchrony of endocrine events in lepidopterans are growth parameters and behavioural or morphological markers (Jones et al., 1981). It is known that there are to major periods of PTTh and ecdysone release in Lepidoptera (Truman and Riddiford, 1974; Riddiford, 1980) and in post-wandering lepidopteran larvae JH promotes the release or the effects of ecdysone (Riddiford, 1980; Safranek and Williams, 1980). Very recently, Shirai et al., (1993) has reported that PTTH is released five times in the 5th larval instars of the silkworm, Bombyx mori. It has been shown that moulting can eventually occur after a long delay in those larvae incapable of further PTTH secretion (due to neck ligation) once the first PTTH release has already occurred, because of leakage of ecdysone from the prothoracic glands (Truman, 1972). Both Smilowitz (1974) and Sparks et al., (1979) used thoracic-abdominal ligations to locate the time of sufficient ecdysone release for successful pupation. Smilowitz (1974) used ecdysis to the pupa as the measured end point while Sparks et al., (1979) used the formation of tanned pupal cuticle beneath the larval cuticle and scored the larvae 1-day after the controls pupated. Smilowitz (1974) placed the time of sufficient ecdysone release as the mid-prepupal stage, while Sparks et al., (1979) placed it earlier at a time associated with cocoon spinning. Since less ecdysone is needed to cause tanning than for the actual moult, Sparks et al., (1979) placed the time of ecdysone release earlier than Smilowitz (1974).

While starvation experiments indicate the critical body weight triggering the first release of PTTH, ligation techniques perhaps evolves the second release of PTTH as vindicated through behavioural or morphological symptoms. Thus, the HCP can precisely define the time of PTTH release into the haemolymph without relying on the PTTH bioassays to do so, although the technique lacks a quantitative dimension (Bollenbacher and Granger, 1985). Starvation of a last-instar larva prior to its attainment of a critical weight (of 5 g. in *Manduca sexta)* was observed to prolong the activity of the CA and delay in pupation (Nijhout and williams, 1974). In considering the evidence for the humoral regulation of PTTH release it must be remembered that any hormone found to affect a response, whether it is JH, 20-hydroxyecdysone etc., is itself controlled by a mechanism integrating intrinsic and/or extrinsic cues (Bollenbacher and Granger, 1985). The effect of seasonal photoperiodism on PTTH release is a well-established phenomenon and this stimulus apparently overrides the input of other environmental stimuli to cue the induction and duration of diapause in insects. Temperature is also an important external cue that can regulate PTTH release, and this effect can be expressed

either in a circadian or seasonal context. Temperature can act either alone or in concert with a particular photoperiod and can affect the rate of development and the induction, reversal or termination of diapause (Danilevski, 1965; Saunders, 1976; Tauber and Tauber, 1976; Beck, 1980). In diapausing pupae of *Manduca sexta* only the release of PTTH is curtailed and elevated temperatures probably evoke the release of previously stored hormone (Bollebnacher and Granger, 1985). The endocrine events involved in the larval-pupal development of *Manduca sexta* has been shown to be a two-step process: the first secretion of PTTH and ecdysone induces the prodormal signs of pupation such as the 'heart exposure', pink-pigment formation, gut purge and wandering, while the second induces pupal cuticle formation (Truman and Riddiford, 1974; Nijhout and williams, 1974). Ligation experiments on *Samia cynthia* yielded the similar results and it was anticipated that PTTH and ecdysone responsible for pupal - cutical formation in *Samia cynthia* must be released sometime in the post-feeding stage (Fujishita and Ishizaki, 1982).

Thus, several factors are known to be involved in the regulation of ecdysteroid production and release by the prothoracic glands. These include a brain ecdysiotropin prothoracicotropic hormone (PTTH) (Gilbert et al., 1980b; Bollenbacher and Bowen, 1983 ; Bollenbacher and Granger, 1985), environmental signals such as photoperiod (Mizoguchi and Ishizaki, 1982) and temperature (Meola and Adkisson, 1977), humoral factors such as lipoproteins (Chino et al.,, 1974), neural signals (Richter and Gersch, 1983), JH levels (Nijhout and williams, 1974; Rountree et al., 1987; Bollenbacher, 1988), a haemolymph factor of fat body origin (Watson et al., 1985; Gray et al., 1987) and ecdysone titre in the haemolymph (Siew and Gilbert, 1971; Sakurai and Williams, 1989). The shutdown of prothoracic gland function associated with the diapause state could be caused by a block at one or more of these points of control, and depending upon the insect species, the mechanism may vary (Gelman et al., 1992).

2.3 Changes in biochemical profiles of different tissues during insect development:

Insect diapause is a phenomenon with lots of species - specific strategies. This period of dormancy normally without any food uptake under extreme and unfavourable conditions requires special adaptations in metabolism. During diapause metabolism takes place at a very low level simply to keep the individual alive. Holometabolous insects, particularly the lepidopteraus, accumulate reserves during

larval development and leave off these in pupae or even in adults. Plasma of insect has highly and widely variable proportions of different inorganic and organic constituents of physiological importance (Wyatt and Pan, 1978).

In lepidopteran development most of the larval organs undergo involution for histolysis during pupal life. The resources, thus available, and the abundant storage of nutrients in the fact body and haemolymph are redistributed for imaginal development. Again, many of the imaginal organs grow from imaginal cell population or imaginal buds. This requires extensive cell division which again, has a direct relevance to the total DNA and RNA contents of different tissues. Among the various nutrients, cholesterol and protein contents are particularly important for histogenesis and morphogenesis. Consequent upon all these developmental stigma the initiatation of imaginal or adult development in a pupa of a species must be characterised by a definite pattern of mobility and profiles of nutrients and their regulating architects.

Cholesterol : In insects cholesterol has a dual role, as structural components of ues and as precursors to essential steroid metabolites and regulators, such as the moulting hormone. "α – ecdysone" first isolated from pupae of the silkworm as the crystalline MH (Butenandt and Karlson, 1954) was identified as a pentahydroxy steroid (Karlson et al., 1963; Huber and Hoppe, 1965). Its precursor was cholesterol. In insects sterol nutrition and metabolism have been reviewed by numerous researchers (Clayton, 1964; Robbins et al., 1971; Dadd, 1973; Svoboda et al., 1975; Svoboda and Thompson, 1985). In fact cholesterol is involved in a variety of functions in insects such as, growth, development, moulting, oogenesis, egg production, hatching etc. (Levinson and Bergmann, 1957; Monroe, 1959, 1960; Kaplanis et al., 1960; Gilmour, 1961; Robbins and Shortino, 1962; Gilbert, 1967; Cooke and Sang, 1970). Insects are unable to synthesize the necessary sterols and thus need an exogenous source (such as diet) to provide for their needs. Many omnivorous and phytophagous species of insects are able to dealkylate and convert C_{28} and C_{29} phytosterols to cholesterol in order to obtain adequate quantities of this essential sterol (Robbins et al., 1971; Svoboda et al., 1975).

Cholesterol turnover during various developmental stages of *Philosamia ricini* has been studied (Pant and Nautiyal, 1974). Further, tissue wise distribution of sterols has been examined in several insects (Casida et al., 1957; Goodfellow and Gilbert, 1967; Chaudhuri et al., 1986; Hurkadli et al., 1989). Ichimasa (1976) examined the sterol contents of ovaries in "diapause" and "non-diapause" silkworm,

Bombyx mori during pupal-adult development. The cholesterol ester of pupal ovary increased from a low to higher concentration between day 3 and 9 in both non-diapause and diapause generation. The diapause ovary contained as much as 200 µg more cholesterol ester per gm. of tissue than that of non-diapause ovary on day 3 and this difference increased to more than 300 µg g^{-1} on day 9. In contrast, the concentrations of free cholesterol in diapause and non-diapause ovaries were similar and decreased from a high amount on day 3 to a lower amount on day 7. Svoboda and Thompson (1985) speculated that the diapuse hormone functions to accumulate cholesterol in pupal ovaries as an ester during oogenesis; however the biological significance of temporal accumulation during oogenesis remains to be resolved.

Protein : Protein metabolism during metamorphosis of holometabolous insects has been the subject of numerous studies (Chen, 1971, 1985). Protein is synthesized in the larval fat body and released into the haemolymph where it continues to accumulate (Munn et al., 1969; Kinnear et al., 1971; Izumi et al., 1981). At the end of the last larval feeding stage when protein synthesis stops, resorption of the accumulated proteins takes place from the haemolymph into the fat body cells and resequestered as dense protein granules (Martin et al., 1971; Price, 1973; Thompson, 1975; Tojo et al., 1981). This storage protein ultimately helps in organogenesis, vitellogenin synthesis and egg maturation (Price, 1973; Tojo et al., 1980; Ogawa and Tojo,1981; Sridhara, 1981) within the pupa. The yolk precursor protein, Vitellogenin is synthesized in the female larval fat body and secreted into the blood for transportation to the growing ovary (Pan et al., 1969; Bradley, 1983; Dhadialla and Wyatt, 1983). Apart from these fat body storage protein hydrolysis of larval tissue also contributes resources to the histogenesis during adult development. In *B. mori* fat body protein content gradually increases from early fifth larval to mid pupal stage with a slight fall during larval - pupal transformation and then gradually decreases till adult emergence (chaudhuri and Medda, 1985b). Tojo et al., (1978) identified two types of storage proteins in the fat body of the saturniid moth, *Hyalophora Cecropia*. Both the proteins accumulate at maximum level at spinning and then are sequestered into fat body so as to make up 60% of the total fat body protein in the female pupa. Vitellogenins synthesized by the fat body are uptaken from the haemolymph by the growing oocytes and are sequestered into yolk proteins (Telfer, 1965; Engelmann, 1979). In *Bombyx mori* vitellogenin is detectable only in the haemolymph in the early half of the pupal period and later increases in the ovary, while keeping a constant level in the haemolymph (Ogawa and Tojo, 1981). The synthetic activity of vitellogenin

by the fact body is elevated during pharate adult development, as in H. cecropia (Pan et al., 1969; Pan, 1971). Vitellogenin isolated from B. mori is very similar to that of H. cecropia (Kunkel and Pan, 1976) and from Philosamia cynthia (Chino et al.,

1976). This suggests a homology of vitellogenin in these species of Lepidoptera (Ogawa and Tojo, 1981).

Patterns of fat body protein synthesis change during insect dormancy. Fat body protein synthesis declines to 3% of its active larval level during diapause in saturniid pupae (Stevenson and Wyatt, 1962). In several insects diapause associated proteins are present in the haemolymph and fat body throughout the diapause period (Brown and Chippendale, 1978, Dortland and dekort, 1978; Brown, 1980; Dillwith et al., 1985; Venkatesh and Chippendale, 1986). These abundant proteins are synthesized before the onset of diapause and characteristically disappear from the haemolymph when development ensues and their most apparent function is as a storage protein (Joplin et al., 1990).

Decline in the fat body protein corresponds to the period of protein uptake by the oocytes and hence correspond to adult development. In B. mori it is reported that ovarian protein content gradually increases with the advancement of age and reach maximum on the last day of pupa and adult stage. But, protein content of the testes increase with the advancement of age and reach a peak during mid pupal stage which sustains upto adult emergence (Chaudhuri and Medda, 1985a; 1986). However, protein profile in different organs of prepupae, pupae and adults of *A. mylitta* is not known. This information is pertinent for the understanding of onset and termination physiology of diapause.

DNA and RNA : Insect DNA is polymerized from deoxynucleoside triphosphates by a battery of enzymes using single-stranded DNA of Template. Deoxynucleoside triphosphates are supplied from two sources : Salvage of the components of degraded DNA and reduction of the corresponding ribonucleoside phosphates. In many groups of insects extensive histolysis occurs, particularly in forms with non-feeding pupae and salvage of deoxynucleotides probably plays a large role in supplying deoxynucleotide precursor for DNA synthesis (Berry, 1985). In the diapausing pupa, many tissues are poised to begin differentiation in response to moulting hormone and would be expected to resume DNA synthesis (Berry, 1981). Several workers have studied the activity of various silkmoth enzymes involved in

DNA metabolism (Berry et al., 1964, 1967; Berry and Firshein, 1967; Firshein et al., 1967; Swindlehurst et al., 1971; Freeman et al., 1972; Moriuchi et al., 1972). Brookes and Williams (1965) measured the activity of thymidine and thymidylate Kinase in *Antheraea pernyi* at various stages of pupal-adult transformation. Oberlander et al., (1965) examined thymidine incorporation into DNA and Uridine incorporation into RNA in the prothoracic glands of saturnud moths and speculated that the pgls lose the ability to synthesize DNA at the larval- pupal moult, while RNA synthesis seems to reflect general synthetic activity. The increase in the activity of these enzymes may reflect DNA degradation more accurately than DNA synthesis (Berry, 1985). Active synthesis of DNA is suspended when *Hyalophora cecropia* pupal enter diapause (Bowers and Williams, 1964; Krishna Kumaran et al., 1967) and resumes only after pharate development is stimulated by the secretion of ecdysone. Most of the insect tissues are polyploid and the total DNA per animal is dependent upon the number and ploidy of constituent cells. Lang et al., (1965) found that total amounts of both RNA and DNA per animal increase steadily during larval stages in the mosquito, decline during pupation and are maintained at steady but lower levels during adult life.

Results of experimental studies (Wyatt and Linzen, 1965; Berry et al., 1967; Barritt and Birt, 1971) support the contention that RNA and protein synthesis are generally suppressed during diapause as are other measurable metabolic activities. Takahashi (1966) measured the RNA-DNA ratio in the fat body of *Philosamia Cynthia ricini* fifth instar larvae after injection of ^{32} P . The ratio of rRNA : DNA decreased as the fifth instar proceeded. The ratio of "sRNA" : DNA remained approximately same, indicating that ribosomes were synthesized in the early 5th instar presumably to support the protein synthesis later on.

Nucleic acid changes in different tissues/organs have been studied in several insects (Clements, 1959; Ishizaki, 1965; Berry et al., 1967; Krishna Kumaran et al., 1967; Chinzei and Tojo, 1972; Price, 1973; Ono et al., 1975; Locke, 1981 ; Chaudhuri and Medda, 1985a, b). In *Hyalophora Cecropia* RNA content enhances in the ovary probably due to elevated RNA level in follicular cells (Pollock and Telfer, 1969). The rate of nucelic acid synthesis in *B. mori* increases during middle and later part of the feeding stage (Akai and Park, 1971). The reduction in RNA and DNA content in normal ovary from pupal stage is supposed to be due to either disintegration of the follicular cells and nurse cells of the ovary (Chinzei and Tojo, 1972) or huge accumulation of organic substances and thereby increase in organ weight or both (Chaudhuri and Medda, 1985a). In testes of *B. mori* the RNA and DNA levels remain unchanged from the third day of final larval instar to the

mid-pupal stage after which there cellular constituents rise and reach maximum level on the last day of pupa or at the adult stage. These changes in nucleic acid contents and the concomitant increase in gonad weight during the pupal period are the positive indications of the enhancement of spermatogenesis or sperm maturation (Chaudhuri and Medda, 1986).

The biochemical profile of cholesterol, proteins, DNA and RNA in haemolymph or in different tissues are not known either in case of diapause or non-diapause generation of *A. mylitta.* This information is essential for an understanding of the diapause physiology of this species particularly in respect of causal relations of these contents with the onset and termination of diapause and diapause development. With this view point attempt has been made to assess the quantitative variations of cholesterol, protein, DNA and RNA contents in the haemolymph, fat body and gonads during different developmental stages of both the generations of *A. mylitta.*

2.4. Effect of vertebrate insulin on the Physiology of insect :

Vertibrate insulin affects early embryonic diapause determination in *Bombyx mori* (Morohoshi and Ohkuma, 1968). When it is injected into female pupae determined to lay diapause eggs, some of the eggs develop without diapause. In the past 20 years, more than 40 neuropeptides have been isolated and identified from insects. Most of them are myotropins. Proctolin was the first insect neuropeptide structurally characterized and isolated from whole body extracts of *Periplaneta americana,* on the basis of its myotropic activity (Starratt and Brown; 1975). Using immunocytochemical techniques, mammalian gastroentero-pancreatic peptides have been reported to occur in the nervous and intestinal system ofinsects (Duve and Thorpe, 1979, 1982, 1988; Fujita et al., 1981; El-Salhy et al., 1983; Kramer, 1980, 1985; Thorpe and Duve, 1984, 1988). A glucagon-like peptide with a molecular weight of 15 KDA has been partially purified from a larval midgut extract of the tobacco hornworm, *Manduca sexta* (Tager and Kramer, 1980). It has been reported that insulin production occurs naturally at extra pancreatic sites (Kramer et al., 1982; Le Roith et al., 1985, 1988). There is substantial evidence for the existence of insulin-like peptides in insects (Tager et al., 1976; Kramer 1980, 1985; Maier et al., 1988). In *Bombyx mori* homology of prothoracicotropic hormone (PTTH) an insect neuropeptide, with vertebrate insulin has already been established (Nagasawa et al., 1984, 1986; Jhoti et al., 1987;

Ishizaki and Suzuki, 1988; Kawakami et al., 1990). A noteworthy effect of vertebrate insulin on insect tissue is that it helps in growth stimulation, moulting hormone induction, lipid mobilization, sugar uptake, cellular internalization, growth of imaginal disks and several cell lines *in vitro* (Seecof and Dewhurst, 1974; Mosna and Barigozzi, 1976; Davis and Shearn, 1977). Very recently it has been reported thatinsulin reduces diapause duration in *Antheraea mylitta* (Sinha et al., 1993).

Insulin has a direct or indirect impact on carbohydrate, lipid and protein metabolism. A lot of turnover of these contents takes place at the initiation of imaginal development i.e. the diapause termination. Permeability of glucose and some amino acids to the cells is promoted by insulin. This is very much needed foradult morphogenesis. Further, insulin raises the rate of DNA transcription in the nucleus ensuring more RNA production and hence promoting more protein synthesis.

The foregoing literature reveals that hormonal convergence cannot be mooted in the vertebrates and invertebrates. Lepidopterans are no exceptions. The vertebrate insulin like neuropeptides such as PTTH (Nagasawa et al., 1986) in *Bombyx mori* may not be greatly different from the PTTH of *A.mylitta.* This provides a hope that vertebrate insulin may induce effects on diapausing pupae similar to those induced by PTTH in diapause termination. As because 20-HE is the finalp component for action towards adult development and diapause termination in *A.mylitta*, and exogenous application of 20-HE to the diapause-destined pre-pupae may have important effect. This is why insulin and 20-HE have been included in the present investigation.

2.5 Effects of exogenous ecdysone on diapause physiology :

It has been observed that diapause can be terminated by injecting ecdysone in larvae or pupae of several species of insects (Sieber and Benz, 1980). Bodnaryk (1977) demonstrated a systematic change in the sensitivity of diapausing pupae of *Mamestra configurata* to injected ecdysteroid. It is well known that excessive amount of ecdysone cause abnormal adult development (Kobayashi et al., 1967 ; Williams, 1970; Judy and Gilbert, 1970) and that the injection of juvenile hormone into pupae results in the production of intermediates possessing

both pupal and adult characters (Gilbert and Schneiderman, 1960). The efficacy of exogenous ecdysteroids in terminating pupal diapause has been well documented in several insects (Williams, 1968; Fraenkel and Hsiao, 1968; Baird, 1972; Zdarek and Denlinger, 1975; Gibbs, 1976; Denlinger, 1976, 1979; Meola and Adkisson, 1977; Walbdauer et al., 1978; Bradfield and Denlinger, 1980; Denlinger et al., 1980; Browning, 1981). It has been showed that 20-hydroxyecdysone is much more efficient than ecdysone in triggering the developmental abilities of diapausing eggs (Gharib et al., 1981).

A single ecdysteroid injection is a poor mimic of the natural release pattern as is often reflected in the high doses required for a response. Dividing a large single dose into several temporally separated smaller doses (Zdarek and Denlinger, 1975; Gibbs, 1976) is usually more effective. A range of developmental responses can be elicited depending on the amount of ecdysteroid injected. Species differences are also to be considered in the responsiveness of pupae to ecdysteroids during different phases of diapause development.

Ecdysteroids injected into the body could initiate development by merely acting on the non-endocrine tissue to promote morphogenesis but such a model seems unlikely since several days of ecdysone exposure are required for completion of adult differentiation (Denlinger, 1985).Exogenous ecdysteroids are rapidly metabolized (Ohtaki et al., 1968; Karlson and Bode, 1969; Zdarek and Fraenkel, 1970) and it is thus unlikely that a threshold level of the hormone would persist for an adequate time. It is much more likely that one of the major actions of exogenous hormone is to exert a stimulatory effect on the intact pupal brain or prothoracic gland (Denlinger, 1985). Several lines of evidence (Williams, 1952; Siew and Gilbert, 1971; Kimura and Kobayashi, 1975) suggest a positive feedback mechanism for ecdysone on the prothoracic gland. In certain instances it is also likely that ecdysteroids exert a stimulatory action on the brain (Agui and Hiruma 1977; Marks et al., 1972).

Many lepidopteran species discontinue spermatogenesis during larval and pupal diapause (Cloutier and Beck, 1963; Chippendale and Alexander, 1973). The fact that exogenous ecdysteroids cause in vitro spermatogenesis renewal in intact testis explanted from diapausing Lepidoptera has been repeatedly confirmed (Yagi et al., 1969; Friedlander, 1989). Preliminary observation reveals that 20-hydroxyecdysone is also able to terminate pupal diapause in *A. mylitta* (Sinha et al., 1994).

Further, a variety of ecdysteroids derived from plant sources are also fully capable of terminating pupal diapause. In fact, in several cases, the phytoecdysteroids are more effective in breaking diapause presumably because they are less vulnerable to enzymatic degradation within the insect's body (Denlinger, 1985).

Jolly and his collaborators studied the impact of six phytoecdysones applied through injection on the termination of pupal diapause in *A. mylitta.* Activation to adult development by these phytohormones differed considerably and also in a dose dependent manner. However,a the resultant adults behaved normally for overall reproductive performance. Cyasterone proved the best promise for the initiation of adult development in diapausing pupae (Jolly et al., 1973). Jolly and his collaborators further studied the impact of six phytohormones after topical application to the freshly moulted diapause destined pupae. The diapause terminating effect of cysterone was confirmed again. Further, the other phytohormones also showed a promise when applied topically at higher doses (Ahsan et al., 1976).

3. MATERIALS AND METHODS

3.1 Comparative Phenology of Both Non-Diapause and Diapause Generations of *A. mylitta.*

3.1.1. Phenology of larvae, pupae and adults.

The insect and rearing condition : All investigations were carried out on the 'Daba' bivoltine brood of *A. mylitta* Drury at Ranchi in the state of Bihar at a longitude, latitude and altitude of 85°18'E, 23°14'N and 708 m MSL respectively. Eggs for both diapause and non-diapause generations were obtained from the Central Tasar Research and Training Institute , Nagri, Ranchi, India. Just after hatching for each generation 40,000 larvae irrespective of sex were maintained in the field on the high bush of Arjun plant (*Terminalia arjuna,* Bedd.), one of the primary food plants of *A. mylitta* under natural environmental conditions. Rearing was done on bushy vegetation of *T. arjuna* made through arboriculture (Fig.6). The rearing was continued in the field from first to fifth larval instars till the formation of cocoon on the food plant after which cocoons were collected from the field and kept at room temperature of insectary. The larval feeding period (phagoperiod) was measured from the day of larval emergence (from egg) to the day of onset of spinning and the non-feeding or non-phagoperiod was counted from the starting of spinning to the onset of pupation. The rearing period in non-diapause generation lasted from middle of July to last week of August and in diapause-destined generation it lasted from first week of October to first week of December.

Body weights and growth indices: The initial body weights for 100 newly hatched larvae (irrespective of sex) were recorded and the average weight was determined. The larval body weight for each instar was recorded immediately before and after each ecdysis to the next instar before the onset of feeding. Sexing could be done only in the 5th (final) instar larvae. Relative growth rate (RGR) of each instar was calculated on green weight following Waldbauer (1968) and Rema Devi et al., (1991) by the formula :

Fig.6 : Photograph of plantation of *Terminalia arjuna*, the primary food plant of *A.mylitta,* raised through arboriculture.

Fig.6

$$\frac{\text{Final larval weight - initial larval weight}}{\text{Mean larval weight x Feeding duration in days}}$$

The larval maximum weight (Lmw) was noted at the fifth instar on larval maturity just after cessation of feeding. In the insectary all cocoons were cut open to record the time of pupation. Cocoons were weighed with and without pupae (empty shell). The shell ratio percentage (SR %) was calculated by

$$\frac{\text{Single shell weight}}{\text{Single cocoon weight (with pupa)}} \times 100$$

Weight of each pupa during the span of pupal life was taken at an interval of 7 day in non-diapause till the emergence of moth (adult). In case of diapause-destined generation carbohydrate profile (Chaudhuri et al., 1993) and anatomical evidence (unpublished observation) suggest that till 150 day of pupal life the development occurs at a very negligible pace. Hence, pupal weight for this generation was initially taken at 30 day interval upto 150 day and thereafter at every 15 day (Table 3). Pupal body become soft on the day before emergence and based on this observation pupal weights were taken on day before emergence. Moth weights were taken just after emergence.

Egg number and hatching percent : This aspect was also studied in the insectary. Generally tropical tasar moths lay eggs continuously spanning over three consecutive days. After proper washing and disinfection the eggs were collected and kept for incubation under natural environmental conditions till hatching which required about 10 days for both the generations (Table 3). Unlaid eggs were counted in each female after completion of oviposition on the third day by dissecting abdomen. Laid and unlaid eggs were counted on the basis of total number of eggs (laid and unlaid) per female. Egg hatchability was calculated by the number of eggs hatched out of total number of eggs laid. For every parameter studied, the data were taken from 100 individuals in each generation.

Environmental records :

Daylength, maximum and minimum temperature and relative humidity(r.h) were recorded daily in the field as well as in the insectary all through the study period for the duration of each larval instar, pupa and adult. Rainfall was also

similarly recorded. Daylength was measured in hours as light : dark cycles of 24 hour periodicity. Relative humidity was measured daily as an average of the records in the morning and in the evening. The data were subjected to student's 't'-test.

3.1.2 Grainage Performance : This was studied on the basis of percentage of moth emergence, sex ratio, mating, fecundity, egg incubation time and hatching percentage. In a year, the moths (adults) emerged from the first week of June to the second week of August) after breaking the overwintering pupal diapause were considered as the first brood, and the second moth emergence (from the first week of September to the third week of October) was regarded as the second brood for this study.

Depending on the time of availability of tasar seed cocoons during the study seasons, selected healthy pupae (a number of 11,000 in first brood and 14,000 in the second brood) were preserved in the model grainage room of the insectary as improvised by the Central Tasar Research Station, Ranchi, India (Kapila *et al.*, 1992) in order to observe their emergence and post-emergence behaviour.

Daily moth emergence (sex wise) , mating percentage and fecundity of each mated female were recorded in every generation. Following oviposition the eggs were incubated in the insectary for observing the time required for incubation together with their hatching performances. Temperature and r.h. in the insectary were recorded daily for every brood.

These daily recorded data were computed as average of every three consecutive days spanning the entire grainage periods. All these average values were subjected to student's 't' test wherever necessary.

3.2 Starvation and Neck Ligation Experiments for Ascertaining the Timings of PTTH Release by the Fifth Instar Larvae :

3.2.1 Starvation: The experiments were conducted on the same population and on the same location as stated under phenology studies (3.1.1) but on separate arjuna plants for both non-diapause and diapause generations. From the very large population only 10,000 healthy , 0-day-old fifth instar larvae were randomly collected, 5000 males and 5000 females for each generation. The larvae of non-

diapause brood attained the fifth stage during August and those of diapause-destined brood attained during October-November under field conditions. Sexing were done by observing the Herold's gland in females and Ishiwata's spot in males. An equal number of same aged larvae of each sex and each generation was also kept in the field from the same population as control. The control larvae were weighed daily from '0'-day to larval maturity when larval maximum weight (Lmw) was recorded just after cessation of feeding. For the purpose of assessment of larval critical weight (Lcw) 60 male and 60 female larvae of each generation were withdrawn from the host plants daily starting with '0'-day old onwards upto the day of gut purge. Such larvae under starvation were kept on leaf-less branches encircled by nets. The larvae were weighed and observed daily for their morphological and/or behavioural changes associated with larval-pupal transformation. The experiment was conducted in two consecutive years.

3.2.2 Neck ligation : The larvae of each sex were ligated behind the head (neck ligation) with a strong fine cotton thread in both non-diapause and diapause generations separately. Neck ligation was performed daily on 25 males and 25 females of each generation from the '0'-day age onwards till the day before larval-pupal transformation for testing the timings of PTTH release. All larvae (control and experimental) in both generations were individually weighed every 24 hr. interval. Morphological and behavioural changes associated with pupal syndrome such as body shrinkage, decremental mobility of anal proleg and gut purge and pupal ecdysis were recorded daily in both control and experimental batches.

Larval critical weight (Lcw), larval maximum weight (Lmw), latent feeding period, pupal critical weight (Pcw) and adult critical weight (Acw) were determined from the starvation experiment following the procedure of Ochieng'-odero (1990a, 1990b). Lcw was regarded as the weight at which 50% of the silkworms were capable of pupation thereby producing functional adults. Pcw and Acw were calculated as follows :

$$Pcw = Lcw - (Lcw \times D_P)$$

and $$Acw = Lcw - (Lcw \times D_A)$$

where, D_P = Constant weight decrease from Lmw to Pw (Pupal weight)
and D_A = Constant weight decrease from Lmw to Aw (adult weight).

Relative silk conversion efficiency were calculated by

$$\frac{\text{Single shell weight (without pupa)}}{\text{Larval weight after gut purge}} \times 100$$

All weights were taken on green weight basis and gated release of PTTH during the period of larval-pupal development was resolved. The different biological performances such as gut purging and wandering behaviour, silk cocoon spinning, larval duration in respect of phagoperiod and non-phagoperiod, formation of pupal cuticle, time required for pupation, pupal characters, adult emergence and number of egg production (laid + unlaid) were recorded from time to time. The ambient environmental data were also recorded all through the experiment.

Data were statistically analysed by student's 't' test wherever necessary.

3.3 Quantitative Status of Cholesterol, Protein, DNA and RNA of Haemolymph, Fat body and Gonads of Pre-pupae, Pupae and Adults of both the Generations

3.3.1 Chemicals and reagents used for estimation:

3.3.1.1 For Cholesterol : Ferric Chloride - Acetic Acid reagent : 50 mg of ferric chloride (E.Merck, AG Darmstadt) was dissolved in 100 ml glacial acetic acid (GR, Sarabhai M. Chemicals, India). The solution was kept in a refrigerator.

Standard Cholesterol solution : 10 mg of cholesterol (E.Merck AG Darmstadt) was dissolved in 100 ml of aldehyde free glacial acetic acid with slight warming and thus a solution having 100 µg of cholesterol per ml. was prepared. This was used as standard solution and kept in the refrigerator.

3.3.1.2 For Protein :

Reagent A. 2% Sodium carbonate (BDH, Glaxo Laboratories, India) in 0.10 (N) sodium hydroxide (E.Merck, India).

Reagent B. A solution of 0.5% cupper sulphate (BDH, Glaxo Laboratories, India) in 1% Potassium Sodium Tartarate (BDH, Glaxo Laboratories, India).

Reagent C: Alkaline cupper sulphate solution . This solution was prepared

by mixing 50 ml. of reagent A with 1 ml of reagent B just before use.

Reagent D :

Diluted Folin Reagent : The Folin reagent was prepared by refluxing gently for 10 hours a mixture consisting of 100 g. of Sodium tungstate (E. Merck, AG., Darmstadt), 25g. of Sodium molybdate (E.marck, AG., Darmstadt), 700 ml.of distilled water, 50 ml. of 85% Phosphoric acid ('Analar', BDH, India)and 100 ml of concentrated Hydrochloric acid (Analar, BDH, India) in a 1.5 litre flask. After boiling , 150g of lithium sulphate (Analar, BDH, India), 50 ml. of distilled water and a few drops of bromine water were added. The mixture was boiled for 15 minutes without condensor to remove excess bromine. It was cooled and diluted to 1 litre and filtered. The acid concentration of the reagent was determined by titration with 1(N) Sodium hydroxide to a phenolpthalein end point. Just before use the reagent was diluted accordingly so as to prepare a diluted Folin reagent of 1(N) of acid strength.

Reagent E. Standard protein solution : Bovine serum albumin Cohn fraction V (Sigma, USA) was used for the preparation of a standard protein solution. A standard solution containing 200 µg of protein per ml. was prepared by dissolving 20 mg of the protein in 100 ml. of distilled water.

3.3.1.3 For DNA : Standard DNA solution: 5 mg of calf thymus DNA (Sigma, U.S.A.) was dissolved in 10 ml of 5% Perchloric acid (GR, E.Merck, AG Darmstadt) at 70° C for half an hour. 5 ml. of this solution was taken and volume was made upto 50 ml with 5% Perchloric acid and thus a solution having 50 µg of DNA per ml was prepared. This was used as standard solution and was kept in the refrigerator at 4°C.

3.3.1.4 For RNA : Standard RNA solution: Yeast RNA (Sigma, U.S.A.) was purified in the laboratory. 10 mg of purified RNA was dissolved in 10 ml of 5% Perchloric acid (GR, E.Merck, AG, Darmstadt) by heating the mixture at 70°C for 30 minutes in a water bath. 5 ml of this solution was taken and the volume was made upto 50 ml with 5% perchloric acid so as to prepare a solution containing 100 µg of RNA per ml. This was used as standard solution and was kept in the refrigerator at 4°C.

3.3.2 Procedure and methods of analysis

Stages of the insect and sample size : Every time 10 individuals of pre-pupae, pupae and moths of each sex and of both the generatioins were sacrificed on different days as required during the experimental period for all the quantitative estimations. In the non-diapause generation pupae were sacrificed on 0, 7, 14 and 21 day while in diapause generation pupae of 0, 40 , 105, 150, 170 and and 200 day old were sacrificed for the estimation.

3.3.2.2 Collection of haemolymph : The haemolymph from pre-pupae was collected by inserting the needle of a microlitre syringe through the second abdominal leg. But the haemolymph of pupal and adult stages was collected by a microsyringe piercing through the anal margin of the wings by applying a gentle pressure on the abdomen. After collection of haemolymph, a few granules of phenylthiourea was added to it in order to prevent blasckening of the haemolymph. The haemolymph was then subjected to centrifuge in cool condition at 3000 rpm for 5 minutes for precipitating the blood cells. The separated plasma was taken for the extraction and estimation of protein and cholesterol.

3.3.2.3 Collection of tissue : The fat body and gonads were quickly and carefully dissected out of the individuals surrounded by ice for making a cold condition. The haemolymph was perfectly soaked. These separated fat body and gonads of males and females were weighed and used for preparation of tissue hamogenate and extraction of cholesterol, protein, RNA and DNA.

3.3.2.4 Preparation of tissue homogenates for

A. Cholesterol : A 5% tissue homogenates of fat body and gonads were

prepared in each case in ferric chloride - acetic acid reagent by using a Potter-Elvehjem all glass homogenizer and kept overnight for precipitation to extract the cholesterol. The tissue samples were then centrifuged for 15 minutes at 3000 rpm and the supernatants were collected for estimation of cholesterol.

B. RNA : A 5% homogenates of the tissues from fat body and gonads of both the sexes were prepared separately in 0.65% cold saline (NaCl) using a Potter-Elvehjem all galss homogenizer. 1 ml. of homogenate was transferred to a centrifuge tube and 1 ml. of 0.6 (N) and 1 ml. of 0.3 (N) Perchloric acid (PCA) were added and mixed thoroughly and kept standing for 10-15 minites. The mixture was then centrifuges at 3000 rpm for 5 minutes. The entire procedure was maintained under cold condition. The supernatant was discarded. The precipitate was washed with 5 ml. of cold 0.3 (N) PCA, mixed thoroughly and again centrifuged. The supernatant was discarded again. The precipitate was then treated with 1 ml. of 0.3(N) KOH and was kept on a water bath at 37°C for 2 hours with occasional stirring and cooled to the room temperature, 2 ml. of cold 0.6 (N) PCA and 2 ml. of cold 0.3 (N) PCA were added to the mixture, kept standing for 15 minites and centrifuged under cool situation. The supernatant was collected for RNA estimation.

C. DNA : The precipitate thus obtained was once again washed with 5 ml of cold 0.3(N) PCA and centrifuged. The supernatant was discarded. The precipitate was then treated with 4 ml. of 0.6(N) PCA, heated at 70°C for about half an hour, cooled to room temperature and then kept at cold (4°C) for 10 minutes after removing the red washing with 1 ml. of 0.6(N) PCA. It was then centrifuged and supernatant was collected for estimation of DNA.

D. Protein : The precipitation left after extraction of RNA and DNA was treated with 5 ml; of alcohol-chloroform - ether mixture (2:1:1), stirred and centrifuged . The supernatant was discarded. The precipitate was then washed by suspension and sedimentation with 5 ml. of absolute alcohol once and finally with 4 ml. of solvent ether. The precipitate was allowed to dry at room temperature. The dried protein thus obtained was dissolved in 5 ml. of 0.3(N) sodium hydroxide and kept overnight at room temperature. The solution thus obtained after thorough mixing was suitably diluted and used for protein estimation.

Calculations : The results of cholesterol, protein, RNA and DNA contents of fat body and gonads of male and female individuals were expressed per 100 mg tissue. The haemolymph (plasma) protein and cholesterol contents were expressed

per ml. of plasma.

Results were statistically analysed using student's 't' test.

3.3.3 Procedure of Estimation :

3.3.3.1 Estimation of Cholesterol - Haemolymph : 50 ml. plasma (from previously isolated stock) was taken in a centrifuge tube and washed with 5 ml. of ferric chloride - acetic acid reagent and kept overnight for precipitation. It was then centfifuged for 15 minites at 3000 rpm. The supernatant was collected for the estimation of cholesterol.

The cholesterol content of haemolymph plasma was determined by the method of Kabara (1962). An aliquot of 4 ml. of the extracted cholesterol was taken in a test tube. The final volume was made to 5 ml. by addition of 1 ml. of ferric chloride - acetic acid reagent. 3 ml. of concentrated suiphuric acid was then added and mixed thoroughly in a cyclomixer. For standard curve, five different concentrations of cholesterol (10 µg, 20 µg, 30 µg, 40 µg, and 50 µg) were used and treated as above. For the reagent blank 5 ml. of ferric chloride - acetic acid reagent was used instead of cholesterol solution and treated as above. The standards and the reagent blank were run simultaneously along with the test samples each time. After mixing the samples the tubes were kept in a cold dark place for 30 minutes. The readings were taken at 560 nm with 'Turner' spectrophotometer (USA).

Fat body and gonad : The cholesterol content of fat body and gonads were determined separately by the same method of Kabara (1962) at 560 nm with 'Turner' spectrophotometer (USA).

3.3.3.2 Estimation of RNA : RNA content of fat body and gonads was estimated by the method of Munro and Fleck (1966) modified by Abalain et al., (1980). Aliquote of 2 ml. for fat body (male and female) and 1 ml each for ovary and testis were taken all through the investigation relating to pre-pupae, pupae of different days/age and freshly emerged adults (moths). Each aliquot was diluted to 3 ml. by 0.3(N) PCA. The samples were read at 260 nm using ultraviolet unit of 'Turner' spectrophotometer (USA).

For standard curve, five different concentrations of purified yeast RNA (10 µg, 20 µg, 30 µg, 40 µg, and 50 µg) were prepared from the standard solution and the volume of each concentration was made to 3 ml with 0.3 (N) PCA. 3 ml. of 0.3(N) PCA was used as reagent blank.

3.3.3.3 Estimation of DNA : DNA content of fat body and gonads was estimated by the method of Munro and Fleck (1966) modified by Abalain et al. (1980). Aliquot of 1 ml. for fat body, ovary and testis of extracted nucleic acid solution was diluted upto 2 ml. with 0.6 (N) PCA and were read at 260 nm using ultraviolet unit of 'Turner' spectrophotometer (USA).

For standard curve, five different concentrations of calf thymus DNA (5 µg, 10 µg, 15 µg, , 20 µg, and 25 µg) were prepared from the stock concentration (standard solution) and the volume of each concentration was made to 2 ml. with 0.6 (N) PCA in each case. 2 ml. of 0.6(N) PCA was used as reagent blank.

3.3.3.4 Estimation of protein:

Haemolymph - From the previously isolated plasma stock 50 µl of plasma was taken in a centrifuge tube and 5 ml. of 0.65% NaCl, 1 ml. of 2/3 (N) H_2SO4 and 1 ml. of 10% sodium tungstate were added. After mixing thoroughly it was centrifuged. The supernatant was discarded. The precipitate was treated with 5 ml. of absolute alcohol, stirred and centrifuged. Again the supernatant was discarded. The precipitate was washed with 4 ml. of solvent ether. The precipitate was allowed to dry at room temperature. The dried protein thus obtained after thorough mixing was suitably diluted and used for protein estimation.

Protein content of haemolymph plasma was determined by the method of Lowry *et al.*, (1951). An aliquot of 0.1 ml diluted solution was taken in a test tube and mixed with 0.9 ml of distilled water. 5 ml. of alkaline copper reagent was added, mixed and kept standing for 15 minutes at room temperature. To this mixture 0.5 ml of diluted Folin reagent was added, mixed well and left for 30 minutes at room temperature for colour development.

For standard curve, five different concentrations of bovine serum albumin viz. 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg were used and for reagent blank, distilled water was used instead of protein solution. The standards and the reagent

blank were run simultaneously with test samples each time. All the samples were read at 750 nm in a 'Turner' spectrophotometer (U.S.A.).

Fat body and Gonads : Protein content of fat body and gonads was determined by the method of Lowry et al., (1951). An aliquot of 0.1 ml. diluted solution was taken in a test tube and mixed with 0.9 ml of distilled water and the protein was estimated similarly as for haemolymph.

3.4 Treatment of Pre-pupae and Early and Late Pupae of Diapausing Generation of *A. mylitta* with Insulin.

Ox pancreas insulin (40 i.u. per ml. which contains 1.67 mg of crystalline insulin) procured from Boots chemical Company, India Ltd. was used for the experiment. Pre-pupae, 40 day old early pupae and 150 day old late pupae of diapausing generation were treated with insulin. A single injection of insulin was given with the help of a Hamilton microlitre syringe to the individuals of each set of experiment. Pre-pupae received separately three doses of insulin each of 1 µl (0.04 i.u.) 5 µl (0.2 i.u.) and 10 µl (0.4 i.u.) per individual. But only two doses of 5 µl and 10 µl of insulin/individual were applied to the 40-day-old and 150-day-old pupae as 1 µl dose of the hormone was found to be ineffective when treated during pre-pupal stage. The control individuals were treated with 10 µl of 0.65% saline. A number of 100 males and 100 females were used for both experimental and control individuals for each dose except the pre-pupae. In the pre-pupae external visible sexual dimorphic characters were extremely inconspicuous. However, sexing was possible in the pupae resulted from the pre-pupae. Pre-pupae were taken out by removing the anterior cap of the silk cocoon and selected irrespective of sex at random with average body weight of 15.76 $\pm$ 1.05 g. 200 pre-pupae (3-day before pupation) were treated separately with each dose of insulin as well as with saline as control.

Pupal duration, moth weight egg production and hatching percentage were all noted separately for each treatment and control.

Total protein, RNA and DNA and cholesterol contents of haemolymph, fat body and gonads of diapausing pupae and adults after insulin treatment were estimated by the methods stated earlier (3.3). Weights of gonads after insulin treatment were also recorded.

The total protein, DNA, RNA and cholesterol contents of different tissues of normal diapausing pupae of different ages and adults were used as controls for this experiment since there was no differences in the status of these bimolecules in different tissues of Sham-operated animals (injected with 0.65% saline) when compared with those of control individuals without any saline injection i.e. normal animals.

3.5 Treatment of Dipause-destined Pre-pupae with 20-Hydroxyecdysone (20-HE)

0-day-old prepupae, after the completion of spinning were taken out by removing the anterior cap of the silk cocoon and were selected at random irrespective of sex and having average body weight of 15.76 $\pm$ 1.05g. 50 prepupae were treated with each of the dosages of 20-HE.

20-hydroxyecdysone (No. H-5142, Sigma Chemical Company, USA, anhydrous molecular weight 480.60) was used in this experiment. The hormone was first dissolved in 96% ethanol and then diluted with distilled water to yield a solution of 10% ethanol. Because of limited availability of 20-HE and incidence of developmental derangements due to progressively higher doses beyond 5 µg/ individual, only 4 dosages of this hormone were applied at the rate of 1, 2, 5 and 10 µl/pre-pupa. The hormone was injected into the abdominal region with the help of a Hamilton microlitre syringe. Every 10 µl of ethanol solution contained each of the desired doses of the hormone. Control insects were administered with 10 µl of 10% ethanol.

The observation was taken on the pupation time(time required by the prepupae to become pupae), pupal duration, emergence percentage, moth weight, morphological derrangements and pupal mortality.

An additional 50 samples of prepupae were treated with similar doses of 20-HE for each treatment along with the control for recording the morphometry and protein content of gonads on 150 day of diapause development. The weights of testes and ovaries of 8-10 individuals in each case were measured on 150 day of diapause development after dissection. Dimension of gonads were taken on the same day using an ocular micrometer under a dissecting binocular microscope. Fecundity and hatching percentage were also recorded from the moths resulted from 20-HE treatments. Gondal protein contents were also estimated.

4. RESULTS

4.1. Comparative Phenology of Larvae, Pupae and Adults of both Non-diapausing and Diapausing Generations:

4.1.1 Span of the two generations and duration of stadia and moulting :

Bimodal generations of *A. mylitta* was observed during the period of investigation. Rearing of the larvae of ND-generation was started on mid-July after the hatching of larvae from eggs and that of D-generation was started on 1st October of the year. Total life span starting from 0-day of 1st instar larva till the death of moth was recorded to be about 70 and 283 days for ND-and D-generations respectively. However, an instarwise longer larval duration was recorded from the 4th instar onwards in diapause-bound generation. No differences was observed between the two generations in the duration of 1st and 2nd larval moults. But, for the 3rd and 4th moults the diapause generation took about 0.5 day more than those of the ND-larvae. The data on the life span for larvae, pupae and adults are given in Table 1. and Fig. 7.

4.1.2 Larval assessment

Compared to the larvae of ND-generation a higher larval body weight was initially recorded from the late 2nd instar larvae of D-generation. This higher weight was significantly different ($P<0.001$) from the corresponding weight of ND-generation. The incremental weight difference is also reflected in the higher weight of initial 3rd instar larvae of D-generation. In the remaining developmental stages the weight differences increased steadily. The relative growth rate (RGR) decreased gradually from first to fifth larval instars in both the generations. But, the diapause-destined larvae exhibited comparatively a lower RGR than that of the ND-generation. (Table 2;

Table 1 . Life span (days) of different developmental stages of A. mylitta in both non-diapause(ND) and diapause(D) generations.

Developmental stages	Sex	Duration of ND-Generation			Duration of D-Generation		
		Feeding	Moulting	Total	Feeding	Moulting	Total
1L		3.00	1.00	4.00	4.00	1.00	5.00
2L		3.50	1.00	4.50	4.00	1.00	5.00
3L		4.00	1.50	5.50	4.50	2.00	6.50
4L		6.00	2.00	8.00	8.00	2.50	10.50
5L	M	12.00	-	12.00	20.00	-	20.00
	F	13.00	-	13.00	22.00	-	22.00
Total larval	M	28.50	5.50	34.00	40.50	6.50	47.00
duration.	F	29.50	5.50	35.00	42.50	6.50	49.00
Spining	M	-	-	3.00	-	-	8.00
duration.	F	-	-	4.00	-	-	9.00
Pre-pupal	M	-	-	4.00	-	-	9.00
life span	F	-	-	4.00	-	-	9.00
Pupal life span	M	-	-	20.00	-	-	208.00
	F	-	-	21.00	-	-	209.00
Moth life	M	-	-	7.00	-	-	9.00
span.	F	-	-	8.00	-	-	10.00
Total duration from 0-day 1st instar larva to the death of adult.	M	-	-	68.00	-	-	281.00
	F	-	-	72.00	-	-	285.00

L = Larval instar , M = Male , F = Female.

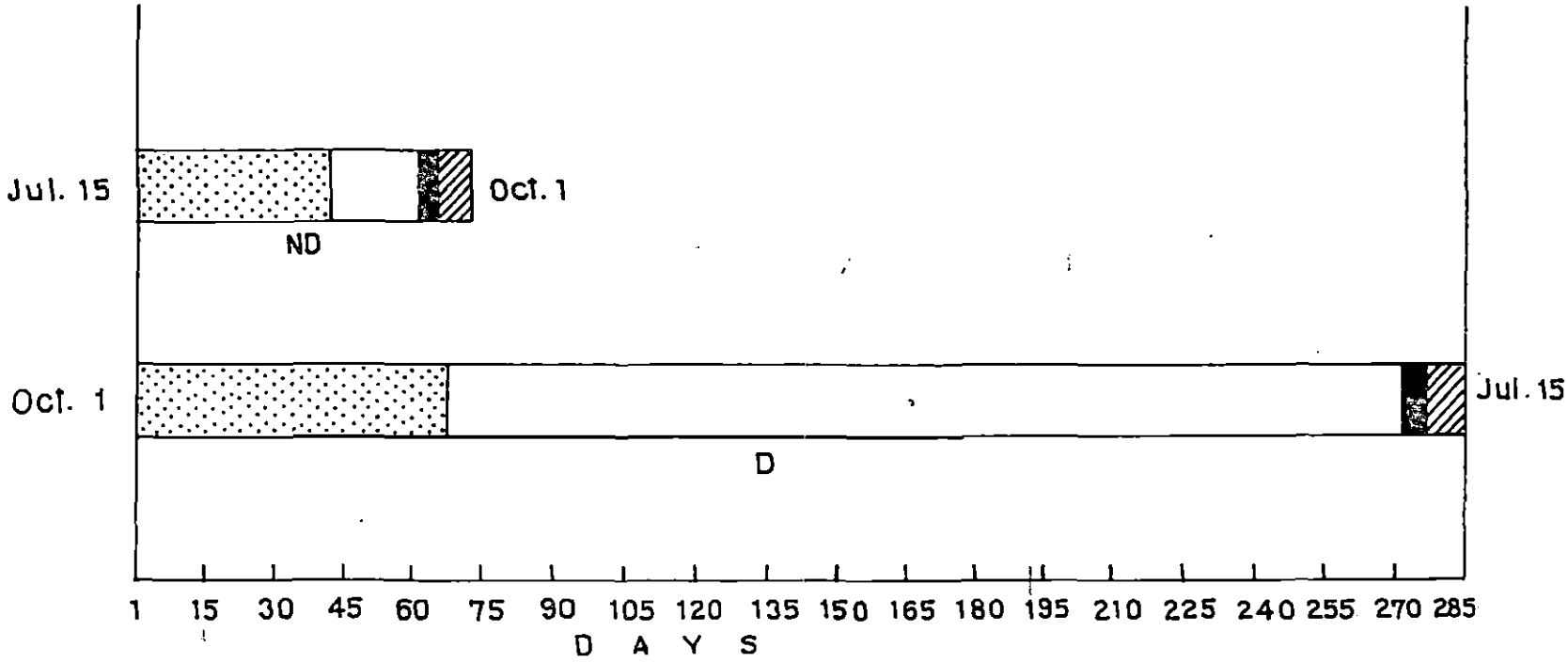


Fig. 7. Life span of A. mylitta in non-diapause (ND) and diapause(D) generation.

Larva (feeding, moulting, spinning and pre-pupal duration)

Pupa.

Moth.

Egg incubation period.

Table 2. Larval weight (mean ± SE) and relative growth rate of *A.mylitta* of non-diapause (ND) and diapause(D) generations.

Larval instars	Sex	Larval weight (g)				Relative growth rate (RGR)	
		ND generation		D-generation		ND generation	D-generation
		0-day	last day	0-day	last day		
1st		0.00842 ±0.00017	0.08474 ±0.00152	0.00881 ±0.00015 NS	0.08699 ±0.00097 NS	0.546	0.408
2nd		0.07854 ±0.00095	0.35170 ±0.00879	0.07885 ±0.00130 NS	0.527 ±0.009 a'	0.423	0.369
3rd		0.31560 ±0.01019	2.12 ±0.07	0.487 ±0.003 a	2.802 ±0.06 a	0.370	0.313
4th		1.90 ±0.03	10.58 ±0.20	2.362 ±0.05 a	11.79 ±0.22 a	0.232	0.166
5th (feeding)	Male	8.98 ±0.16	32.57 ±0.36	9.97 ±0.16 a	35.78 ±0.40 a	0.095	0.056
	Female	11.66 ±0.22	40.83 ±0.40	12.74 ±0.20 a	45.46 ±0.36 a	0.085	0.051
5th (non-feeding)	Male	*20.22 ±0.26	**10.23 ± 0.10	*23.18 ± 0.24 a	**14.03 ± 0.12 a		
	Female	*25.78 ±0.48	**13.56 ±0.12	*28.05 ±0.20 a	**17.34 ± 0.17 a		

* Just after gut purging.
** Pre-pupa.

't' - test probability differences between the two generations.
a = P<0.001.
NS=Non significant.

Fig 8). Thus, the larval developmental rate was faster in the ND-over the D-generation.

4.1.2. Pupal assessment

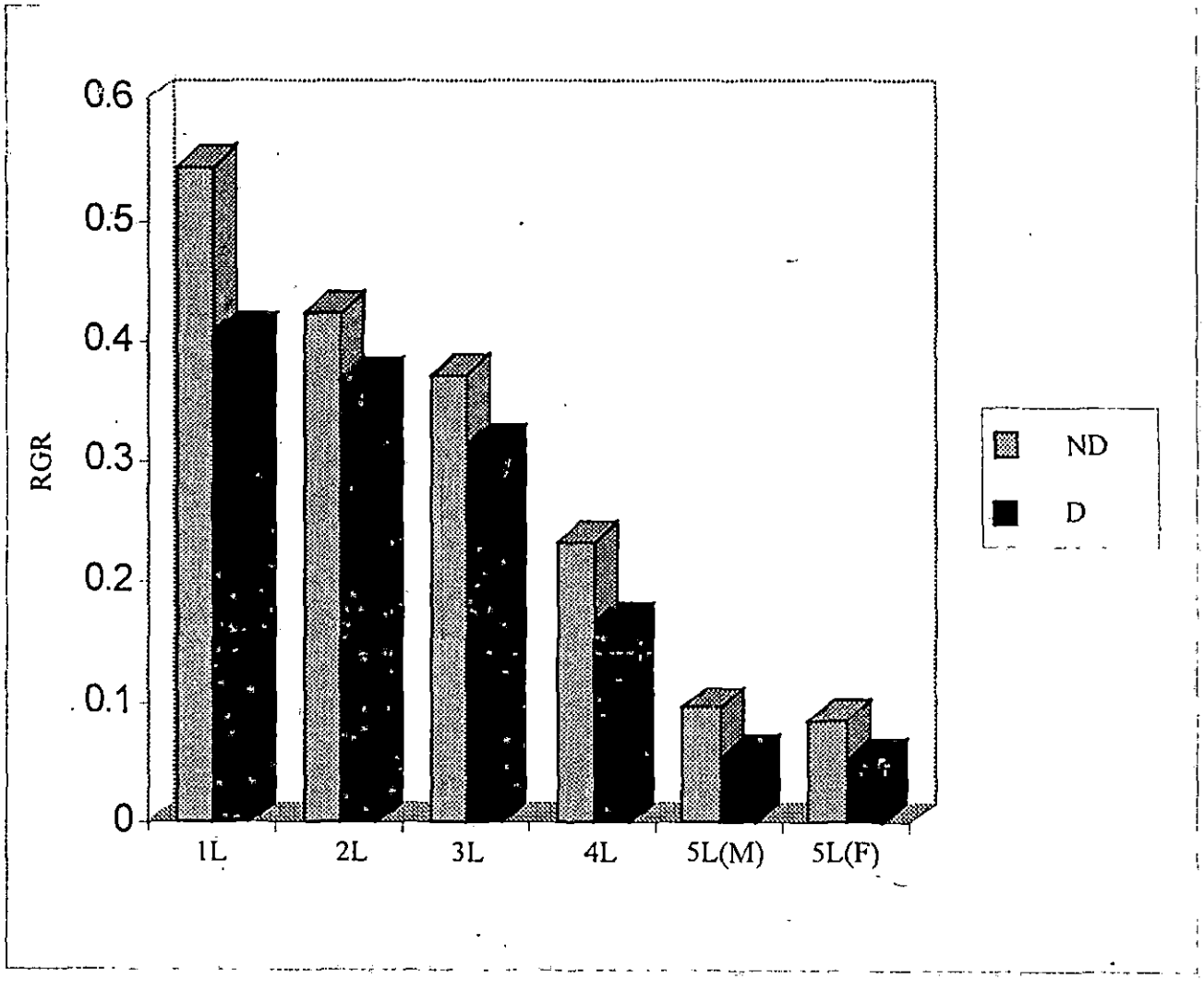
Pupal life span was found to be significantly longer and pupal weight as well as cocoon weight and shell weight in both the sexes of D-generation were higher over these values of the ND-generatio(Table 3). However, a gradual reduction in pupal weight from 0-day onwards till moth eclosion was observed in both the sexes of the two generations. In both the generations pupal weight, cocoon weight and shell weight remained higher in females than these measurements in males except the S.R% which was found to be reverse (Table 3).

4.1.3. Adult weight and egg production

Male and female moth weights as well as fecundity and total egg production (laid + unlaid) per female were found to be significantly lower ($P<0.001$) in the D-generation than those in the ND-generation (Table 3). However, no significant difference was observed in egg incubation period and hatching percentage between the two generations (Table 3).

4.1.4. Influence of environmental factors on the two generations :

Prevailing ambient daylength, temperature, r.h. and rainfall were quite different during the life span of the two generations. The fluctuations in the different environmental parameters in field condition as well as in the insectary are presented monthwise (Table 4) covering the entire period of the observation, together with the ambient conditions experienced by each developmental stage of life history (Table 5, Fig 9). Short day length of less than 12 hrs. (11.65 hrs) in combination with low (minimum)) temperature of less than 20°C (17.68°C) was availed from third instar onwards in case of diapause-destined generation. The minimum average temperature availed during 3rd instar larvae of diapausing -destined generation remained significantly lower ($P<0.001$) than the temperature experienced by the 1st and 2nd larval instar while no significant difference was observed in between



3. 8. Relative growth rate (RGR) of *A.mylitta* in non-diapause (ND) and diapause (D) generation. L=Larval instar, M=Male, F=Female .

Table 3 . Pupal and moth weights, cocoon characters and reproductive performance of *A. mylitta.* The data are mean ± SE.

Pupal age (day)	Weights (g)			
	Non-diapause		Diapause	
	Male	Female	Male	Female
0	9.12 ±0.15	12.89 ±0.10	12.12 ±0.13	15.20 ±0.16
7	8.77 ±0.06	12.03 ±0.06	-	-
14	8.31 ±0.04	11.23 ±0.11	-	-
30	-	-	11.75 ±0.15	14.71 ±0.11
60	-	-	11.60 ±0.11	14.61 ±0.08
90	-	-	11.41 ±0.09	14.51 ±0.07
120	-	-	11.29 ±0.08	14.32 ±0.07
150	-	-	11.00 ±0.05	14.10 ±0.06
165	-	-	10.70 ±0.05	13.63 ±0.06
180	-	-	10.30 ±0.06	12.92 ±0.07
Day before emergence	7.91 ±0.03	9.98 ±0.05	10.12 ±0.05	12.79 ±0.04
Moth	2.97 ±0.06	6.89 ±0.12	2.55 ±0.07	6.32 ±0.16
Cocoon	10.36 ±0.20	13.59 ±0.27	13.90 ±0.19	17.31 ±0.15
Shell	1.37 ±0.02	1.53 ±0.03	2.17 ±0.05	2.45 ±0.06
S.R.(%)	13.34 ±0.30	11.32 ±0.30	15.61 ±0.43	14.15 ±0.34
Egg (laid)	-	257 ±11	-	179 ±5
Production(unlaid)	-	23 ±3	-	36 ±4
(No.) (total)		280 ±10	-	215 ±6
Egg incubation period (days)	-	9.36 ±0.17	-	9.44 ± 0.16
Hatching (%)	-	80.42 ±1.39	-	77.63 ±2.14

Table 4. Environmental conditions experienced by *A.mylitta* during the study period. Each value represents the monthly average ± SE except the rainfall which is total of a month.

Month	Field					Insectary		
	Light(L) : Dark(D) (hrs.)	Max.Temp. (°C)	Min.Temp. (°C)	r.h.(%)	Total rainfall(mm)	Max.Temp. (°C)	Min.Temp. (°C)	r.h.(%)
July	13.33 : 10.67	29.11	22.01	87.95	215.25	27.87	23.81	84.74
	±0.02 ±0.02	±0.39	±0.11	±0.74		±0.25	± 0.13	± 1.06
August	12.86 : 11.14	27.88	21.72	90.74	355.25	26.09	23.55	90.82
	±0.03 ±0.03	±0.24	±0.09	±0.46		±0.21	±0.16	±0.47
September	12.24 : 11.76	28.05	20.35	89.78	245.50	25.87	23.67	81.15
	±0.03 ±0.03	±0.26	±0.22	±0.43		±0.16	±0.18	±1.28
October	11.60 : 12.40	27.05	16.07	84.08	34.50	26.26	20.29	73.92
	±0.04 ±0.04	±0.25	±0.62	±0.78		±0.10	±0.30	±1.17
November	11.05 : 12.95	25.14	9.04	77.47	0.75	20.70	14.07	60.95
	±0.03 ±0.03	±0.28	±0.51	±0.94		±0.19	±0.48	±1.24
December	10.77 : 13.23	21.56	7.41	78.38	45.00	20.13	11.97	61.23
	±0.009 ±0.009	±0.44	±0.47	±5.57		±0.15	±0.32	±1.47
January	10.92 : 13.07	22.00	6.81	73.56	-	15.74	13.68	66.29
	±0.02 ±0.02	±0.62	±0.47	±0.78		±0.39	±0.46	±1.52
February	11.43 : 12.57	24.24	7.93	74.26	17.50	18.43	16.57	63.84
	±0.04 ±0.04	±0.41	±0.39	±0.95		±0.22	±0.23	±1.34
March	12.04 : 11.96	31.98	15.91	61.32	4.50	26.26	23.56	48.68
	±0.04 ±0.04	±0.47	±0.58	±1.16		±0.62	±0.51	±1.45
April	12.71 : 11.29	32.10	19.98	62.87	18.50	30.04	27.63	40.65
	±0.04 ±0.04	±0.77	±0.34	±1.69		±0.37	±0.36	±1.36
May	13.21 : 10.79	32.12	20.75	70.65	67.00	30.00	26.73	53.17
	±0.02 ±0.02	±0.89	±0.39	±1.90		±0.60	±0.62	±3.11
June	13.49 : 10.51	29.04	21.85	81.07	138.75	30.17	27.20	66.03
	±0.005 ±0.005	±0.47	±0.28	±1.09		±0.41	±0.41	±2.71

Table-5. Environmental factors experienced by different developmental stages of *A.mylitta* in both outdoor and the insectary. Values are mean ± SE (except rainfall)

Developmental stages	Daylength (hrs.) (L : D)		Max. Temp.(°C)		Min. Temp .(°C)		r.h.(%)		Total rainfall(mm)	
	Non-diapause	Diapause	Non-diapause	Diapause	Non-diapause	Diapause	Non-diapause	Diapause	Non-diapause	Diapause
IL	13.33 : 10.67 ±0.005 ±0.005	11.89 : 12.11 ±0.02 ±0 .02	27.48 ±0.59	27.44 ±0.60	21.53 ±0.23	19.12 ±0.34	91.13 ±1.19	85.60 ±1.99	56.25	11.50
2L	13.27 : 10.73 ±0.009 ±0.009	11.85 : 12.15 ±0.02 ±0.02	26.80 ±1.19	27.33 ±0.62	21.52 ±0.23	19.57 ±0.41	92.37 ±1.63	89.17 ±0.85	73.00	12.50
3L	13.22 : 10.78 ±0.013 ±0.013	11.65 : 12.35 ±0.02 ±0.02	28.50 ±0.51	27.20 ±0.59	21,78 ±0.08	17.68 0.18	90.67 ±1.28	86.11 ±0.45	38.50	10.50
4L	13.12 : 10.88 ±0.014 ±0.014	11.46 : 12.54 ±0.03 ±0.03	28.07 ±0.25	27.30 ±0.34	21.67 ±0.16	13.02 ±0.58	89.64 ±0.38	81.09 ±0.93	21.25	-
5L(feeding period)	12.93 : 11.07 ±0.019 ±0.019	11.18 : 12.82 ±0.02 ±0.02	28.22 ±0.28	25.97 ±0.31	22.01 ±0.09	11.10 ±0.52	91.00 ±0.69	79.68 ±1.06	139.00	0.75
5L (non-feeding period i.e., spinning to pupation).	12.72 : 11.28 ±0.015 ±0.015	10.91 : 13.09 ±0.02 ±0.02	27.36 ±0.66	23.84 ±0.18	21.61 ±0.12	6.44 ±0.45	90.75 ±0.97	75.21 ±0.91	158.25	-
Pupa in insectary	12.43 : 12.57 ±0.224 ±0.224	12.08 : 11.92 ±0.38 ±0.38	25.70 ±0.16	24.39 ±2.16	23.65 ±0.23	21.05 ±2.37	85.65 ±1.73	57.13 ±3.45	187.25	291.25
Moth in insectary	12.15 : 11.85 ±0.013 ±0.013	13.45 : 11.55 ±0.01 ±0.01	26.29 ±0.26	29.10 ±0.29	24.28 ±0.17	24.20 ±0.24	73.57 ±3.94	79.05 ±1.32	85.75	47.50

L = Larval instar.

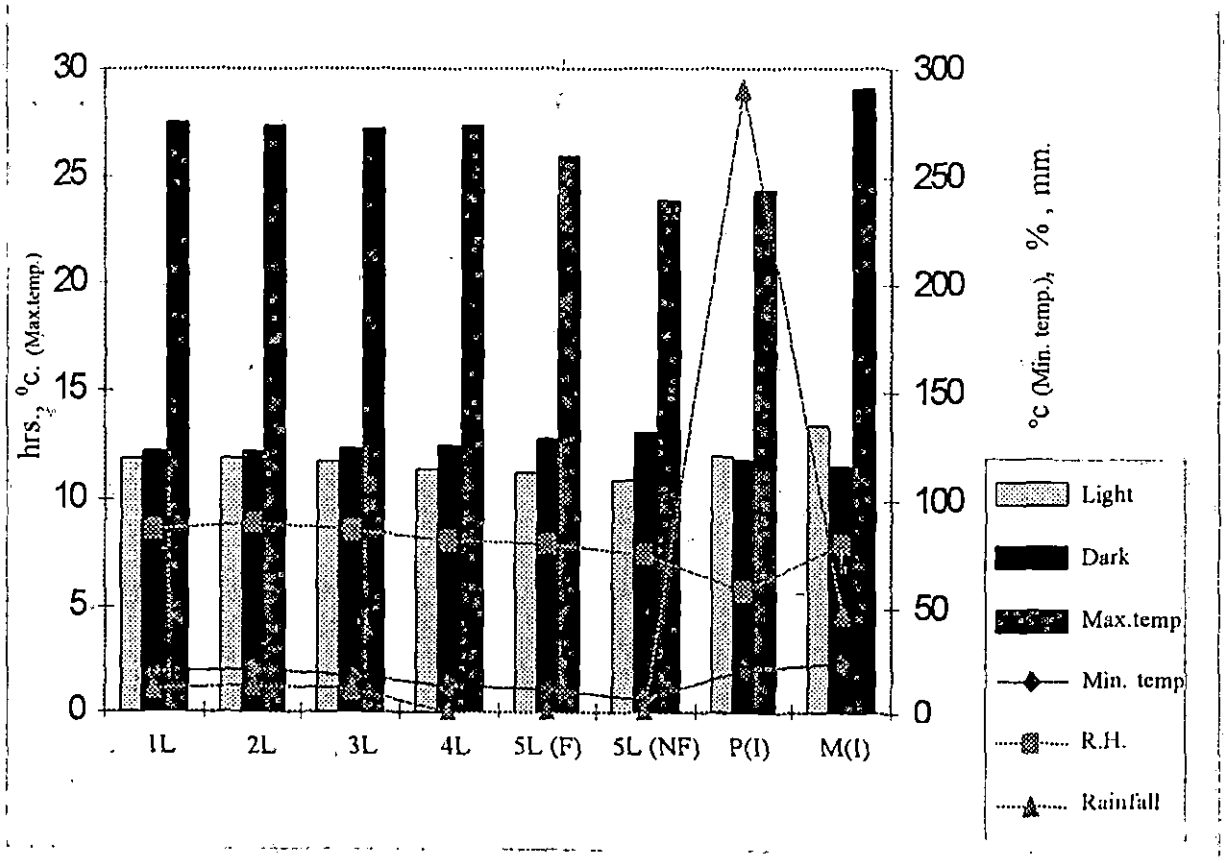


Fig. 9. Environmental factors experienced by different developmental stages of *A. mylitta* in diapause generation in both outdoor and the insectary. L=Larval instar, F=Feeding period, NF=Non-feeding period (spinning to pupation), P(I)=Pupa in insectary, M(I)=Moth in insectary.

first and 2nd instar. However, average maximum temperature was found to be significantly different among the different instars of the same generation and among the corresponding instars of the two generations with the single exception in feeding period of 5th larval instar (table 5).It is to be noted that r.h. % and total rainfall was recorded to be lower in diapause-destined generation than that of the non-diapause one. From the photoperiodic record it was observed that from April upto onwards upto about 150 day of pupal age the daylength suddenly increased above 12 hrs. The average minimum and maximum temperature recorded for this month inside the insectary were found to be a little above 27°C and 30°C respectively. This sudden change in daylength and temperature may influence the initiation of the termination of pupal diapause. The moth emergence and egg laying occurred under long daylength and high temperature in both the generations during or after monsoon.

4.1.5. Grainage performance

Moth Emergence : In the first generation normal moth emergence commenced on the first week of June and continued upto the second week of August covering a period of about 69 days. But in case of the second brood emergence period extended over 42 days from the first week of September upto the third week of October (Table 6 & 7). The time of adult eclosion as recorded from daily observation was mostly between the periods of late photophase and late scotophase in case of both the broods.

In case of first brood, most of the moth emergence took place during the period from fourth week of June to fourth week of July showing a peak on the third week of July, while in the second brood it occurred from second to fourth week of September showing a peak during the third week. Subsequent to the peak a very sharp decline occurred in the daily emergence rhythm. This was consistent in both the generations. However, in ND-generation a total of 85.91% emergence took place in a peak span of 21 days. Whereas in case of D-generation the value was 72.20% spanning a peak period of 30 days (Table 6 & 7). The overall percentage of moth emergence was, however, higher in the second brood than in the first. Moreover, male moths emerged in higher numbers than in females of both

Table 6. Environmental (insectary) conditions and grainage performance of the first brood of *A.mylitta*

Observations at every 3 day from June 1 to August 8.	Temperature(°C) Max.	Min.	Mean ±S.E	R.h% (Mean±S.E)	Emergence (%) Male	Female	Total	Spontaneous mating (%)	Facundity (nos.) (Mean±S.E)	Egg incubation period in days (Mean±S.E)	Hatching (Mean±S.
June 3	31.66	26.33	28.33±0.31	74.33±1.02	0.028	0.019	0.047	50.00	120±5	8.00±0.50	40.50±3.9
6	30.66	24.66	28.91±0.41	66.99±5.03	0.084	0.065	0.149	14.29	161±8	8.00±0.35	47.11±4.6
9	32.00	25.66	29.08±0.49	61.99±3.93	0.084	0.056	0.140	33.33	146±4	9.00±0.35	47.87±3.3
12	31.50	25.50	29.91±0.27	55.50±2.49	0.112	0.103	0.215	15.00	206±8	8.62±0.35	63.58±4.3
15	32.33	24.33	27.04±0.21	78.58±1.19	0.065	0.084	0.149	21.35	209±8	9.33±0.27	73.22±2.7
18	26.00	23.66	24.79±0.16	89.33±0.94	0.261	0.233	0.494	14.00	222±5	9.00±0.21	71.59±2.5
21	27.00	24.33	26.49±0.24	88.16±1.38	1.195	1.008	2.203	20.37	230±5	9.58±0.15	74.60±2.9
24	26.33	24.33	25.00±0.12	89.83±1.08	1.185	0.971	2.156	17.31	234±3	10.08±0.23	76.88±2.4
27	26.33	23.33	25.62±0.21	89.08±1.01	0.355	0.299	0.654	73.75	247±4	10.42±0.19	78.02±2.0
30	26.00	23.66	25.08±0.24	85.33±1.17	2.632	1.596	4.228	43.27	236±4	10.50±0.26	82.61±1.2
July 3	27.00	23.66	24.91±0.17	91.08±0.60	3.669	2.091	5.760	41.52	239±5	10.25±0.46	81.02±2.6
6	25.33	22.66	24.62±0.28	91.74±0.07	2.931	1.727	4.658	48.11	225±6	10.00±0.43	80.29±2.3
9	25.33	22.66	24.54±0.26	91.74±0.07	2.931	2.567	5.498	34.54	235±7	10.33±0.48	78.97±2.8
12	25.00	23.33	24.54±0.16	91.58±0.07	4.574	2.016	6.590	50.00	242±7	10.33±0.33	75.74±2.8
15	25.00	23.00	24.58±0.18	90.58±0.90	6.768	2.688	9.456	60.42	243±6	10.42±0.51	78.06±2.9
18	26.00	23.00	25.12±0.45	87.75±1.88	6.820	4.250	11.070	67.91	237±5	10.33±0.34	82.06±2.0
21	25.00	22.50	24.25±0.37	91.62±0.21	5.320	6.530	11.850	56.57	244±7	10.50±0.40	84.71±3.2
24	25.66	23.00	24.83±0.25	91.91±0.07	2.690	5.250	7.940	23.49	231±5	10.44±0.28	75.50±2.3
27	25.66	23.33	24.95±0.31	91.24±0.30	1.940	3.210	5.150	27.03	233±4	10.35±0.42	76.07±3.3
30	25.50	22.50	24.64±0.14	90.06±0.46	0.887	0.840	1.727	37.78	220±6	10.25±0.29	70.50±2.5
Aug. 2	26.66	23.50	25.12±0.31	84.89±2.69	0.150	0.140	0.290	33.33	202±5	9.70±0.30	61.22±3.4
5	26.50	23.50	25.45±0.28	85.08±1.99	0.080	0.120	0.200	23.08	199±4	9.15±0.35	53.00±3.4
8	27.66	24.50	26.12±0.45	85.99±1.91	0.050	0.030	0.080	33.33	172±6	9.25±0.35	48.50±4.1
AVERAGE :	27.22 ±0.52	23.78 ±0.22	25.87 ±0.37	84.54 ±2.14	44.82	35.89	80.71	43.02	214 ±7	9.73 ±0.16	69.50 ±2.81

Table 7. Environmental (insectary) conditions and grainage performance of the second brood of *A.mylitta*

Observations at every 3 day from Sept.4 to Oct. 15.	Temperature(°C) Max.	Min.	Mean ±S.E	R.h% (Mean±S.E)	Emergence (%) Male	Female	Total	Spontaneous mating (%)	Facundity (nos.) (Mean±S.E)	Egg incubation period in days (Mean±S.E)	Hatching (%) (Mean±S.E)
Sept. 6	26.67	24.67	26.16±0.27	86.92±1.17	0.022	0.00	0.022	0.00	0	0	0
9	27.00	25.00	26.67±0.31	85.67±1.08	0.584	0.007	0.591	0.00	0	0	0
12	27.67	25.33	26.75±0.19	82.16±1.88	6.815	4.540	11.355	53.33	245±6	9.50±0.35	70.57±2.55
15	27.33	25.00	26.42±0.43	83.91±2.07	8.439	5.350	13.789	38.97	252±8	9.50±0.35	86.96±2.42
18	28.67	25.67	27.37±0.32	78.00±2.02	8.826	6.478	15.304	54.51	264±6	9.55±0.46	85.44±4.36
21	27.67	25.33	26.75±0.28	82.25±0.17	8.102	6.225	14.327	40.06	256±3	9.50±0.42	78.73±3.19
24	27.00	25.00	26.62±0.14	83.83±0.04	7.868	5.222	13.090	40.95	241±7	10.00±0.20	82.80±3.20
27	27.67	25.00	27.04±0.26	79.66±1.01	7.600	5.185	12.785	17.62	228±6	10.35±0.25	67.45±2.33
30	27.33	24.67	26.50±0.37	83.42±0.56	2.585	2.677	5.262	15.96	235±7	11.70±0.29	64.22±3.77
Oct 3	26.83	24.66	25.30±0.22	87.08±1.71	0.346	0.418	0.764	10.34	230±5	12.00±0.30	62.50±5.39
6	25.16	23.33	24.62±0.18	89.75±2.39	0.130	0.072	0.202	0.00	0	0	0
9	26.00	23.50	25.06±0.12	83.75±0.30	0.043	0.050	0.093	42.86	225±4	12.00±0.27	64.50±6.33
12	25.83	24.00	24.43±0.27	84.16±1.88	0.043	0.036	0.079	20.00	227±6	12.50±0.27	60.10±5.00
15	25.00	23.00	24.08±0.31	8216±1.36	0.007	0.00	0.007	0.00	0	0	0
Average :	26.84 ±0.28	24.58 ±0.23	25.99±0.28	83.76±0.80	51.41	36.26	87.67	41.51	240±4	10.66±0.39	72.33±0.30

the broods (Table 6 & 7). Emergence rhythm was very slow with the advancement of night, peak was around midnight, than the rate declined gradually till dawn. Daytime emergence is sporadic.

Mating : Spontaneous mating took place at night , particularly around midnight in most of the cases and continued also to the early morning. Mating of most of the moths took place during the period from fourth week of June to third week of July and second to fourth week of September in case of first and second brood respectively after which mating efficacy declined almost to the negligible number. Average spontaneous mating percentage was slightly higher in the first brood than in the second brood though it was only a little over 40% in both the cases (Table 6 & 7).

Fecundity : A lower fecundity was recorded in the first generation during several early (first to third week of June) and late (fourth week of July to second week of August) days of moth emergence, while maximum oviposition was noted in the third week of June to fourth week of July.

In the second brood fecundity remained higher only during the first half of moth emergence period (second and fourth week of September). Thereafter, the fecundity gradually declined.

Relative to the second brood the total fecundity was lower in the first brood (Table 6 & 7).

Egg incubation period : In general, the egg incubation period was not uniform in a population of *A. mylitta.* The daily data revealed a gradual increase in the egg incubation period in each brood. However, in the first brood, egg incubation time decreased at the last phase of the study season (August) and the average egg incubation period was shorter than that of the second brood (Table 6 & 7).

Hatching Performance : Hatching percentage of the eggs of both the broods was almost the same and showed a similar pattern. The eggs obtained from the females emerged during the early and late days of the total emergence span suffered from relatively poor hatchability (Table 6 & 7).

4.2. Determination of Critical Weight of Fifth Instar Larvae and Timings of PTTH Release for Larval-Pupal Transformation

4.2.1 Growth index for control 5th instar larvae

Growth rate was faster and RGR was higher in ND-generation than in the D-generation. Males showed faster RGR than the females of both the generations (Table 10). Overall gain in larval weight during 5th instar was 264.99% in male and 251.22% in female in case of ND-generation and 281.64% and 271.19% respectively in males and females of D-generation (Table 8 & 9) .

4.2.2 Behavioural changes and morphological markers for pupation

Under ambient field conditions the duration of fifth larval instar was 21 days and 39.50 days (irrespective of sex) in ND and D broods respectively. Invariably the phagoperiod including the latent feeding duration, and the non-phagoperiod were longer in diapause-destined generation. Sex specific variation was observed in respect of only latent feeding period irrespective of generations, female took more time than its male counterpart. It is interesting to note that there was no sex-specific differences in non-phagoperiod. After attainment of final (maximum) weight the larvae started moving aimlessly and egested very frequently first solid faeces and later on semisolid ones for 1-1.5 days. Simultaneously the larvae consumed very frequently little amount of leaves. Because of much higher rate of egestion than ingestion and elimination of water through semisolid faeces the body weight declined sharply. Withing about another 6 hrs the gut purging was complete after final elimination of liquid excreta. The maximum body weights after complete gut purging was reduced by about 36% in both the sexes in case of ND-brood and by 33.94% in male and 37.16% in female in case of D-generation.

Spinning continued for 3.25 days and 7.75 days in ND and D-destined broods respectively. A shrinkage of body occurred gradually with the advancement of spinning. Further, during last phase of silk spinning,

Table 8. Daily body weight (g) of non-diapausing brood of *A.mylitta* during the feeding period of 5th larval instar. Values are mean ±S.E. (n=100).

Day / Sex	0	1	2	3	4	5	6	7	8	9	10	11	12	13	
M	8.94 ±0.25	9.87 ±0.34	11.69 ±0.48	15.53 ±0.58	16.03 ±0.60	18.59 ±0.60	20.18 ±0.65	22.40 ±0.87	24.06 ±0.65	26.90 ±0.90 (Lcw)	27.52 ±0.77	30.18 ±0.79	32.63 ±0.61	-	-
F	11.82 ±0.22	14.22 ±0.48	17.14 ±0.74	21.74 ±0.85	25.84 ±0.85	28.37 ±0.85	30.39 ±0.95	31.84 ±0.77	32.05 ±0.36	34.37 ±0.64 (Lcw)	36.58 ±0.93	39.14 ±0.36	40.68 ±0.34	41.55 ±0.40	-

	Percentage increase in weight													Overall increase in wt.(%)
M	10.40	18.44	32.85	3.22	15.97	8.54	11.00	7.41	11.80	2.30	9.67	8.12		264.99
F	20.30	20.53	26.84	18.86	9.79	7.12	4.77	0.66	7.24	6.43	6.99	3.93	2.14	251.52

M = Male ; F = Female.

Table 9. **Daily body weight (g) of diapause-destined brood of *A.mylitta* during the feeding period of 5th larval instar. Values are mean ± S.E.. (n=100).**

Day	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
Sex																								
Male	9.97 ±0.23	10.88 ±0.21	12.98 0.19	14.45 ±0.34	15.94 ±0.49	16.36 ±0.36	17.02 ±0.50	19.36 ±0.49	21.48 ±0.48	22.41 ±0.56	23.64 ±0.41	24.33 ±0.43	24.68 ±0.43	25.33 ±0.40	26.18 ±0.54	27.88 ±0.64 (Lcw)	30.44 ±0.49	32.87 ±0.57	34.66 ±0.54	36.6 ±0.29	38.05 ±0.65	-	-	
	12.74 ±0.26	13.97 ±0.26	14.86 ±0.31	16.16 ±0.26	18.77 ±0.26	21.48 ±0.31	23.95 ±0.32	25.13 ±0.34	26.81 ±0.35	28.01 ±0.48	29.87 ±0.51	31.00 ±0.44	32.05 ±0.40	32.75 ±0.44	33.15 ±0.51	35.29 ±0.54 (Lcw)	36.26 ±0.50	38.03 ±0.58	40.31 ±0.55	42.4 ±0.53	44.66 ±0.40	45.97 ±0.33	47.29 ±0.42	
									% increase in weight															overall increase in wt (%)
		9.13	19.3	11.32	10.31	2.63	4.03	13.75	10.95	4.33	5.49	2.92	1.44	2.63	3.36	6.49	9.18	7.98	5.44	5.6	3.96			281.64
		9.65	6.37	8.75	16.15	14.44	11.5	5.05	6.56	4.51	6.6	3.78	3.39	2.21	1.22	6.45	2.75	4.88	3.36	5.18	5.33	2.93	2.87	271.19

Table 10. Relative growth rate (RGR) during the fifth larval instar of *A. mylitta*.

Brood / Sex	Non-diapause(ND)	Diapause(D)	Percentage decrease in D-brood with comparison to ND - brood.
Male	0.095	0.058	38.95
Female	0.085	0.052	38.82

anal prolegs ceased their mobility and finally all the abdominal prolegs became immobile with the attainment of pre-pupal stage. The anal prolegs closed completely after the end of spinning. Irrespective of sexes the duration of pre-pupa was about 4 and 9 days in ND and D generations respectively. (Table 11).

4.2.3 Larval starvation and subsequent biological performances

Starvation stress applied to the larvae of ND-generation upto 6 day age imposed failure to spin and pupate and the larvae eventually died. For the diapause generation this was true upto the age of 12-day. The result was consistent in both the sexes. However, depending on the age of the larvae subjected to starvation and the nature of the two generations the larval survival span varied from 10 to 25 days. Starvation from the age of 9 day onwards and 15 day onwards in ND and D-generation respectively, more than 50% of the starved larvae could ecdyse into pupae showing all the prodorms of pupation and finally could able to emerge as functional adults. Below that age, the functional adults could develop only in negligible numbers (Tables 12 & 13, Figs. 10, 11 and 12). Thus, the larval critical weight(L_{cw}) was attained on the 9th and 15th day of 5th stage larval life for ND and D generation respectively.

In case of ND generation the latent feeding period was 3 and 4 days for males and females respectively. In case of D-generation this period was 5 and 7 days for males and females respectively.

The quality indices such as L_{cw}, larval maximum weight (Lmw), cocoon characters, relative silk conversion efficiency, pupal critical weight (Pcw), pupal maximum weight (Pmw), adult critical weight (Acw) and adult maximum weight (Amw) with relation to the weights of the gut-purged larvae were all related to the length of latent feeding period in both the generations (Table 14 & 15). The indices other than Amw and fecundity of D generation were superior to those of ND-generation. Irrespective of the day of starvation the time required after 4th moult till the onset of spinning did not noticeably differ from the control larvae. However, the overall duration of the 5th stage

Table 11. Span of different functional components during 5th instar larval life of *A. mylitta*

Functional components of 5th stage larval life	Non-diapause(Day)		Diapause (Day)	
	Male	Female	Male	Female
Time required to attain Lcw.	9.00	9.00	15.00	15.00
Latent feeding period	3.00	4.00	5.00	7.00
Total Phagoperiod	12.00	13.00	20.00	22.00
Wandering and gut purging.	1.25	1.25	1.75	1.75
Spinning	3.25	3.25	7.75	7.75
Prepupa	4.00	4.00	9.00	9.00
Total non-phagoperiod	8.50	8.50	18.50	18.50
Total 5th larval life	20.50	21.50	38.50	40.50

Table 12. Effect of starvation on spinning, pupation and moth eclosion performances in non-diapause generation of *A.mylitta*. Control larvae were starved 8-10 hrs. before gut purge. Percentage in each case was calculated out of total population (n=60).

Day of Starvation	Sex	Larval body weight(g)	% of larvae capable to spin	% of larvae capable to pupate	% of larvae capable to emerge as moth
7	M	22.40 ±0.87	8.33	6.67	3.33
	F	31.84 ±0.77	10.00	8.33	5.00
8	M	24.06 ±0.65	15.00	15.00	11.67
	F	32.05 ±0.36	13.33	11.67	10.00
9*	M	26.90 ±0.90	90.00	81.67	68.33
	F	34.37 ±0.64	85.00	75.00	56.66
10	M	27.52 ±0.77	93.33	86.67	75.00
	F	36.58 ±0.93	88.33	80.00	61.67
11	M	30.18 ±0.79	96.67	93.33	78.33
	F	39.14 ±0.36	91.67	90.00	75.00
12	M	32.63 ±0.61	100.00	98.33	90.00
	F	40.68 ±0.34	100.00	93.33	81.67
13	F	41.55 ±0.40	100.00	98.33	90.00
12 (control)	M	26.25 ±0.85	100.00	100.00	93.33
13 (control)	F	31.64 ±0.68	100.00	96.67	91.67

M = Male ; F = Female ; * - Day of critical weight.

Table 13. Effect of starvation on spinning, pupation and moth eclosion performances in Diapause-destined generatio of *A.mylitta*. Control larvae were starved 8-10 hrs. before gut purge. Percentage in each case was calculate out of total population (n=60).

Day of Starvation	Sex	Larval body weight(g)	% of larvae capable to spin	% of larvae capable to pupate	% of larvae capable to emerge as moth
13	M	25.33 ±0.40	6.67	5.00	0.00
	F	32.75 ±0.44	8.33	6.67	0.00
14	M	26.18 ±0.54	13.33	11.67	3.33
	F	33.15 ±0.51	11.66	8.33	3.33
15*	M	27.88 ±0.64	78.33	75.00	51.67
	F	35.29 ±0.54	73.33	70.00	56.67
16	M	30.44 ±0.49	80.00	76.67	55.00
	F	36.26 ±0.50	76.67	71.67	53.33
17	M	32.87 ±0.57	88.33	85.00	55.00
	F	38.03 ±0.58	81.67	75.00	50.00
18	M	34.66 ±0.54	95.00	93.33	63.33
	F	40.31 ±0.55	86.67	81.67	53.33
19	M	36.60 ±0.29	96.67	96.67	68.33
	F	42.40 ±0.53	90.00	86.67	66.67
20	M	38.05 ±0.65	98.33	98.33	73.33
	F	44.66 ±0.40	96.67	91.67	70.00
21	F	45.97 ±0.33	96.67	95.00	68.33
22	F	47.29 ±0.42	100.00	98.33	75.00
20(Control)	M	29.17 ±0.40	100.00	96.67	75.00
22(Control)	F	33.90 ±0.51	100	93.33	73.33

M = Male , F = Female , * - Day of critical weight.

Fig.10 :Photograph showing the results of starvation from different days of non-diapause 5th stage larvae of *A.mylitta*. a.Dead larvae fed upto 8th day. b. Larvae fed upto 9th day, attained Lcw and showing pupal syndrome (prepupa). c. Pupae resulted from the larvae that attained Lcw (as in b).

Fig.11: Photograph of cocoon shells (silken part of cocoon) of *A.mylitta* produced by the 5th stage larvae of non-diapause generation allowed to feed upto different days. a. upto 9th day feeding (age for Lcw), b. upto 11th day feeding, c. upto 13th day (normal feeding period).

Fig.10

Fig.11

Fig. 12 : Photograph of female moths of non-diapause generation of A.*mylitta*. resulted from the larvae fed for normal duration of 13 day. (a) and from the larva fed upto 9 day, the day for attaining Lcw(b).

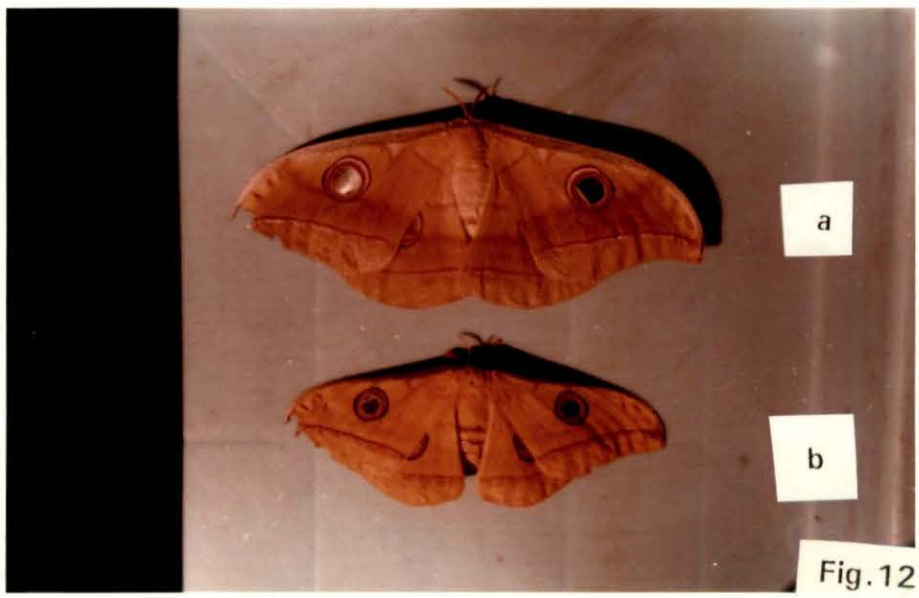


Fig. 12

Table 14. Effect of starvation on biological peformmances and quality indices of non-diapause generation of *A.mylitta*. Control larvae were starved 8-10 hours before comple tion of gut purging. All weights are in (g) and duration in (Day). The values are mean ± SE.

Day of starvation.	Sex	Body wt. on day of starvation.	Body wt.after GPR	Time took for onset of spinning	Spinning duration	Pre-pupal duration	Total 5th larval duration	Relative silk conversion efficiency(%)	Cocoon Characters				Pupal duration	Moth wt.	Egg Production (No.)		
									Coccon wt.	Pupal wt.	Shell wt.	S.R. (%)			Laid	Unlaid	Total
	M	27.10 ±0.61	17.10 ±0.24	3.75 ±0.10	2.75 ±0.15	3.25 ±0.11	18.75 ±0.21	4.561	7.97 ±0.15	7.19 ±0.18	0.78 ±0.03	9.78 ±0.17	17.50 ±1.20	2.39 ±0.07	-	-	-
9	F	34.70 ±0.44	22.65 ±0.21	4.75 ±0.15	2.75 ±0.10	3.25 ±0.14	19.25 ±0.31	3.797	10.71 ±0.19	9.85 ±0.16	0.86 ±0.04	8.03 ±0.10	17.25 ±1.18	5.50 ±0.12	23 ±4	37 ±3	60 ±5
	M	28.09 ±0.53	17.74 ±0.20	3.25 ±0.07	2.75 ±0.14	3.25 ±0.10	19.25 ±0.33	5.411	8.74 ±0.11	7.73 ±0.06	0.96 ±0.06	10.98 ±0.15	18.25 ±0.90	2.55 ±0.08	-	-	-
10	F	36.84 ±0.48	23.24 ±0.21	4.25 ±0.20	2.75 ±0.17	3.25 ±0.16	20.25 ±0.21	3.915	11.39 ±0.10	10.48 ±0.07	0.91 ±0.03	7.99 ±0.07	18.00 ±1.02	5.81 ±0.09	62 ±6	40 ±4	102 ±7
	M	30.53 ±0.36	18.95 ±0.19	2.00 ±0.05	3.00 ±0.14	3.75 ±0.12	19.75 ±0.20	6.227	9.68 ±0.07	8.50 ±0.10	1.18 ±0.04	12.19 ±0.15	19.25 ±0.98	2.74 0.05	-	-	-
11	F	38.98 ±0.51	24.01 ±0.13	2.75 ±0.15	3.00 ±0.09	3.75 ±0.15	20.50 ±0.32	5.206	12.06 ±0.09	10.81 ±0.12	1.25 ±0.05	10.36 ±0.18	18.75 ±1.01	6.26 ±0.10	102 ± 9	70 ±7	172 ±11
	M	32.80 ±0.74	20.39 ±0.24	0.75 ±0.03	3.25 ±0.06	4.00 ±0.11	20.00 ±0.24	6.327	10.26 ±0.08	8.97 ±0.16	1.29 ±0.02	12.57 ±0.22	20.75 ±1.07	2.93 ±0.05	-	-	-
12	F	40.72 ±0.56	24.58 ±0.20	2.00 ±0.04	3.00 ±0.17	3.75 ±0.10	20.75 ±0.31	5.574	12.98 ±0.09	11.61 ±0.15	1.37 ±0.04	10.55 ±0.16	20.00 ±0.96	6.54 ±0.05	152 ±7	58 ±6	210 ±5
13	F	41.77 ±0.38	25.12 ±0.15	0.75 ±0.02	3.25 ±0.12	4.00 ±0.16	21.00 ±0.26	5.732	13.41 ±0.12	11.97 ±0.20	1.44 ±0.06	10.74 ±0.20	21.25 ±1.05	6.98 ±0.06	232 ±8	35 ±6	267 ± 9
12 (Control)	M	26.44 ±0.73	20.46 ±0.21	0.51 ±0.04	3.25 ±0.10	4.00 ±0.14	20.76 ±0.28	6.598	10.34 ±0.20	8.99 ±0.15	1.35 ±0.04	13.05 ±0.30	21.00 ±0.84	3.01 ±0.06	-	-	-
13 (Control)	F	31.86 ±0.61	25.29 ±0.10	0.53 ±0.03	3.25 ±0.16	4.00 ±0.15	21.78 ±0.22	5.783	13.64 ±0.08	12.16 ±0.12	1.48 ±0.03	10.85 ±0.26	22.00 ±1.18	7.24 ±0.10	249 ±10	24 ±4	273 ±11

M = male ; F= female.

Table 15. Effect of starvation on biological pcformmances and quality indices of diapause destined generation of *A.mylitta*. Control larvae were starved 8-10 hours before comple tion of gut purging. All weights are in (g) and duration in (Day). The values are mean ± SE.

Day of starva-tion.	Sex	Body wt. on day of star-vation.	Body wt.after GPR	Time took for onset of spinning	Spinning duration	Pre-pupal duration	Total 5th larval duration	Relative silk conversion efficiency(%)	Cocoon Characters				Pupal duration	Moth wt.		Egg Production (No.)		
									Coccon	Pupal	Shell	S.R.		wt.	wt.	Laid (%)	Unlaid	Total
	M	28.22 ±0.55	18.25 ±0.31	5.75 ±0.15	4.75 ±0.25	5.00 ±0.24	30.50 ±0.41	4.493	8.13 ±0.18	7.31 ±0.28	0.82 ±0.06	10.08 ±0.30	56.80 ±3.21	2.07 ±0.09		-	-	-
15	F	35.44 ±0.73	23.43 ±0.21	8.00 ±0.19	4.75 ±0.27	5.00 ±0.16	32.75 ±0.64	3.841	10.88 ±0.20	9.98 ±0.30	0.90 ±0.04	8.27 ±0.20	62.50 ±3.15	4.86 ±0.12		12 ±2	32 ±4	44 ±6
	M	30.11 ±0.58	19.77 ±0.32	5.25 ±0.20	5.25 ±0.20	5.25 ±0.20	31.75 ±0.52	5.817	9.13 ±0.14	7.98 ±0.22	1.15 ±0.04	12.59 ±0.16	78.20 ±3.86	2.31 ±0.08		-	-	-
16	F	37.02 ±0.72	23.87 ±0.25	7.75 ±0.21	5.25 ±0.19	5.75 ±0.14	34.75 ±0.61	5.027	11.43 ±0.12	10.23 ±0.19	1.20 ±0.05	10.49 ±0.15	84.50 ±2.70	5.09 ±0.10		25 ±3	37 ±4	62 ±6
	M	32.69 ±0.54	20.65 ±0.20	3.75 ±0.15	6.00 ±0.19	5.75 ±0.16	32.50 ±0.73	6.634	10.04 ±0.15	8.67 ±0.25	1.37 ±0.06	13.64 ±0.20	115.00 ±8.65	2.39 ±0.05		-	-	-
17	F	38.33 ±0.71	24.16 ±0.20	6.00 ±0.16	6.00 ±0.16	6.75 ±0.15	35.75 ±0.44	5.877	11.84 ±0.18	10.42 ±0.18	1.42 ±0.08	11.99 ±0.30	132.14 ±4.55	5.21 ±0.08		41 ±2	40 ±3	81 ±5
	M	34.20 ±0.44	21.67 ±0.28	3.00 ±0.10	6.25 ±0.23	6.75 ±0.24	34.00 ±0.38	6.691	10.89 ±0.15	9.44 ±0.20	1.45 ±0.04	13.31 ±0.22	167.20 ±5.32	2.42 ±0.05		-	-	-
18	F	40.07 ±0.58	24.84 ±0.32	4.75 ±0.07	6.25 ±0.24	7.00 ±0.16	36.00 ±0.55	6.583	12.72 ±0.22	11.06 ±0.28	1.66 ±0.08	13.05 ±0.27	157.08 ±5.40	5.85 ±0.14		52 ±4	80 ±6	132 ±8
	M	36.18 ±0.36	22.24 ±0.27	2.00 ±0.07	7.00 ±0.30	8.00 ±0.32	36.00 ±0.36	8.183	11.78 ±0.12	9.96 ±0.15	1.82 ±0.07	15.45 ±0.24	185.33 ±4.95	2.51 ±0.08		-	-	-
19	F	42.04 ±0.66	25.39 ±0.22	3.75 ±0.15	6.75 ±0.27	7.25 ±0.19	36.75 ±0.54	7.286	13.83 ±0.14	11.98 ±0.22	1.85 ±0.04	13.37 ±0.30	162.81 ±6.20	6.08 ±0.12		50 ±3	102 ±8	152 ±9
	M	37.86 ±0.49	22.45 ±0.20	1.50 ±0.06	7.75 ±0.19	9.00 ±0.35	38.25 ±0.72	8.775	12.07 ±0.12	10.10 ±0.15	1.97 ±0.06	16.32 ±0.18	198.00 ±5.90	2.53 ±0.07		-	-	-
20	F	44.49 ±0.53	26.36 ±0.30	3.00 ±0.14	7.75 ±0.21	7.75 ±0.22	38.50 ±0.58	7.777	14.84 ±0.12	12.79 ±0.20	2.05 ±0.13	13.81 ±0.20	170.72 ±4.59	6.13 ±0.08		68 ±5	82 ±4	150 ±8
21	F	45.88 ±0.58	27.29 ±0.42	2.25 ±0.06	7.75 ±0.15	8.25 ±0.30	39.25 ±0.67	8.354	15.38 ±0.15	13.10 ±0.24	2.28 ±0.10	14.82 ±0.21	190.33 ±4.00	6.29 ±0.10		159 ±4	26 ±7	185 ±5
22	F	47.21 ±0.44	27.63 ±0.29	1.50 ±0.05	7.25 ±0.30	9.00 ±0.15	39.75 ±0.44	8.469	15.83 ±0.14	13.49 ±0.21	2.34 ±0.09	14.78 ±0.24	208.66 ±2.70	6.47 ±0.20		168 ±5	36 ±4	204 ±8
20 (Control)	M	29.17 ±0.40	22.83 ±0.28	0.53 ±0.03	7.75 ±0.26	9.00 ±0.24	38.28 ±0.58	9.111	12.33 ±0.12	1025 ±0.15	2.08 ±0.06	16.87 ±0.30	205.12 ±4.02	2.74 ±0.06		-	-	-
22 (Control)	F	33.90 ±0.51	27.78 ±0.30	0.58 ±0.04	7.75 ±0.24	9.00 ±0.36	40.33 ±0.66	8.603	15.91 ±0.10	13.52 ±0.16	2.39 ±0.07	15.02 ±0.28	211.83 ±2.07	6.56 ±0.15		178 ±7	34 ±6	212 ±9

M = Male, F = Female.

larvae slowly increased with the advanced age of larvae subjected to starvation. The starved larvae of early ages took lesser time for completion of spinning. Simultaneously the prepupal duration was also shortened slightly. The observed and predicted values of critical and maximum weights of pupae and adults were very close signifying the accuracy of the experimental results. This was true for both the broods (Table 16).

4.2.4. Ligation

The effect of neck ligation experiment on the gut purging and pupal moult was studied on 'O'- day old 5th stage larvae to the pre-pupae 10-12 hrs before pupation. The results observed were consistent in both the sexes and the broods. Ligation upto the age of 8 day in ND and 14 day in the D-generation debarred the larvae to show any prodormal sign of pupation (except in negligible number of individuals) and such larvae died eventually.(Figs. 13 &14). In case of ligation from the age of 9th or 15th day of the respective broods onwards upto the day of normal gut purge in almost all the ligated larvae gut purging occurred, first surge within 2-24 hrs after ligation followed by intermittent surges which ended within 24-72 hrs after ligation depending on the age of the larvae ligated. The body of the silk-worms had undergone shrinkage together with immobility of anal prolegs when ligated during this period (Table 17, Fig.15). Silkworms when ligatured from the age of 5 hrs after gut purge and upto 3-4 day before pupation, had undergone larval-pupal intermoult in few cases though exhibited all other prodormal signs of pupation such as body shrinkage and loss of mobility of anal prolegs Anteriorily these developed pre-pupal and posteriority (specially abdomen) pupal morphology (Fig. 16). Ultimately these silkworms in both the generations were able to ecdyse into apparently headless pupae (Fig. 17). Moreover, most of the larvae ligated at relatively late-age i.e. more approaching to pupation day survived upto 2-3 months irrespective of sex and generation and ultimately died. Further, the headless pupae could not grow and emerge out as moths and died within 10-60 days.

Table 16. **Quality indices of different stages of *A.mylitta* during two generations. All weights are in grams. Values are means ± S.E. where applicable. Predicted values are in parentheses**

Stages	Non - diapause		Diapause	
	Male	Female	Male	Female
Lcw	26.90 ±0.90	34.37 ±0.64	27.88 ±0.64	35.29 ±0.54
Lmw	32.63 ±0.61	41.55 ±0.40	38.05 ±0.65	47.29 ±0.42
D_p (Decrease of wt. in pupa).	0.724	0.707	0.731	0.714
D_a (Decrease of wt. in adult)	0.908	0.826	0.928	0.861
Pmw	(9.00) 8.99 ±0.15	(12.17) 12.16 ±0.12	(10.26) 10.25 ±0.15	(13.53) 13.52 ±0.16
Pcw	(7.42) 7.19 ±0.18	(10.07) 9.85 ±0.16	(7.50) 7.31 ±0.28	(10.09) 9.98 ±0.30
Amw	(3.00) 3.01 ±0.06	(7.23) 7.24 ±0.10	(2.74) 2.74 ±0.06	(6.57) 6.56 ±0.15
Acw	(2.47) 2.39 ±0.07	(5.98) 5.50 ±0.12	(2.01) 2.07 ±0.09	(4.90) 4.86 ±0.12

Fig.13: Neck-ligated 5th stage larvae of non-diapause gen eration of *A.mylitta*. Ligated larva rolled up the body compared with that of normal one above.

Fig. 14: Neck-ligated 5th stage larvae of non-diapause gen eration of *A.mylitta*. Larvae ligated upto the age of 8th day showing no prodormal sign of pupation and died eventually.

Fig.13

Fig.14

Table 17. **Effect of neck ligation of 5th instar larvae on the expression of prodormal signs for pupation in the two gen erations of *A.mylitta*. Positive responses are expressed by (+) and the negative ones by (-).**

Ligation time	GPR and wandering	Body shrinkage	Immobility of anal prolegs.	Immobility of abdominal prolegs.	Pupal cuticle formation	Larval-pupal intermediates	Pupation
0-day to the day of Lcw.	-	-	-	-	-	-	-
0-hr. of Lcw to 0-hr. of GPR.	+	+	+	-	-	-	-
5-hr. after GPR to 3-4 day before pupation.	+	+	+	+	+ (partial)	+	-
2-3 day before pupation to the day of pupation (before 10-12 hrs.)	+	+	+	+	+	-	+

Fig.15 : Neck-ligation of 5th stage larvae on different ages. a-c. 5-hr after GPR to 3-4 day before pupation. d and e. 3-2 day before pupation.

Fig.16: Larval-pupal intermediates resulted from ligation of larvae 5-hr after GPR to 3-4 day before pupation, corresponding to a-c in figure 15.

Fig.17: Head less pupae resulted from ligation of larvae 3-2 day before pupation, corresponding to d and e in figure 15.

Fig.15

Fig.16

Fig.17

4.2.5 Critical period for gated release of PTTH

On the basis of above findings a sequential model for three step gated release of PTTH is presented in Figures 18 & 19 during the fifth larval life span and upto the larval-pupal moult. The first release appeared to be on the day after attaining the Lcw, second 5 hrs after complete gut purge (GPR) and third step within 2-3 days before pupation.

4.2.6 Climatological conditions encountered by the larvae during starvation and neck ligation experiment

With respect to the max. temp., min. temp., r.h.% , L:D hrs and total rainfall there were considerable difference during the life of the two broods. Rainfall was almost nil during D-generation. L:D hrs were reversed in the two broods. The temperature and r.h.% values were lower in the D-brood (Table 18).

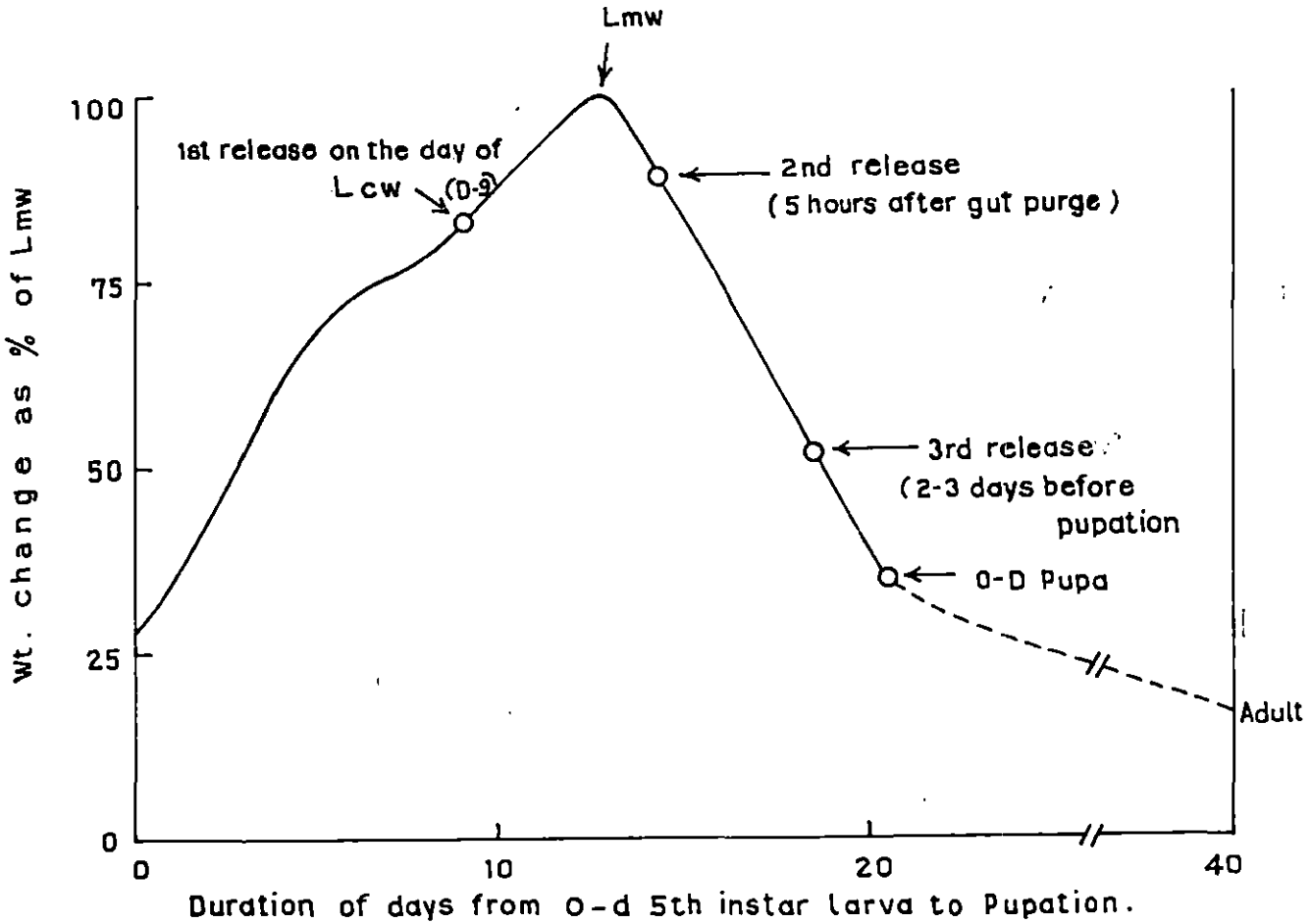


Fig. 18. Model of sequential gated release of PTTH in non-diapause generation of A. mylitta. Lcw = larval critical wt., Lmw = larval maximum wt.

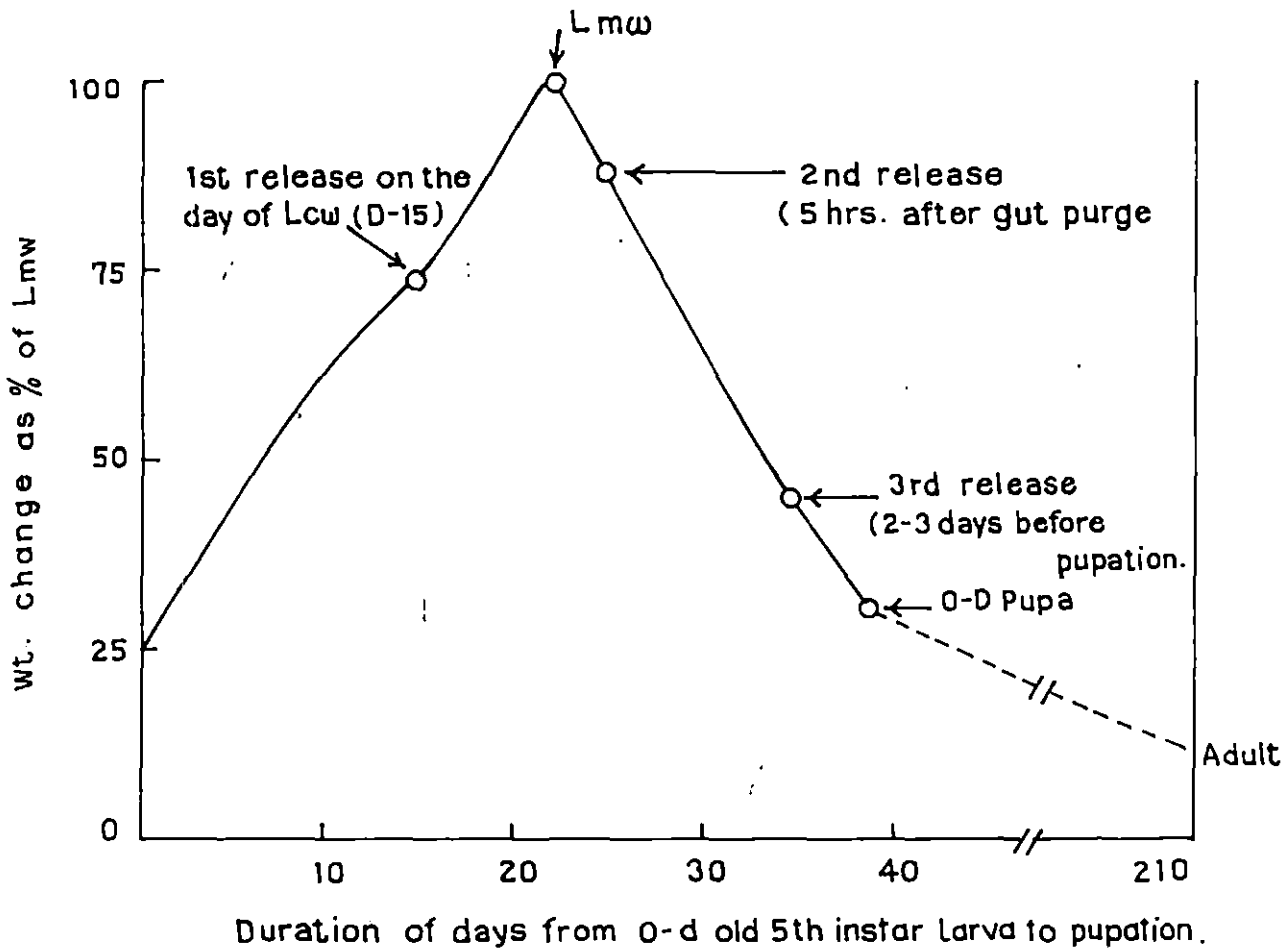


Fig. 19. Model of Sequential gated release of PTTH in diapause-destined generation of A. mylitta. Lcω = larval critical wt, Lmw = larval maximum wt.

Table 18. Climatological conditions availed by the 5th stage larvae during starvation and ligation experiments in the two broods of *A.mylitta*. Values are mean ± S.E.

Brood	Period of 5th larval life	Max . temp. (°C)	Min. temp. (°C)	R.H (%)	L : D (hrs.)	Total rainfall (mm)
ND	Feeding	28.22 ±0.28	22.01 ±0.09	91.00 ±0.69	12.93 : 11.07 ±0.019 ±0.019	139.0
	Non-feeding (upto pupation)	27.36 ±0.66	21.61 ±0.12	90.75 ±0.97	12.72 : 11.28 ±0.015 ±0.015	158.7
D	Feeding	26.65 ±0.31	12.34 ±0.50	80.57 ±0.93	11.33 : 12.67 ±0.03 ±0.03	-
	Non-feeding (upto pupation)	24.90 ±0.37	8.80 ±0.51	77.62 ±1.14	11.02 : 12.98 ±0.02 ±0.02	0.75

ND - Non - diapause; D - Diapause.

4.3. Profile of Cholesterol, Protein, DNA and RNA Contents in Some Tissues of Pre-pupae, Pupae and Adults of Non-Diapause and Diapause Generations

4.3.1. Cholesterol content in haemolymph, fat body and gonad

4.3.1.1. Haemolymph cholesterol :

The cholesterol content of haemolymph plasma of male and female *A.mylitta* of non-diapause generation increased significantly ($P<0.001$) from pre-pupal stage to pupa reaching the peak on day 14 of pupal development. Thereafter, it declined sharply ($P<0.001$) just before pupal - adult moult and in freshly emerged adults ($P<0.01$). Likewise , cholesterol titre in diapause generation increased from pre-pupal age to 15 day old pupa showing the peak level after which gradual decrease in cholesterol level occurred till adult emergence on or about 210 day indicating a slower rate of cholesterol deposition in haemolymph from fat body or other tissue sources and later subsequent utilization and/or transportation for growth and adult development. Compared with the values of males, the degree of rise and fall of cholesterol content in female was higher in most of the cases. However, female haemolymph contained significantly more cholesterol than male counterparts upto 14 day and 150 day of pupal development in non-diapause and diapause generation respectively. Thereafter, cholesterol content was found at lower level upto adult emergence in both the generations (Table 19 & 20, Figs. 20 & 21).

4.3.1.2. Fat body cholesterol :

Cholesterol concentration in non-diapause and diapause generation sharply declined ($P<0.001$) from pre-pupa to 0-day pupa (particularly in female) i.e. during larval-pupal transformation indicating the utilization of the

said biomolecules for pupal organogenesis. Thereafter, gradual increase in cholesterol content was found in non-diapause generation reaching the peak level on 14-day old pupa. On the contrary, the elevation in cholesterol level is very sharp in diapause generation from 0-day pupa to 40-day old pupa and ultimately touched the peak on 150 day of pupal development. However, a reduction in cholesterol titre was recorded in fat body cells on 14-day and 170-day onwards upto adult emergence in non-diapause and diapause generation respectively. The decrease in cholesterol concentration during the later phase of pupal development in both the generations again confirmed the transportation and/or utilization of the cholesterol for pupal - adult transformation. Further, female fat body contained significantly higher cholesterol (µg/100 mg tissue) from pre-pupa to 14-day old pupa in non-diapause generation but only from pre-pupa to 0-day pupa in diapause generation thereafter it was found to be low. Thus, it is evident that the transportation and/or utilization of cholesterol in female fat body started more earlier in diapause generation and continued for a longer period i.e. upto adult emergence than that of non-diapausing ones. However, the reason is unknown (Tables 19 & 20, Figs. 20 & 21).

4.3.1.3. Gonad cholesterol

In gonad (testis and ovary), the cholesterol content showed a specific pattern of variation in both the generations where a gradual increase of the biomolecules upto adult emergence was recorded. Ovary showed always a higher cholesterol titre than testis. It is interesting to point out that in the initial stages i.e.from pre-pupa to 0-day pupa cholesterol level in both the tissues of testis and ovary remained significantly higher ($P<0.02$ - $P<0.001$) in diapausing generation compared to that of non-diapausing ones. But, afterwards it remained low in both the sexes of diapausing generation upto adult emergence (with few exception). This again established the slower rate of cholesterol metabolism (particularly deposition of this biomolecule in gonad) in diapausing generation while the rate was very faster in the non-diapausing animals (Tables. 19 & 20, Figs. 20 & 21).

Table 19. Cholesterol content in different tissues of non-diapausing *A.mylitta* during pre-pupal pupal and adult stages of both the sexes. Each value represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Haemolymph (µg/ml)		Fat body (µg/100mg)		Gonad (µg/100mg)	
	M	F	M	F	M	F
Pre-pupa	295.49 ±6.27	365.33 ±9.44 c	392.79 ±9.60	901.26 ±7.38 c	128.42 ±4.06	375.00 ±9.11 c
0-day pupa	482.87 ±6.55	914.31 ±9.13 c	275.70 ±4.15	525.32 ±11.15 c	185.75 ±4.96	425.48 ±8.14 c
7-day pupa	685.71 ±12.68	1015.72 ±18.87 c	457.01 ±25.59	684.14 ±28.55 c	457.63 ±9.10	528.33 ±20.30 b
14-day pupa	1110.20 ±55.34	1806.87 ±44.66 c	1492.60 ±32.87	1613.15 ±35.70 a	875.08 ±9.33	1045.40 ±25.31 c
21-day pupa	622.74 ±18.32	543.66 ±22.50 a	1165.06 ±17.39	652.70 ±8.62 c	925.78 ±12.34	1686.80 ±11.49 c
Freshly emerged adult (22 day)	543.90 ±15.42	472.81 ±19.75 a	766.03 ±12.11	418.64 ± 9.31 c	982.61 ±10.29	1875.18 ±35.81 c

't' - test probability differences (male vs. female) : M = Male ; F = Female.
a = P<0.02 ,
b = P<0.01,
c = P<0.001.

Table 20. Cholesterol content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represent Mean±S.E (n=10).

Tissue / Stage of insect	Haemolymph (µg/ml)		Fat body (µg/100mg)		Gonads (µg/100mg)	
	M	F	M	F	M	F
Pre-pupa	338.48 ±5.57	404.15 ±4.94 c	431.06 ±3.37	1023.57 ±6.72 c	186.77 ±3.86	439.98 ±14.21 c
0-day pupa	409.12 ±5.75	494.26 ±11.35 c	310.96 ±7.95	683.84 ±12.39 c	207.24 ±9.68	450.38 ±18.44 c
40-day pupa	740.46 ±16.19	850.86 ±28.15 b	2095.19 ±68.92	1752.88 ±36.09 c	262.89 ±8.95	692.86 ±11.53 c
105-day pupa	778.94 ±10.86	1094.63 ±42.78 c	2567.85 ±86.30	1944.67 ±56.95 c	508.12 ±19.36	1206.18 ±35.97 c
150-day pupa	2172.46 ±52.39	2350.84 ±69.40 a	3765.49 ±68.33	2525.30 ±65.56 c	720.83 ±20.59	1325.60 ±24.35 c
170-day pupa	1922.64 ±42.11	1882.00 ±55.10 NS	2850.94 ±62.44	1878.86 ±51.90 c	815.16 ±15.30	1414.49 ±29.05 c
200-day pupa	1725.72 ±33.43	1573.50 ±48.79 a	1469.08 ±16.44	713.25 ±15.25 c	913.88 ±31.30	1576.77 ±39.40 c
Freshly emerged adult (210-day)	1619.41 ±25.33	922.37 ±38.09 c	965.12 ±31.79	515.58 ±23.43 c	924.35 ±26.30	1751.77 ±56.32 c

't'-test probability differences (Male vs Female) :

a = P<0.05, M = Male ; F = Female.
b = P<0.01,
c = P<0.001,
NS = Not significant.

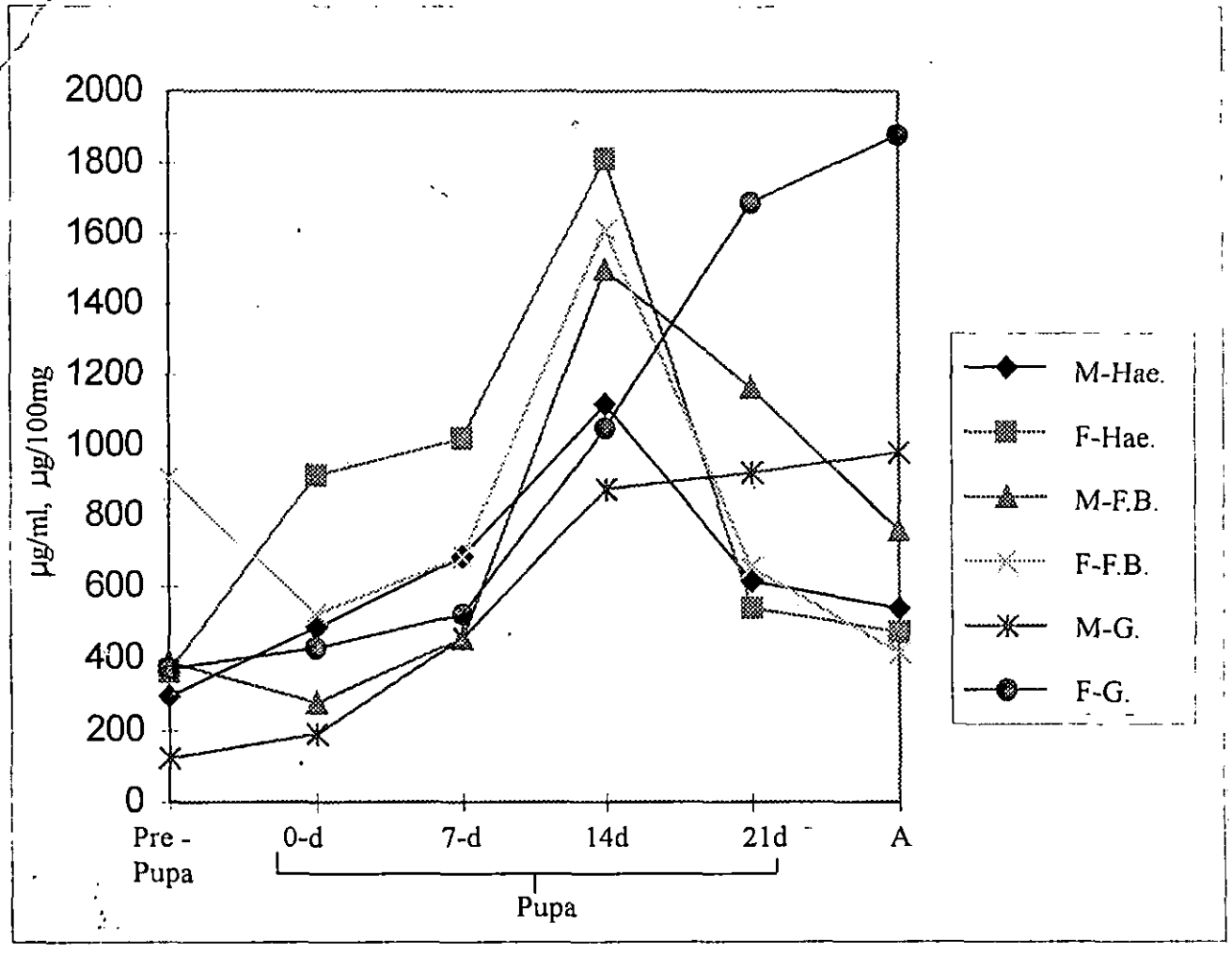


Fig.20. Cholesterol content in different tissues of non-diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes.M= Male, F= Female, A = Adult, Hae.= Haemolymph (µg/ml) F.B.= Fat Body (µg/100mg) , G= Gonad (µg/100mg) .

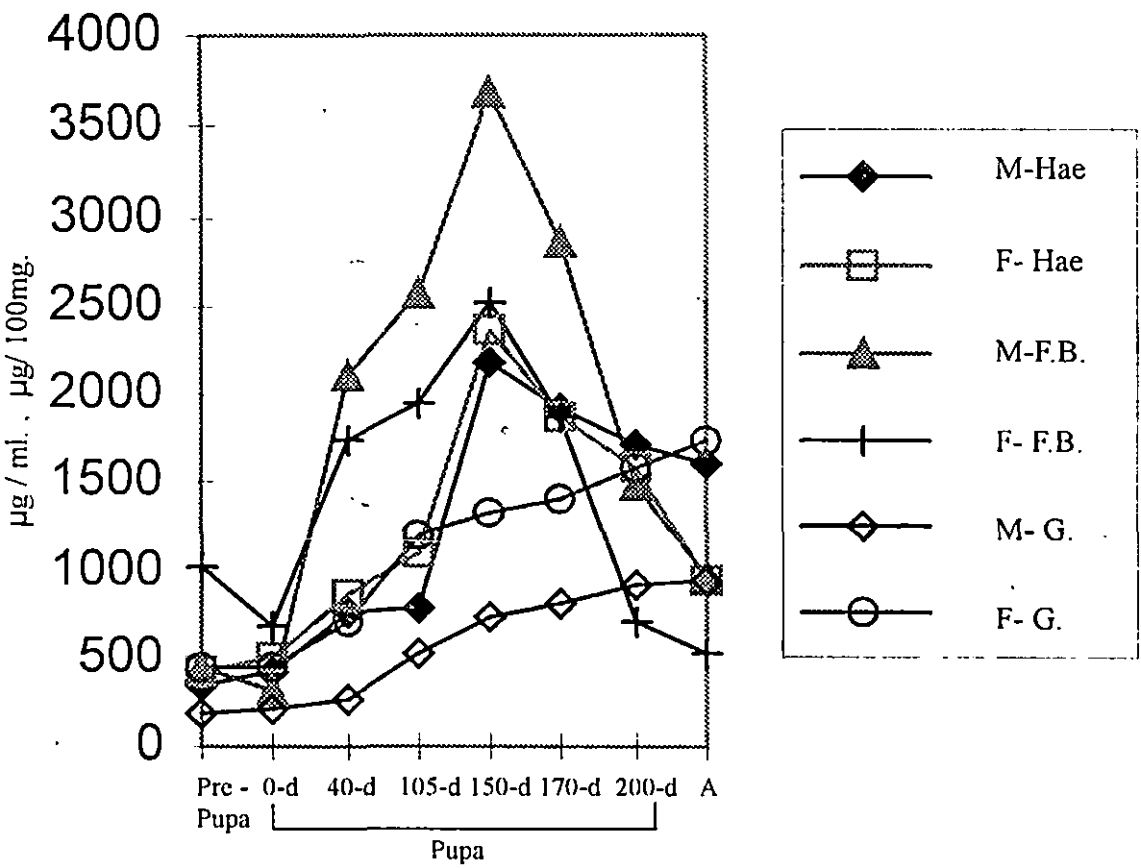


Fig.21. Cholesterol content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, Hae=Haemolymph (µg / ml), F.B.=Fat Body (µg/100mg) , G=Gonad (µg / 100mg), A=Adult.

4.3.2. Total protein content in haemolymph , fat body and gonads

4.3.2.1. Haemolymph protein

Age-dependent changes in haemolymph protein concentration in non-diapause and diapause generations were found to be quite different. In the non-diapause brood animals showed gradual and significant decrease after pre-pupal stage i.e. starting from 0-day pupa to adult emergence. But, in diapause-destined insects peak level of total protein concentration was attained in the pre-pupae and then the protein level decreased slowly but significantly ($P<0.001$) in the 0-day pupa. Thereafter, a sharp decrease in protein titre was recorded in 40 day of pupal age followed by a steep rise in 105 day old pupa. However, this level was maintained upto 150 day during diapause development. Interestingly, after 150 day the total protein content again declined in the subsequent developmental steps gradually and significantly ($P<0.01$ - $P<0.001$) upto the day of adult emergence reaching there at minimum level. During different developmental steps (pre-pupa to adult emergence) the fall and rise in protein level strongly support that the deposition, transportation and utilization of this biomolecule helps to maintain and later on to terminate the diapause state. Thus, 150 day of pupal age may be considered for critical phase for diapause termination in *A.mylitta*. Further, irrespective of sex diapausing taser insects contained higher protein in haemolymph than the non-diapausing ones and females contained significantly more amount of protein than that of males in both the generations (Tables 21 & 22, Figs. 22 & 23).

4.3.2.2 Fat body protein

In the non-diapausing brood total protein content of male and female fat body gradually elevated significantly ($P<0.05$ - $P<0.01$) from pre-pupal stage to 7-day pupa attaining the peak level. Thereafter, protein concentration steadily declined upto adult emergence. In the diapausing generation the fat body protein level increased very slowly upto 150 day of pupal age during diapause development preceded by a significant fall ($P<0.001$) on 105 day old pupa. Then, from 170 day onwards steady decrease

in fat body protein concentration was recorded upto adult emergence. Male and female insects showed age and stage specific pattern of variation in this biomolecule in both the generations which was found to be quite different from each other. However, diapausing generation showed higher level of fat body protein compared to non-diapausing one and females contained more fat body protein then male counterparts in all the cases. The rise and fall in fat body protein concentration in both sexes revealed the synthesis of protein in fat body, then its transportation to the haemolymph and subsequent re-sequestration to the fat body (Tables21 & 22, Figs. 22 & 23).

4.3.2.3. Gonad protein

In general, gonadal protein concentration of male and female insects were found to increase significantly ($P<0.001$) from pre-pupa to adult emergence covering the entire gamut of pupal development in non-diapause and diapause-destined generations. In both the generations the rate of increase of protein level in testis was recorded very low. While in ovary the rise in protein level was very slow only upto 0-day and 40-day old pupa of non-diapause and diapause generation respectively. Again a quantum increase in ovarian protein level was revealed on 7th day of pupal age in non-diapause and 105 day in case of diapausing pupae. Thereafter, the rate of increase of this biomolecule was found to be slow and gradual upto adult stage which showed ultimately the peak level in both the generations. Female gonad contained higher amount of protein throughout the life span with exception in prepupa to 0-day old pupa in case of non-diapause and from pre-pupa to 40-day old pupa for the diapausing generation. It reveals that ovarian maturation took place steadily from 7th day and 105 day onwards in non-diapause and diapause generations respectively and corresponding fluctuations in protein and cholesterol concentration in fat body and haemolymph (stated earlier) also support this observation. Further, male and female gonads in diapausing generation contained higher amount of protein compared to that of non-diapausing ones in most of the cases. (Tables 21 & 22, Figs. 22 & 23).

Table21. Total protein content in different tissues of non-diapausing *A. mylitta* during pre-pupal pupal and adult stages of both the sexes. Each values represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Haemolymph (mg/ml)		Fat body (mg/100mg)		Gonad (mg/100mg)	
	M	F	M	F	M	F
Pre-pupa	22.29 ±0.30	30.20 ±0.12 a	4.12 ±0.09	5.66 ±0.18 a	2.20 ±0.10	0.95 ±0.04 a
0-day pupa	20.56 ±0.10	26.21 ±0.19 a	4.63 ±0.15	6.82 ±0.21 a	3.16 ±0.21	1.55 ±0.20 a
7-day pupa	13.98 ±0.22	21.43 ±0.38 a	5.08 ±0.12	7.60 ±0.25 a	3.85 ±0.16	6.74 ±0.42 a
14-day pupa	12.24 ±0.31	17.61 ±0.44 a	4.11 ±0.21	6.75 ±0.14 a	4.39 ±0.25	10.15 ±0.38 a
21-day pupa	11.10 ±0.11	15.82 ±0.15 a	3.16 ±0.22	4.33 ±0.20 a	5.26 ±0.12	13.03 ±0.39 a
Freshly emerged adult (22-day)	9.62 ±0.18	12.10 ±0.31 a	2.34 ±0.10	3.69 ±0.11 a	5.98 ±0.14	14.14 ±0.50 a

't'-test probability differences (Male vs. Female) M = Male ; F = Female.

a = $P < 0.001$

Table 22. Total protein content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represent Mean±S.E (n=10).

Tissue / Stage of insect	Haemolymph (mg/ml)		Fat body (mg/100mg)		Gonads (mg/100mg)	
	M	F	M	F	M	F
Pre-pupa	28.45 ±0.29	34.19 ±0.32 a	4.46 ±0.13	6.85 ±0.22 a	2.58 ±0.04	1.15 ±0.07 a
0-Day pupa	25.52 ±0.26	30.43 ±0.35 a	5.10 ±0.12	7.90 ±0.21 a	3.97 ±0.06	1.83 ±0.10 a
40-day pupa	4.86 ±0.08	6.74 ±0.33 a	5.65 ±0.17	8.94 ±0.25 a	4.08 ±0.20	2.96 ±0.15 a
105-day pupa	14.28 ±0.17	22.07 ±0.34 a	4.05 ±0.11	6.20 ±0.15 a	4.24 ±0.17	8.52 ±0.20 a
150-day pupa	14.65 ±0.15	22.52 ±0.42 a	6.08 ±0.10	11.85 ±0.12 a	4.97 ±0.16	10.41 ±0.22 a
170-day pupa	12.17 ±0.18	16.42 ±0.34 a	5.40 ±0.14	8.77 ±0.11 a	5.32 ±0.14	11.30 ±0.25 a
200-day pupa	11.45 ±0.10	15.08 ±0.15 a	4.02 ±0.13	7.48 ±0.20 a	5.84 ±0.10	13.54 ±0.35 a
Freshly emerged adult (210 day)	10.14 ±0.22	13.29 ±0.36 a	2.97 ±0.10	4.76 ±0.14 a	6.70 ±0.17	15.68 ±0.40 a

't'-test probability differences (Male vs Female) : M = Male, F = Female

a = P<0.001

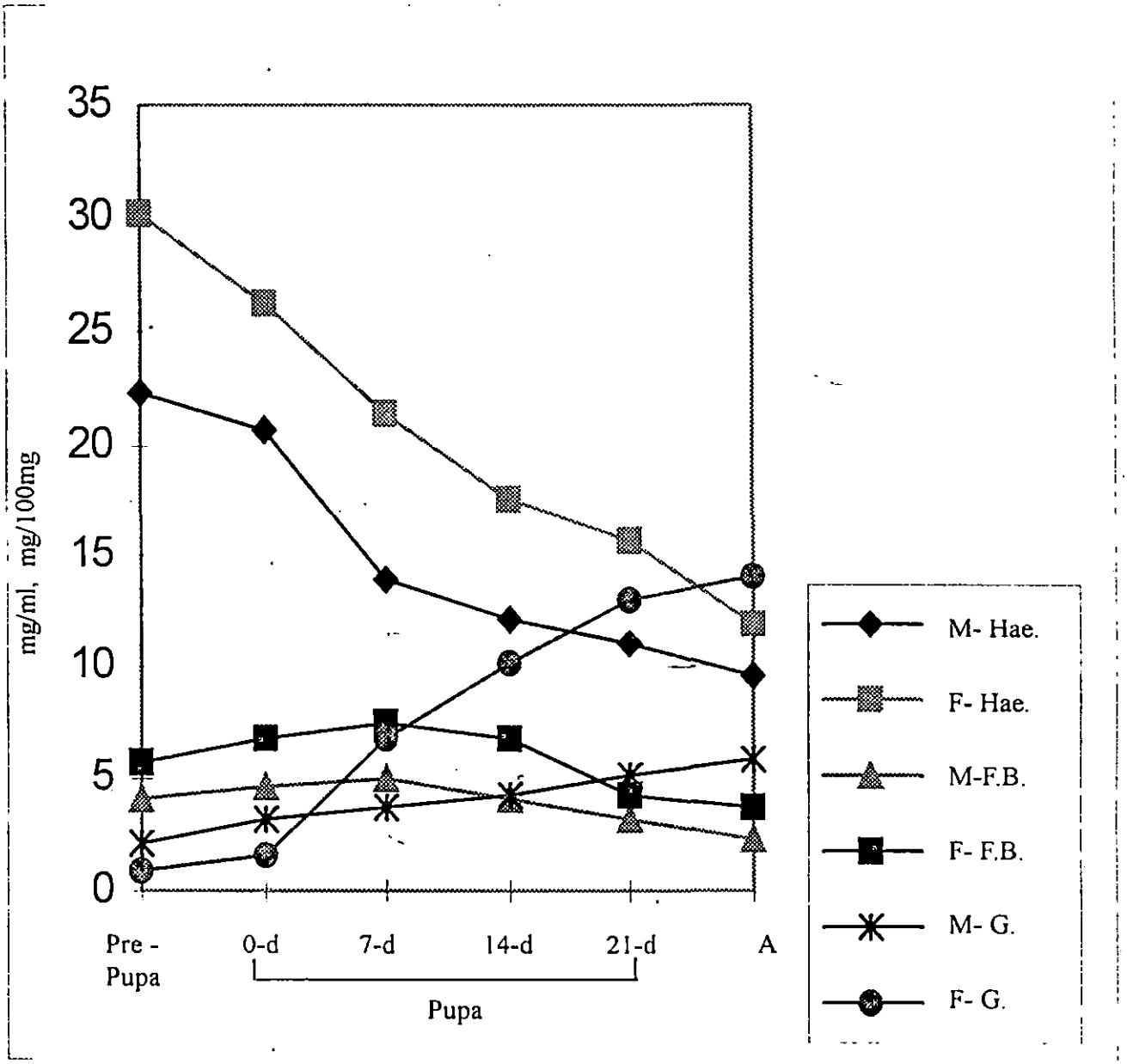


Fig .22. Total protein content in different tissues of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, A = Adult, Hae.= Haemolymph (mg/ml) F.B.= Fat Body(mg/100mg) , G= Gonad (mg/100mg).

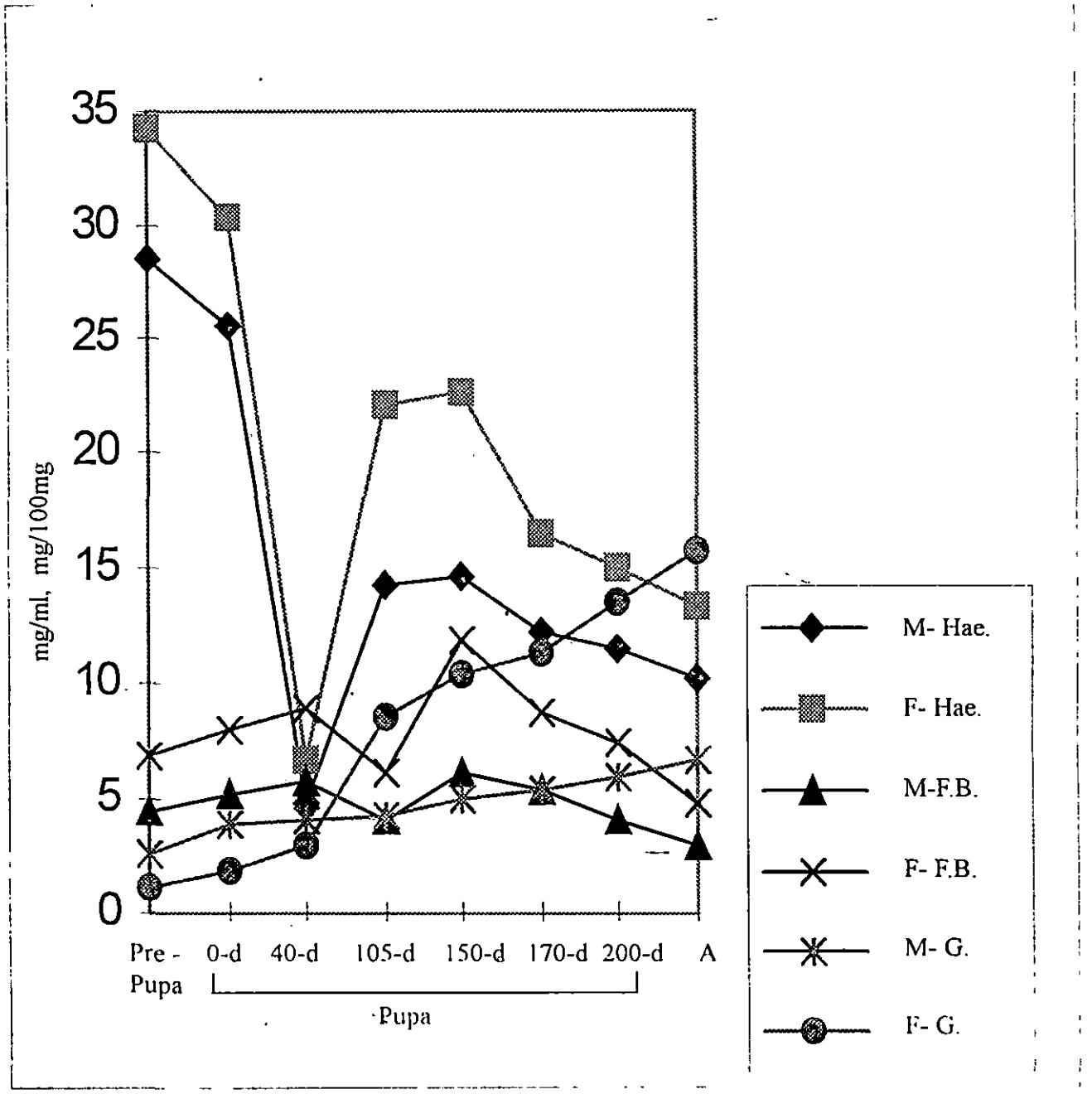


Fig .23. Total protein content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, A = Adult, Hae.= Haemolymph (mg/ml) F.B.= Fat Body(mg/100mg) , G= Gonad (mg/100mg).

4.3.3. DNA content in fat body and gonad

4.3.3.1. Fat body DNA

Pattern of age-dependent variation in DNA content in fat body is not similar between the two generations. In case of non-diapause brood DNA concentration in male and female fat body suddenly increased from pre-pupa to 0-day pupa i.e. after larval-pupal transformation, then gradually declined upto 21 day old pupa and a same level was maintained till adult emergence. Female fat body showed significantly higher value ($P<0.05$ to $P<0.001$) in DNA concentration throughout the pupal phase, but exhibited lower level in DNA titre ($P<0.001$) in pre-pupal stage compared to that of male. Further, there was no significant difference of this biomolecule in freshly emerged adults.

In diapausing generation variation in DNA content in fat body between the two sexes is different in pre-pupa, pupa and adult stages. In male, DNA content in fat body cells showed peak level twice throughout the life span studied from pre-pupa to adult emergence. First, peak of DNA level was observed on 0-day pupa followed by a gradual fall upto 105-day while second peak was recorded on 150 day of pupal age following gradual and significant fall ($P<0.001$) upto 200 day of pupal age and same level was maintained till adult emergence. In female gradual increment of DNA concentration was found upto 150 day pupa exhibiting first peak while second peak was noted on 200-days of pupal development preceeded by a drastic fall on 170-day old pupa. However, no significant difference in DNA concentration of female fat body was observed between 200 day old pupa and adult moths. Unlike in non-diapause brood, female fat body contained less amount of DNA from pre-pupa to 170-day old pupa but it was recorded to be higher only at late phase of life span i.e. 200 day old pupa and freshly emerged adults. It is pertinent to mention here that frequent rise and fall in fat body DNA concentration particularly in diapausing brood irrespective of sexes actually reflects the alteration of metabolic state of fat body tissue particularly in relation to protein and RNA synthesis for maintaining and terminating the diapause state of this insect (Tables23 & 24, Figs. 24 & 25).

4.3.3.2 Gonad DNA

In gonad also the pattern of variation in DNA concentration was different in between the two generations like fat body cells as found earlier. In the non-diapause brood DNA concentration in testis was found to increase sharply from pre-pupal stage onwards to 7th day old pupa and then drastically reduced to a minimum level on day of adult emergence (22 day). The female gonad also maintained the same trend like testis with the difference that ovary always contained high level of DNA. The DNA concentration in diapausing testis was increased day by day from pre-pupal stage and reached maximum level on the day of adult emergence (210 day). A steep rise in DNA level was recorded between 40 to 105 day of pupal age. But in case of female, the DNA titre in ovary increased day by day and touched the peak level on 150 day pupa; thereafter gradual reduction in DNA content was recorded upto the day of adult eclosion. It should be mentioned here that ovary of pre; pupa, 0-day old pupa, 200 day old pupa and freshly emerged adult showed low amount of DNA than their male counterparts which is very unlike from that of non-diapause generation. However, the cause of such variation in DNA concentration is not known. But, it can be presumed the this pattern of variation in DNA level only reflects the RNA and protein synthesis according to the stage and/or age-specific requirement for development and growth during diapause (Table 23 & 24, Figs. 24 & 25).

4.3.4. RNA content in fat body and gonad

4.3.4.1. Fat body RNA

The pattern of variation in RNA content of fat body is similar tin the male and female pre-pupae, pupae and freshly emerged adults of non-diapause generation. In both sexes RNA content in fat body increased significantly ($P<0.001$) from pre-pupa to 0-day old pupa followed by gradual reduction in RNA level upto 7 day pupa. Thereafter, a very sharp decrease in RNA concentration was recorded till adult emergence and reached the minimum level. Females always contained significantly higher amount of RNA in fat body than the males.

In both sexes of diapause generation the pattern of variation in RNA titre of fat body during the different developmental stages is different from that of non-diapausing insects. However, in this brood male and female fat body followed similar pattern of variation in RNA content in every developmental stages studied. The RNA concentration in fat body of two sexes first significantly decreased ($P<0.01$ to $P<0.001$) from peak level in pre-pupa to 40-day old pupa. Thereafter, the concentration of this biomolecule was found to be increased significantly ($P<0.001$) in both the sexes and again touched the peak on 150 day old pupa after which a significant drastic fall ($P<0.001$) in RNA level was recorded in the two sexes from 150 day onwards upto the day of adult emergence and was found lowest amount in freshly emerged male and female moths. Unlike non-diapause generation female fat body contained more amount of RNA than in the male fat body from pre-pupa to 40-day old pupa and then from 200 day of pupal age to freshly emerged adults (210 day). However, RNA level in female fat body was found to be significantly lower in 105-day, 150-day and 170-day old diapausing pupae. (Tables 25 & 26, Figs. 26 & 27).

4.3.4.2. Gonad RNA

In both non-diapause and diapause generations male and female gonads followed the same pattern of variation in RNA concentration from pre-pupal stage to the day of adult emergence. RNA level in both testis and ovary increased gradually and significantly ($P<0.02$ to $P<0.01$) from pre-pupal age to 7-day pupa and 105-day pupa of non-diapause and diapause generation respectively. Thereafter a gradual and significant fall in RNA content ($P<0.001$) was recorded and reached the lowest level in freshly emerged male and female adults of the two generations. Ovary always contained significantly higher ($P<0.01$ to $P<0.001$) amount of RNA than testis from pre-pupa to the day of adult eclosion in both the generations. Thus, it is evident that in case of non-diapause generation day 7 pupa and for diapausing generation 105-day old pupa is the critical phase for the initiation of gonadal development and maturation which is evident from the variations of DNA and protein concentrations of the same tissues (Tables 25 & 26, Figs 26 & 27).

Table 23. DNA content in different tissues of non-diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Fat body (µg/100mg)		Gonad (µg/100mg)	
	M	F	M	F
Pre-pupa	235.43 ±6.68	170.31 ±7.10 d	42.15 ±0.20	65.06 ±0.45 d
0-day pupa	265.39 ±6.10	334.10 ±6.19 d	108.20 ±2.91	141.60 ± 2.88 d
7-day pupa	241.60 ±3.39	272.05 ±5.10 d	281.78 ±3.66	392.52 ±8.04 d
14-day pupa	208.51 ±4.64	224.18 ±4.10 a	202.26 ±2.75	254.06 ±5.61 d
21-day pupa	197.70 ±5.69	218.51 ±5.14 b	192.99 ±3.71	210.14 ±2.82 c
Freshly emerged adult (22-day)	200.92 ±6.49	208.15 ±7.08 NS	180.64 ±2.17	201.39 ±4.16 d

't'-test probability differences (Male vs Female) :

a = P<0.05,
b = P<0.02,
c = P<0.01,
d = P<0.001,
NS = Not significant.

M = Male ; F = Female.

Table 24. DNA content in different tissues of diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each value represents Mean± S.E. (n=10)

Tissue / Stage of insect	Fat body (µg/100mg) M	Fat body (µg/100mg) F	Gonads (µg/100mg) M	Gonads (µg/100mg) F
Pre-pupa	246.87 ±7.34	105.72 ±6.86 c	28.67 ±1.09	25.25 ±0.94 a
0-day pupa	271.06 ±6.74	98.07 ±4.26 c	62.51 ±1.78	49.41 ±1.50 c
40-day pupa	239.50 ±4.10	131.15 ±5.24 c	86.24 ±3.42	182.53 ±6.28 c
105-day pupa	182.98 ±7.45	155.34 ±7.10 b	232.90 ±2.67	227.12 ±4.50 NS
150-day pupa	365.84 ±8.60	280.49 ±6.11 c	250.82 ±3.09	304.30 ±5.40 c
170-day pupa	206.35 ±11.69	178.74 ±3.81 a	262.40 ±2.33	271.61 ±7.94 NS
200-day pupa	175.33 ±6.28	266.13 ±9.34 c	285.94 ±3.12	187.63 ±6.45 c
Freshly emerged adult (210 day)	168.34 ±6.83	277.55 ±14.86 c	306.63 ±8.18	180.59 ±5.52 c

't'-test probability differences (Male vs Female) :

a = P<0.05, M = Male ; F = Female.
b = P< 0.02,
c = P<0.001,
NS = Not significant.

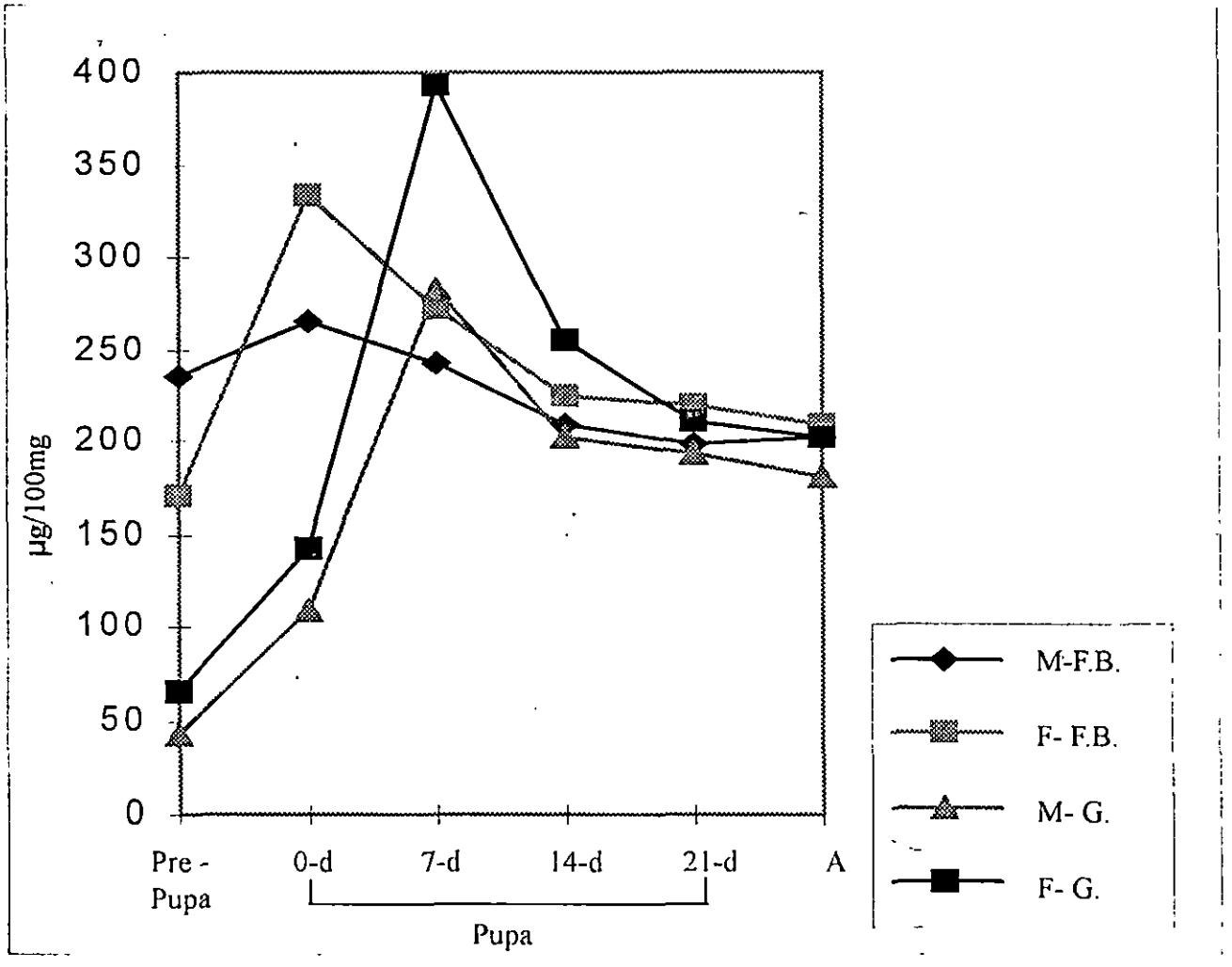


Fig. 24. DNA content in different tissues of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M= Male, F= Female, A = Adult, F.B.= Fat Body (µg/100mg), G = Gonad (µg/100mg).

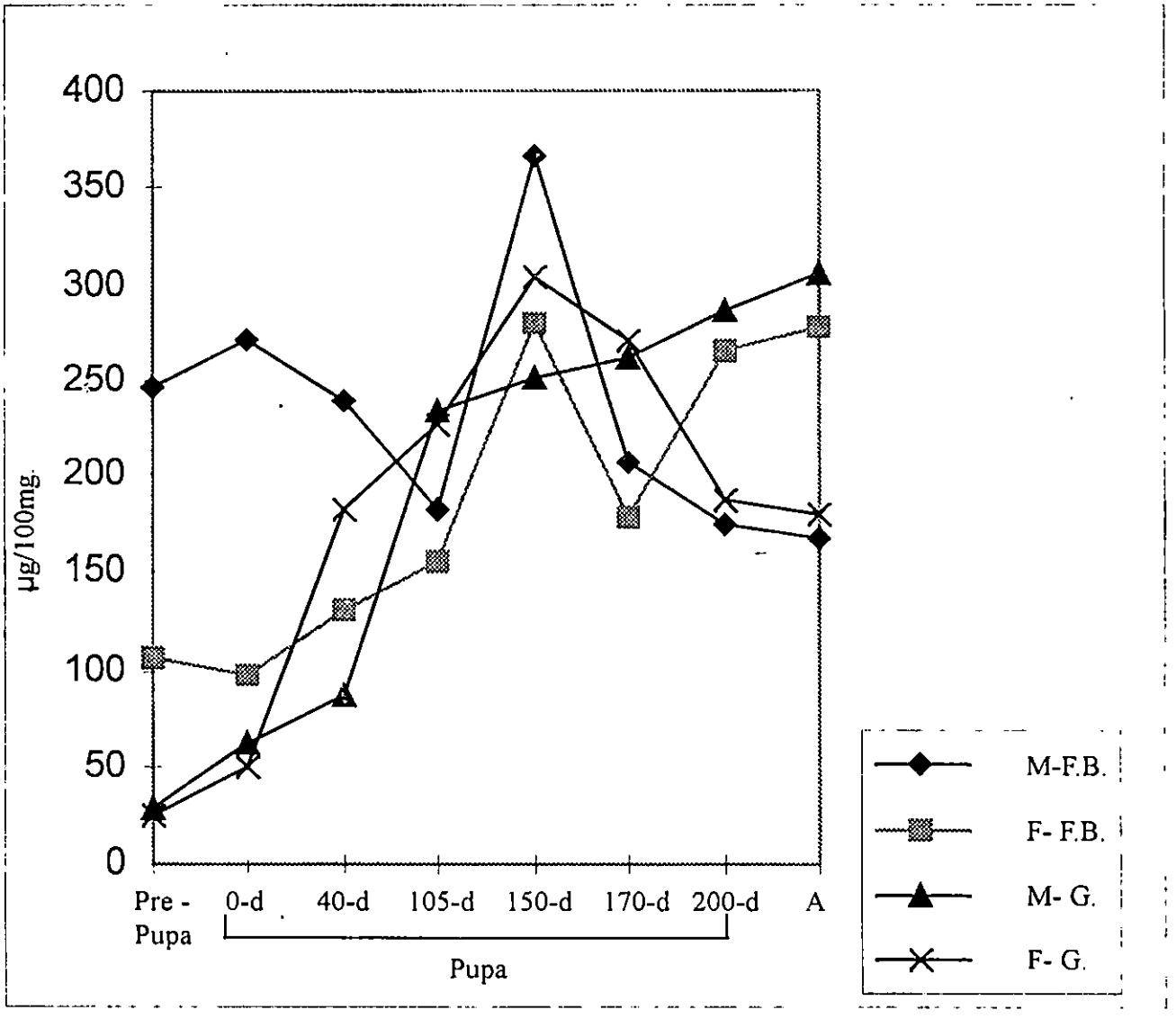


Fig. 25. DNA content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A =Adult, F.B. = Fat body (µg/100mg), G = Gonad. (µg/100mg).

Tabl 25. RNA content in different tissues of non-diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each values represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Fat body (µg/100mg)		Gonad (µg/100mg)	
	M	F	M	F
Pre-pupa	3003.50 ±50.66	3215.00 ±32.49 a	278.86 ±8.22	365.71 ±7.70 b
0-day pupa	3285.49 ±45.05	3418.80 ±40.50 a	312.10 ±8.40	472.63 ±8.15 b
7-day pupa	2807.14 ±35.40	3022.25 ±38.52 b	565.66 ±7.14	1092.88 ±15.31 b
14-day pupa	1815.86 ±39.10	2038.55 ±42.26 a	418.00 ±8.49	610.40 ±9.42 b
21-day pupa	618.30 ±20.90	724.02 ±18.21 a	202.70 ±6.15	408.12 ±12.44 b
Freshly emerged adult (22-day)	385.49 ±13.07	478.72 ±12.14 b	152.60 ±4.10	346.80 ±8.40 b

't'-test probability differences (Male vs Female) : M = Male , F = Female.

a = P< 0.01,
b = P <0.001.

Table 26. RNA content in different tissues of diapausing *A.mylitta* during pre-pupal, pupal and adult stages of both the sexes. Each value represents Mean± S.E. (n=10)

Tissue / Stage of insect	Fat body (µg/100mg) M	Fat body (µg/100mg) F	Gonads (µg/100mg) M	Gonads (µg/100mg) F
Pre-pupa	3116.87 ±44.65	3385.80 ±50.58 b	305.35 ±9.15	412.27 ±6.60 c
0-day pupa	2824.41 ±46.96	3174.23 ±28.54 c	355.06 ±9.31	510.07 ±9.35 c
40-day pupa	1768.23 ±62.85	1950.20 ±50.70 a	547.64 ±25.20	879.20 ±32.65 c
105-day pupa	2739.06 ±48.31	2318.34 ±87.40 c	718.46 ±16.63	1279.52 ±23.85 c
150-day pupa	3820.45 ±38.62	2635.80 ±42.45 c	595.70 ±25.59	855.83 ±21.52 c
170-day pupa	1510.50 ±43.74	1254.66 ±31.20 c	274.68 ±7.44	653.10 ±14.62 c
200-day pupa	614.26 ±30.30	743.05 ±32.39 a	187.30 ±8.62	325.01 ±15.08 c
Freshly emerged adult (210-day)	406.27 ±17.56	502.46 ±20.52 b	121.81 ±6.93	308.65 ±11.46 c

't'-test probability differences (Male vs Female) : M=Male , F=Female.

a = P<0.02,
b = P<0.01,
c = P<0.001.

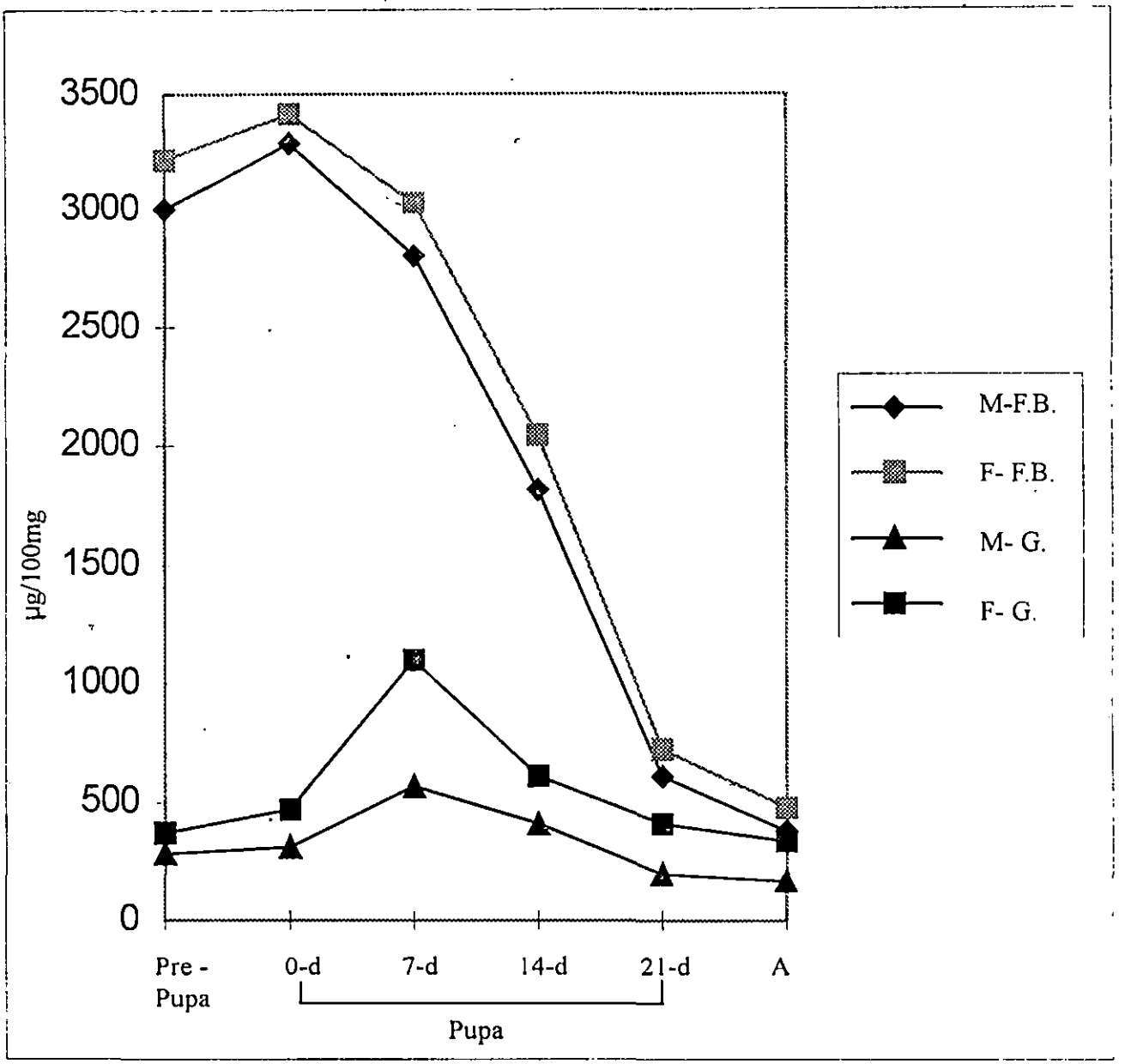


Fig. *26*. RNA content in different tissues of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A =Adult, F.B. = Fat body (µg/100mg), G = Gonad. (µg/100mg).

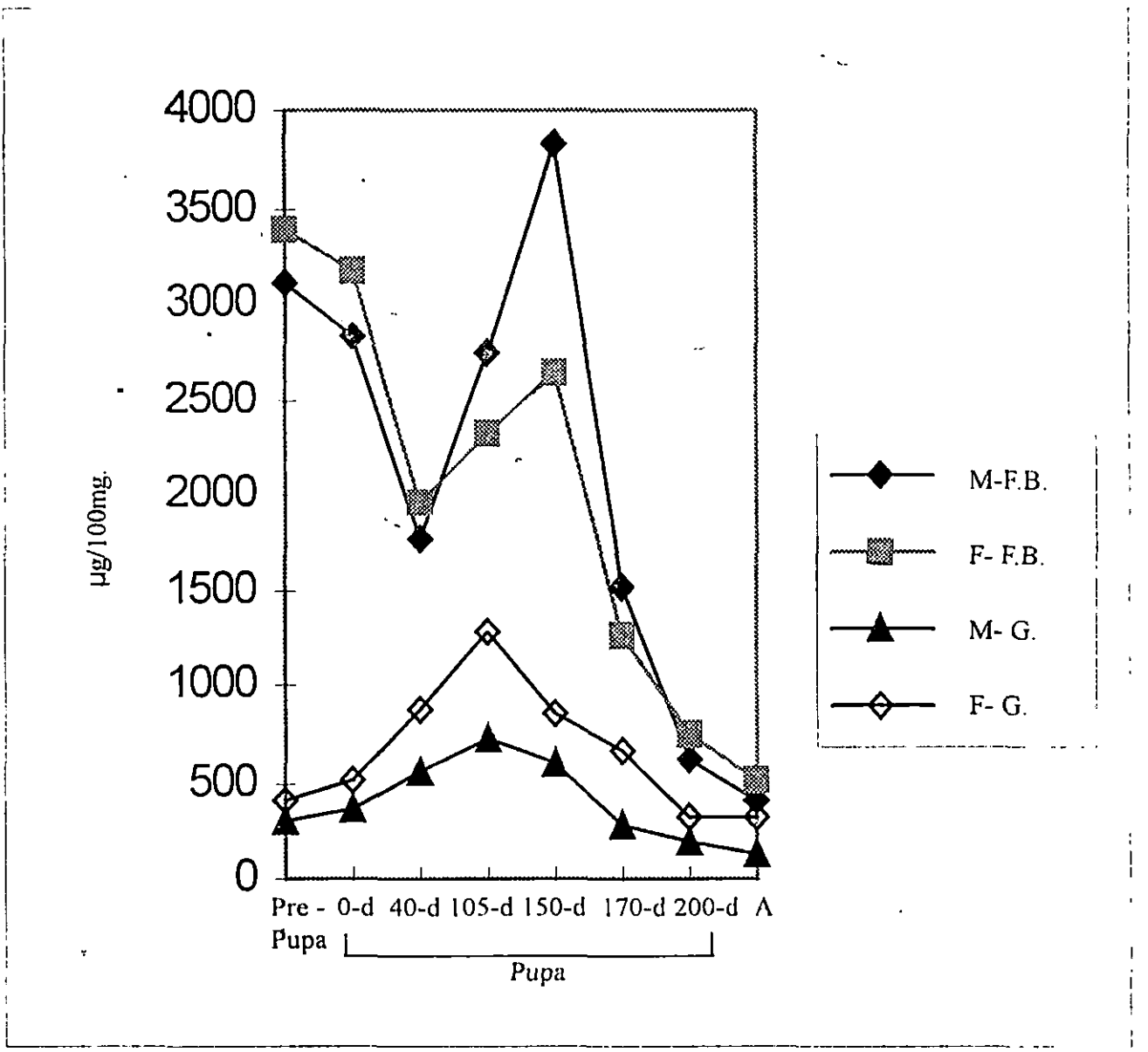


Fig. 27. RNA content in different tissues of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A =Adult, F.B. = Fat body (µg/100mg), G = Gonad. (µg/100mg).

4.3.5. Weight of gonads of pre-pupae, pupae and adults on non-diapause and diapause generations

Weight of gonad in male and female insects of both non-diapause and diapause generations gradually increased from pre-pupa to adult stage and reached the peak level in the adult moths. It was interesting to note that ovarian weight suddenly increased at about 23.50 times from 0-day to day 7 pupa in non-diapause generation. But, curiously enough the increase in ovarian weight on 200 day old diapausing pupae was found to be 470 times heavier over that of 170 day old pupal ovary. so, it revealed that ovarian growth rate is faster in non-diapause than that of in diapausing moths (Tables 27 & 28, Figs. 28 & 29).

Table 27. Weight of gonads of non-diapausing *A.mylitta* during pre-pupal , pupal and adult stages. Each value represents Mean ±S.E. (n=10).

Tissue / Stage of insect	Weight of gonads (mg) Testis	Ovary
Pre-pupa	18.10 ±0.15	4.14 ±0.20 a
0-day pupa	22.21 ±0.12	12.78 ±0.16 a
7-day pupa	24.92 ±0.14	300.34 ± 4.12 a
14-day pupa	28.15 ±0.17	3968.30 ± 32.24 a
21-day pupa	36.10 ±0.50	5310.26 ±72.43 a
Freshly emerged adult (22-day)	38.20 ±0.61	5419.95 ±88.54 a

't'-test probability differences (Male vs Female) :
a = P<0.001

M = Male ; F = Female.

Table 28. Weight of gonads of diapausing *A. mylitta* during pre-pupal, pupal and adult stages. Each mean value represents Mean ± S.E. (n=10).

Tissue / Stage of insect	Weight of gonads (mg)	
	Testis	Ovary
Pre-pupa	15.19 ±0.21	3.15 ±0.14 a
0-day pupa	15.57 ±0.23	3.32 ±0.18 a
40-day pupa	17.20 ±0.44	4.85 ±0.22 a
105-day pupa	18.62 ±0.80	5.14 ±0.17 a
150-day pupa	20.28 ±0.32	5.75 ±0.11 a
170-day pupa	22.44 ±0.50	6.58 ±0.26 a
200-day pupa	27.86 ±0.41	3094.77 55.93 a
Freshly emerged adult (210 day)	28.58 ±0.59	3311.80 ±97.39 a

't'- test probability differences (Male vs Female) : M = Male, F = Female.

a = P<0.001.

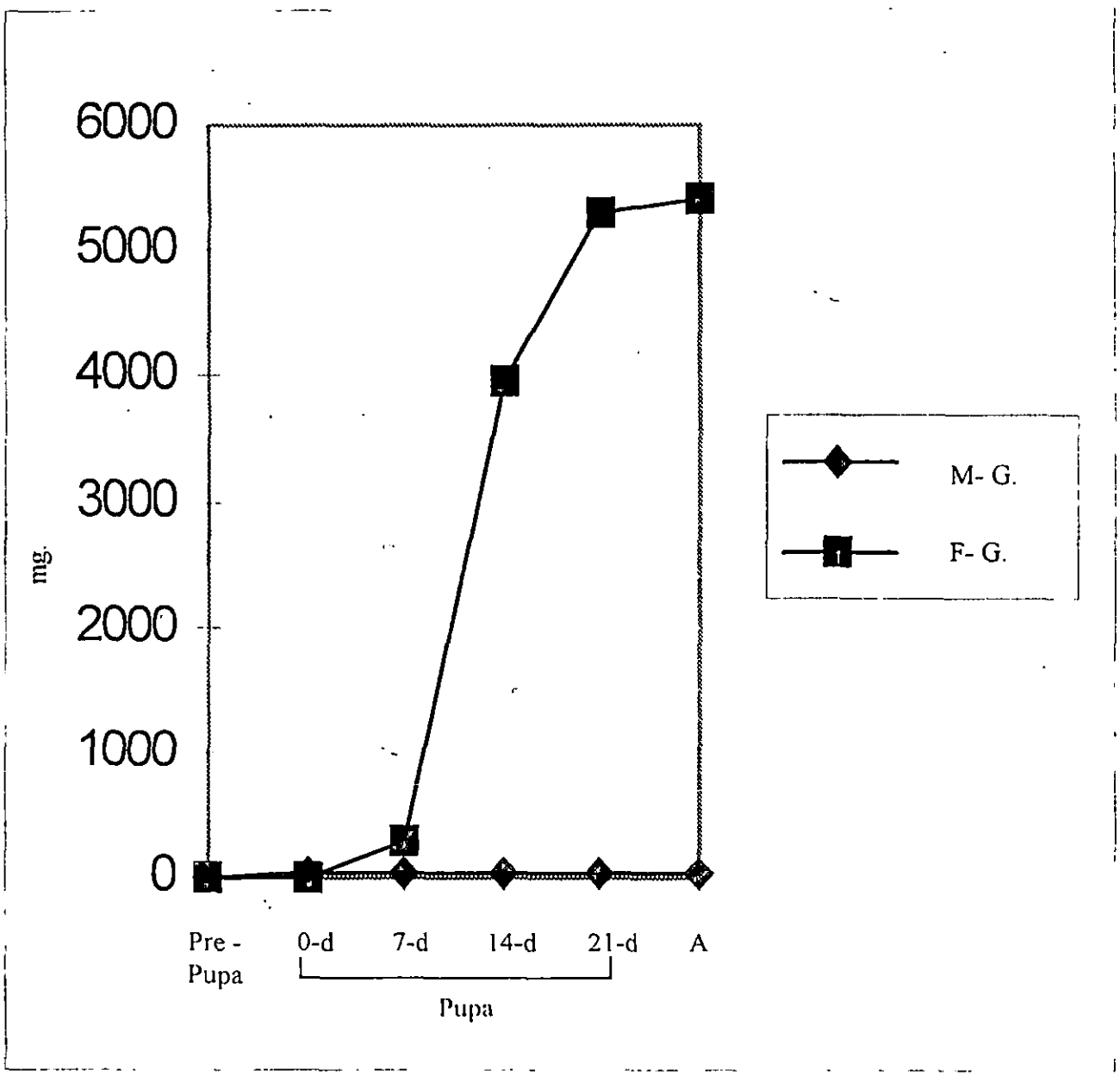


Fig.28. Weight of gonads of non- diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A = Adult, G = Gonad. (mg.)

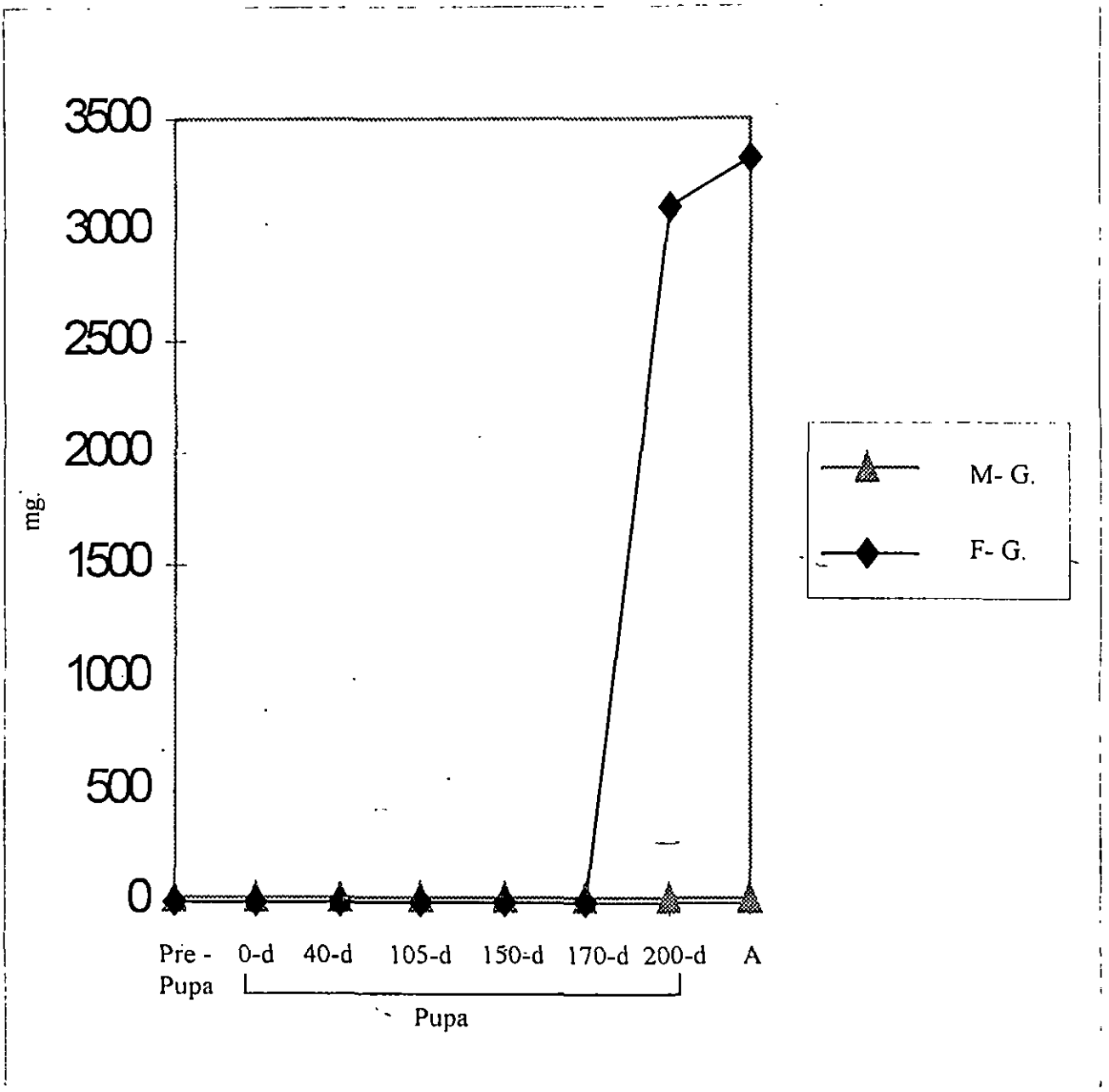


Fig.29- Weight of gonads of diapausing *A. mylitta* during pre-pupal, pupal and adult stages of both the sexes. M=Male, F= Female, A = Adult, G = Gonad. (mg.)

4.4. Effect of Insulin on Diapause Generation of *A.mylitta*.

4.4.1 Treatment of insulin with diapausing pre-pupae of *A.mylitta*.

Pupal duration : Treatment with Insulin at the doses of 5 µl and 10 µl/pre-pupa significantly shortened the female pupal life span by more than 12 days. However, pupal period in male was significantly reduced by about 11 days only at the dose of 10 µl/pre-pupa (Table 29 & 32)

Moth weight : Moth weight in both the sexes was not significantly enhanced in any of the doses of the applied hormone in comparison to that of control. However, there is a trend in increasing male and female moth weights with 5 µl and 10 µl of insulin although the data were not found to be significant (Table 29).

Egg production and hatching : Eggs laid by a single mother moth (fecundity) as well as the total number of egg production (laid + unlaid) were not significantly influenced by the insulin but an increasing trend was observed in higher doses (5 and 10 µl) although the data were not significant. However, the hatching percentage was also not influenced in any case by insulin in comparison to their control counterparts (Table 29).

4.4.2. Treatment of 40 day old diapausing pupae with insulin

Pupal life span : Pupal life span was significantly shortened in both the sexes after application of insulin at the doses of 5 and 10 µl/pupa in comparison to that of control (Table 30 & 33).

Moth weight : Compared to control all the doses of insulin significantly enhanced both male and female moth weights (Table 30).

Egg production : All the doses of insulin significantly increased the number of eggs laid by a mother moth i.e. fecundity as well as the total egg production (laid + unlaid) when compared to that of control (Table 30).

Hatching performance : No significant effect was observed on hatching percentage due to application of any of the doses of the hormone when compared with the same values of control individuals (Table 30).

4.4.2. Treatment of 150 days old diapausing pupae with insulin

Pupal duration : In case of control the female pupae took more time for emergence than the males. Both the doses of the hormone were able to reduce the average duration of pupal life. (Table 31 & 34).

Moth weight : Female moth weight was significantly increased only with 5 µl dose of insulin while the male moth weight was in no way affected by either of the doses of insulin. (Table 31).

Egg production : Insulin at the dose of 5 µl/pupa significantly increased the total number of egg production (laid + unlaid) as well as fecundity in this insect. But, the dose of 10 µl/pupa significantly decreased the fecundity as well as total egg production. (Table 31).

Hatching performance :

No significant change in hatching percentage was observed in either of the doses of insulin after treatment in comparison with that of control (Table 31).

4.2.3 Effect of insulin on cholesterol content in haemolymph, fat body and gonad of diapausing *A.myllita* during pupal and adult stages of development

In haemolymph : Irrespective of sexes the cholesterol content of haemolymph increased significantly in case of both 5 µl and 10 µl doses of insulin on 150 and 170 day pupa but in adult stage, it decreased significantly compared to that of control. It is interesting to note that 10 µl dose of insulin showed more decreased cholesterol titre ($P<0.02$) during adult phase in both the sexes

Table 29. Effect of single injection of different doses of insulin on diapausing pre-pupae (3 days before pupation) of *A.mylitta*. Each value represent mean±S.E (n=50).

Treatment	Pupal life span(days)		Single moth wt.(g)		Egg production (no.)			Hatching(%)
	M	F	M	F	Laid (Fecundity)	Unlaid	Total	
Control	208.14 ±3.74	215.17 ±3.28	2.53 ±0.13	6.46 ±0.30	147 ±11	31 ±6	178 ±13	78.58 ±5.36
1 µl/pre-pupa.	203.86 ±1.77 NS	209.68 ±2.89 NS	2.50 ±0.12 NS	6.69 ±0.26 NS	138 ±9 NS	48 ±9 NS	186 ±8 NS	83.60 ±2.88 NS
5 µl/pre-pupa.	202.44 ±3.56 NS	201.93 ±3.01 b	2.63 ±0.07 NS	6.94 ±0.29 NS	191 ±19 NS	32 ±6	223 ±20 NS	83.25 ±3.12 NS
10µl/pre-pupa.	197.30 ±2.84 a	203.12 ±2.12 b	2.67 ±0.16 NS	7.08 ±0.20 NS	195 ±22 NS	16 ±3	211 ±17 NS	84.68 ±2.42 NS

't'-test probability differences (Control vs Treatments) :

a = P<0.05,
b = P<0.01,
NS= Not significant. M=Male . F=Female.

Table 30. Effect of single injection of different doses of insulin on 40-day old diapausing pupae of *A.mylitta*. Each value represent mean±S.E (n=50).

Treatment	Pupal life span(days)		Single moth wt.(g)		Egg production (no.)			Hatching(%)
	M	F	M	F	Laid (Fecundity)	Unhid	Total	
Control	212.87 ±2.04	215.22 ±1.50	2.37 ±0.10	5.57 ±0.26	153 ±16	13 ±2	166 ±13	80.76 ±4.56
5µl/pupa	203.56 ±1.65 c	201.30 ±2.11 c	2.81 ±0.17 a	6.58 ±0.22 b	239 ±13 c	23 ±3	262 ±18 c	87.92 ±2.14 NS
10 µl/ pupa.	201.84 ±2.42 c	204.60 ±1.67 c	2.79 ±0.16 a	6.71 ±0.12 c	265 ±9 c	43 ±16	308 ±16 c	86.93 ±3.19 NS

't'-test probability differences (Control vs Treatments) :

a = P<0.05 ,
b = P<0.01,
c = P<0.001,
NS= Not significant.

M = Mule F = Female.

Table 31. Effect of single injection of different doses of insulin on 150-day old diapausing pupae of *A.mylitta*. Each value represent mean±S.E (n=50).

Treatment	Pupal life span(days)		Single moth wt.(g)		Egg production (no.)			Hatching(%)
	M	F	M	F	Laid (Fecundity)	Unlaid	Total	
Control	53.87 ±1.06	58.57 ±2.90	2.38 ±0.08	4.43 ±0.24	154 ±24	29 ±7	183 ±20	78.45 ±3.24
5 µl/pupa	50.37 ±1.02 a	50.95 ±1.19 a	2.54 ±0.08 NS	5.71 ±0.30 b	218 ±17 a	31 ±6	249 ±19 a	84.41 ±2.23 NS
10 µl/pupa	49.27 ±1.80 a	51.78 ±1.07 a	2.37 ±0.12 NS	5.01 ±0.30 NS	73 ±9 b	45 ±10	118 ±12 b	77.20 ±2.93 NS

T-test probability differences (Control vs Treatments)

a = P<0.05, M= Male ; F = Female.
b = P<0.01,
NS= Not significant

Table 32. Rhythm of moth emergence in *A. mylitta* following insulin application to the diapause-destined pre-pupae (n=160).

Observation at every 3 day from May. 25 to July,29.	Control		1 µl/pre-pupa		5 µl/pre-pupa		10 µl/pre-pupa	
	No.	%	No.	%	No	%	No.	%
May 27	0	0	0	0	3	1.875	4	2.50
30	0	0	0	0	0	0	1	0.625
June 2	1	0.625	4	2.50	1	0.625	3	1.875
5	1	0.625	2	1.25	3	1.875	9	5.625
8	4	2.50	4	2.50	7	4.375	11	6.875
11	4	2.50	5	3.125	11	6.875	13	8.125
14	6	3.750	10	6.25	14	8.75	18	11.25
17	5	3.125	19	11.875	13	8.125	24	15.00
20	9	5.625	19	11.875	18	11.25	18	11.25
23	14	8.75	17	10.625	16	10.00	9	5.625
26	12	7.50	15	9.375	14	8.75	8	5.00
29	12	7.50	11	6.875	8	5.00	4	2.50
July 2	11	6.875	7	4.375	4	2.50	1	0.625
5	10	6.25	5	3.125	1	0.625	0	0
8	12	7.50	3	1.875	0	0	1	0.625
11	8	5.00	1	0.625	1	0.625	4	2.50
14	5	3.125	0	0	1	0.625	1	0.625
17	2	1.25	1	0.625	0	0	0	0
20	1	0.625	2	1.25	1	0.625	0	0
23	0	0	1	0.625	1	0.625	0	0
26	1	0.625	0	0	0	0	0	0
29	1	0.625	0	0	0	0	0	0

Table 33. Rhythm of moth emergence in *A.mylitta* following insulin application to the 40-day old diapausing pupae(n=200) .

Observation at every 3 day from May, 26to July,30.		Control		5 µl/ pupa		10 µl/pupa	
		No.	%	No	%	No.	%
May	28	1	0.50	1	0.50	2	1.00
	31	0	0	2	1.00	4	2.00
June	3	0	0	2	1.00	1	0.50
	6	2	1.00	2	1.00	5	2.50
	9	0	0	4	2.00	6	3.00
	12	2	1.00	8	4.00	10	5.00
	15	5	2.50	15	7.50	19	9.50
	18	8	4.00	28	14.00	33	16.50
	21	16	8.00	31	15.50	34	17.00
	24	13	6.50	29	14.50	22	11.00
	27	19	9.50	11	5.50	12	6.00
	30	12	6.00	4	2.00	4	2.00
July	3	14	7.00	4	2.00	2	1.00
	6	16	8.00	0	0	0	0
	9	11	5.50	0	0	1	0.50
	12	10	5.00	1	0.50	1	0.50
	15	9	4.50	2	1.00	1	0.50
	18	3	1.50	3	1.50	1	0.50
	21	1	0.50	0	0	0	0
	24	0	0	0	0	0	0
	27	1	0.50	0	0	0	0
	30	2	1.00	0	0	0	0

Table 34. Rhythm of moth emergence in *A.mylitta* following insulin application to the 150-day old diapausing pupae(n=100) .

Observation at every 3 day from May, 26to July,30.		Control		5 µl/ pupa		10 µl/pupa	
		No.	%	No	%	No.	%
May	28	0	0	0	0	0	0
	31	0	0	0	0	0	0
June	3	0	0	2	2.00	2	2.00
	6	0	0	1	1.00	2	2.00
	9	1	1.00	1	1.00	2	2.00
	12	1	1.00	4	4.00	5	5.00
	15	2	2.00	3	3.00	7	7.00
	18	5	5.00	13	13.00	15	15.00
	21	9	9.00	18	18.00	19	19.00
	24	13	13.00	26	26.00	14	14.00
	27	14	14.00	15	15.00	12	12.00
	30	17	17.00	3	3.00	9	9.00
July	3	11	11.00	2	2.00	3	3.00
	6	6	6.00	3	3.00	0	0
	9	5	5.00	1	1.00	2	2.00
	12	1	1.00	0	0	1	1.00
	15	3	3.00	2	2.00	1	1.00
	18	1	1.00	1	1.00	0	0
	21	1	1.00	0	0	1	1.00
	24	2	2.00	0	0	0	0
	27	1	1.00	0	0	0	0
	30	0	0	0	0	0	0

However, there was uniformity in the pattern of variation of cholesterol in 150 day pupa, 170 day pupa and adult stages. The cholesterol content first elevated significantly ($P<0.001$) on 150 day and then declined significantly ($P<0.001$)) on 170 day pupa and adults ($P<0.001$) in both the sexes of control and treated individuals showing a specific pattern of variation (Table 35).

In Fat Body : In male and female fat body significant enhancement in cholesterol level occurred in both 5 µl and 10 µl doses of insulin on 150 day and 170 day of pupal age while, it was decreased significantly only in the resultant female adults not in the males when compared with that of control. The magnitude of the effect of the insulin remained almost the same in both the doses. Further, the pattern of variation in cholesterol content of fat body in both the sexes was the same as in haemolymph in case of both control and treated groups (Table 35).

In gonad : In case of both control and treatments the cholesterol contents of male and female gonads increased significantly after 40 day of pupal age and reached a peak on adult stage. Compared to control treatment with both the doses (5 and 10 µl) of insulin resulted significantly in higher amount of cholesterol in testis and ovary during the subsequent pupal life and adult stage of development (Table 35).

4.4.4. Effect of insulin on the total protein content in haemolymph, fat body and gonal of diapausing *A.mylitta* during pupal and adult development

In haemolymph : In control and treatments total protein content of haemolymph of each sex first increased on 150 day of pupal age and then declined on 170 day pupa and finally in the adult. However, both 5 and 10 µl doses of insulin significantly enhanced protein level in the two sexes on 150 day and 170 day pupae while the hromone lowered the level of protein in each sex at the adult stage in comparison to those of control individuals. No dose response effect was observed in this case. (Table 36).

In Fat Body : A gradual increase in protein concentration was observed in control and both doses of insulin treated lots, the increase was at the peak on 150 day and then gradually declined upto adult stage establishing a specific pattern of variation of this bimolecule. Insulin (5 and 10 µl/pupa) induced a significant enhancement ($P<0.01$ to $P<0.001$) in protein titre particularly on 150 day and 170 day pupae compared to that of control while the lowest level of the biomolecule was recorded in adult stage. Moreover female fat body contained more amount of protein that male fat body throughout the pupal and adult life span (Table 36)

In Gonad : A specific pattern of variation was observed in gonad protein level in control insects where same was gradually increased upto adult stage with the peak in concentration. Exogenous insulin significantly enhanced the protein titre in testis and ovary throughout the period under study (pupal and adult stages). The specific pattern of variation in protein concentration remained unaltered after insulin treatment. In general, female gonads showed higher amount of protein than male gonads of pupal and adult stages (except in 40 - day old pupa) (Table 36).

4.4.5. Effect of insulin on the DNA content in fat body and gonad of diapausing *A.mylitta* during pupal and adult development

In Fat Body : DNA content of male fat body initially enhanced significantly ($P<0.001$) on 150-day pupa followed by a significant reduction ($P<0.001$) on 170-day old diapausing pupa and reached minimum level at adult stage; while in female, DNA content first increased significantly ($P<0.001$) on 150-day and then declined ($P<0.001$) on 170-day of pupal development but again significantly enchanced ($P<0.001$) in adult stage in both control and treated lots. However, compared to control, the two doses of insulin induced and increase in DNA concentration significantly in fat body of both the sexes of 150 day and 170 day pupae but not in the adults. No dose dependent effect of insulin was observed in DNA titre irrespective of the sex (Table 37).

In Gonad : In control lots DNA content of testis gradually increased significantly from 150 day pupa onwards and touched the peak on the day of

adult emergence; while in ovary it increased on 150 day pupa showing the peak followed by a significant reduction ($P<0.001$) on 170 day old pupa and adults. Both 5 µl and 10 µl doses of insulin expressed significantly higher DNA content in both the sexes over controls in each stage. Further, the age-dependent pattern of DNA in gonad remained unaltered by insulin treatment at any of the doses used. The insulin treated groups also followed the same pattern of variation in DNA concentration like control insects.(Table 37).

4.4.6. Effect of insulin on RNA content in fat body and gonad of diapausing *A.mylitta* during pupal and adult development

In Fat Body : A sharp rise in fat body RNA concentration was recorded in both sexes on 150 day pupa followed by a drastic fall on 170 day and more so during adult stage. Insulin at the doses of 5 and 10 µl/pupa was able to enhance the RNA content only during the pupal stage (150 and 170 day) while no effect of the hormone was recorded in the adult stage. Age-dependent changes in RNA titre remained unaltered after insulin treatment (Table. 38)

In Gonad : RNA levels in control animals was found to be same from 40 to 150 day old diapausing pupae in testis and ovary, then declined from 150 day onwards and reached a minimum level on the day of adult eclosion. Ovary showed higher RNA concentration than testis during pupal and adult stages. Insulin (5 and 10 µl/pupa) significantly elevated the RNA titre from 40-day to 150-day and then from 150 day onwards declined upto the adult stage when compared with RNA content of control lots. It should be mentioned here that insulin was able to change slightly the specific pattern of variation where peak level in RNA concentration was recorded on 150 day and then declined to adult stage (Table 38).

4.4.7. Effect of insulin on gonad weight of diapausing *A.mylitta* during pupal and adult development

Weight of both male and female gonads increased significantly ($P<0.01$ - $P<0.001$) due to the two doses of insulin than that of gonads of

the control individuals at pupal and adult stages. In both control and treatments the weights of testis and ovary gradually increased significantly from 150 day pupa onwards to adult emergence. The rise in the ovarian weight was very sharp approaching the adult stage. Further, during pupal stage (40 to 170 day) ovarian weight remained very low in comparison to testis weight reflecting the commencement of ovarian maturation during very late phase of pupal age. In this case also, no dose response relationship was observed like all other biochemical parameters in this experiment (Table 39).

Table 35. **Cholesterol content in haemolymph , fat body and gonads of pupae and adults of diapausing generation of *A.mylitta* following administration of insulin to 40- day old pupae. Each value represents mean ± S.E. of 10-15 observations.**

Treatment	Sex	Haemolymph(µg/ml)				Fat body (µg/100mg)				Gonad (µg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	740.56 ±16.19	2172.46 ±52.39	1922.64 ±42.11	1619.41 ±25.33	2095.19 ±68.92	3765.49 ±68.33	2850.94 ±62.44	965.12 ±31.79	262.89 ±8.95	720.83 ±20.59	815.16 ±15.30	924.35 ±26.30
	F	850.86 ±28.15	2350.84 ±69.40	1882.00 ±55.10	922.37 ±38.09	1752.88 ±36.09	2525.30 ±65.56	1878.86 ±51.90	515.58 ±23.43	692.86 ±11.53	1325.60 ±24.35	1414.49 ±29.05	1751.77 ±56.32
5 µl /pupa	M	698.37 ±24.35 NS	2385.00 ±62.83 a	2079.86 ±45.74 a	1438.50 ±35.36 c	1986.55 ±73.40 NS	3993.15 ±41.26 b	3081.64 ±45.10 b	1020.52 ±32.26 NS	271.35 ±6.91 NS	855.60 ±22.24 c	965.18 ±15.94 c	1056.23 ±25.51 b
	F	902.41 ±39.70 NS	2592.44 ±42.70 b	2151.23 ±55.88 b	718.35 ±25.19 c	1810.97 ±44.15 NS	2875.39 ±49.67 c	2220.58 ±62.63 c	310.20 ±16.74 c	673.49 ±16.32 NS	1468.35 ±26.47 c	1720.87 ±40.69 c	1962.30 ±45.27 b
10 µl /pupa	M	719.50 ±34.23 NS	2422.91 ±51.15 b	2110.87 ±55.49 b	1292.80 ±48.72 c	2148.36 ±57.40 NS	3981.50 ±38.14 b	3150.44 ±55.36 b	983.70 ±46.50 NS	254.90 ±9.05 NS	872.40 ±22.36 c	951.83 ±20.59 c	1082.25 ±28.90 c
	F	837.60 ±19.22 NS	2588.23 ±60.95 a	2227.69 ±41.34 c	632.48 ±20.77 c	1697.10 ±33.64 NS	2930.49 ±52.61 c	2375.10 ±45.22 c	350.40 ±25.81 c	711.36 ±19.20 NS	1446.15 ±25.42 b	1761.44 ±50.39 c	2009.67 ±58.94 b

't'-test probability differences (Compared to Control) :
a = P<0.02 , b = P<0.01, c = P<0.001, NS = Not significant

M = Male ; F = Female.

Table 36. **Total protein content in haemolymph , fat body and gonads of pupae and adults of diapausing generation of *A.mylitta* after injection of insulin to 40-day old pupae. Each value represents mean ± S.E. of 10-15 observations.**

Treatment	Sex	Haemolymph(mg/ml)				Fat body (mg/100mg)				Gonad (mg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	4.86 ±0.08	13.56 ±0.15	12.17 ±0.18	10.14 ±0.22	5.35 ±0.17	6.08 ±0.10	5.40 ±0.14	2.97 ±0.10	4.08 ±0.20	4.97 ±0.10	5.32 ±0.14	6.70 ±0.17
	F	6.74 ±0.33	19.20 ±0.42	16.42 ±0.34	13.29 ±0.36	8.94 ±0.25	11.85 ±0.12	8.77 ±0.11	4.76 ±0.14	2.96 ±0.15	10.41 ±0.22	11.30 ±0.25	15.68 ±0.40
5µl/ pupa	M	4.93 ±0.10 NS	14.68 ±0.21 c	13.22 ±0.15 c	9.18 ±0.25 b	5.60 ±0.19 NS	7.10 ±0.15 c	4.82 ±0.11 b	2.23 ±0.12 c	3.97 ±0.18 NS	5.36 ±0.14 a	6.08 ±0.10 c	7.55 ±0.12 c
	F	6.59 ±0.28 NS	24.42 ±0.30 c	18.19 ±0.22 c	11.10 ±0.30 c	9.25 ±0.16 NS	13.25 ±0.18 c	6.47 ±0.20 c	3.10 ±0.19 c	3.04 ±0.11 NS	12.15 ±0.32 c	13.29 ±0.42 c	17.50 ±0.25 c
10µl/ pupa	M	5.01 ±0.07 NS	14.02 ±0.25 c	13.39 ±0.23 c	9.09 ±0.18 c	5.49 ±0.15 NS	7.22 ±0.20 c	4.75 ±0.16 b	2.11 ±0.10 c	4.21 ±0.14 NS	5.40 ±0.15 a	6.28 ±0.15 c	7.59 ±0.14 c
	F	7.43 ±0.30 NS	25.05 ±0.35 c	18.77 ±0.32 c	10.85 ±0.37 c	9.18 ±0.27 NS	13.26 ±0.24 c	6.39 ±0.25 c	3.27 ±0.22 c	3.10 ±0.12 NS	12.06 ±0.38 c	13.46 ±0.30 c	17.68 ±0.36 c

't'-test probability differences (Compared to Control) :
a = P<0.05 , b = P<0.01, c = P<0.001,
NS = Not significant
M = Male ; F = Female.

Table 37. **DNA content in fat body and goads of pupae and adults of diapausing generation of A.mylitta following insulin injection to 40 day old pupae. Each value represents mean ± SE of 10-15 observations.**

Treatment	Sex	Fat body (µg/100mg)				Gonad (µg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	239.50 ±4.10	365.84 ±8.60	206.35 ±11.69	168.34 ±6.83	86.24 ±6.42	250.82 ±8.09	262.40 ±5.33	306.63 ±8.18
	F	131.15 ±5.24	280.49 ±6.11	178.74 ±3.81	277.55 ±14.86	182.53 ±6.28	304.30 ±5.40	271.61 ±7.94	180.59 ±5.42
5µl/ pupa	M	228.41 ±5.38 NS	462.50 ±8.19 b	280.95 ±6.42 c	155.90 ±8.32 NS	78.06 ±4.23 NS	298.34 ±4.57 b	289.07 ±3.24 b	361.25 ±6.87 b
	F	122.53 ±7.11 NS	351.88 ±9.41 b	197.80 ±4.54 a	274.65 ±7.50 NS	185.33 ±7.60 NS	363.01 ±6.44 b	326.54 ±8.92 b	224.46 ±7.30 b
10µl/ pupa	M	245.26 ±6.90 NS	475.64 ±10.18 b	285.41 ±8.59 c	164.42 ±7.21 NS	91.65 ±5.39 NS	294.28 ±7.95 b	302.49 ±10.05 b	375.41 ±8.20 b
	F	135.60 ±8.14 NS	368.71 ±7.70 b	201.50 ±3.56 c	266.10 ±8.48 NS	176.40 ±9.21 NS	358.20 ±6.82 b	315.18 ±7.22 b	236.19 ±6.89 b

't'-test probability differences (Compared to Control) :
a = P<0.02 , b = P<0.01, c = P<0.001,
NS = Not significant
M = Male : F = Female.

Table 38. **RNA content in fat body and goads of pupae and adults of diapausing generation of A.mylitta following insulin injection to 40 day old pupae. Each value represents mean ± SE of 10-15 observations.**

Treatment	Sex	Fat body (µg/100mg)				Gonad (µg/100mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult	40-day pupa	150-day pupa	170-day pupa	Adult
Control	M	1768.23 ±62.85	3820.45 ±38.62	1510.50 ±43.74	406.27 ±17.56	547.64 ±25.20	595.70 ±25.59	274.68 ±7.44	121.81 ±6.93
	F	1950.20 ±110.70	2635.80 ±42.45	1154.56 ±31.20	502.46 ±20.52	879.20 ±32.65	855.83 ±21.52	653.10 ±14.62	308.65 ±11.46
5µl/ pupa	M	1747.81 ±55.26 NS	3943.20 ±27.60 a	1715.34 ±33.70 b	417.92 ±11.50 NS	590.15 ±24.48 NS	716.42 ±17.60 c	322.90 ±8.64 c	178.59 ±6.20 c
	F	2069.40 ±59.23 NS	2866.30 ±62.80 b	1297.41 ±34.90 b	556.40 ±41.35 NS	866.84 ±25.36 b	943.61 ±20.42 b	712.90 ±15.18 b	375.44 ±8.10 b
10µl/ pupa	M	1820.16 ±67.30 NS	4010.23 ±39.54 b	1764.29 ±41.80 c	412.75 ±18.42 NS	610.20 ±22.40 NS	736.78 ±15.32 c	331.84 ±9.40 c	190.08 ±7.50 c
	F	1988.71 ±120.45 NS	2920.66 ±51.32 c	1328.73 ±43.20 b	497.41 ±19.25 NS	872.33 ±28.00 NS	950.74 ±18.46 b	722.66 ±15.71 b	362.80 ±7.95 c

't'-test probability differences (Compared to Control) :
a = P<0.02 , b = P<0.01, c = P<0.001,
NS = Not significant
M = Male ; F = Female.

Table 39. Gonadal weights of pupae and adults of diapausing generation of *A.mylitta* after the treatment of 40-day old pupae with insulin. Each value represent mean ± S.E. of 10-15 individuals.

Treatment	Sex	Weight of Gonad (mg)			
		40-day pupa	150-day pupa	170-day pupa	Adult
Control	Male	17.20 ±0.44	20.28 ±0.32	22.44 ±0.50	28.58 ±0.59
	Female	4.85 ±0.22	5.75 ±0.11	6.58 ±0.26	3311.80 ±97.39
5µl /pupa	Male	16.81 ±0.56 NS	21.64 ±0.40 a	24.08 ±0.33 a	31.10 ±0.44 a
	Female	4.94 ±0.31 NS	6.99 ±0.20 b	8.41 ±0.45 b	4140.49 ±102.85 b
10 µl/pupa	Male	17.58 ±0.39 NS	22.04 ±0.30 b	24.22 ±0.18 a	31.64 ±0.35 b
	Female	5.06 ±0.25 NS	7.46 ±0.35 b	9.50 ±0.60 b	4390.15 ±115.16 b

't'-test probability differences (Control vs Treatment)

a = P<0.01, b = P<0.001, NS = Not significant.

4.5. Effect of Exogeneous 20-HE on the Diapause-destined Generation when Applied to the Pre-pupae

4.5.1. On larval - pupal transformation

All the doses of 20-HE caused pupation earlier than in the control, pupation time was shortened progressively with the higher dosages (Table-40). Irrespective of the doses and sex, the pupal mortality did not differ from that of control. The doses other than 1 µg/pre-pupae led to prothetelic development in the pupae such as exposed antennae of pupal-imaginal intermediates and the short crumpled wings . Such developmental derangement was of higher frequency in higher dosages of 20-HE.

4.5.2. Diapause duration and adult development

Irrespective of the sexes 20-HE shortened significantly the duration of pupal diapause with the rise of dosages.Defective pupae were produced due to 20-HE. All the defective pupae resulted from a 2 µg/prepupa dose could develop into normal adults. But, in case of other two higher doses of the hormone only a few of the defective pupae could grow into normal moths, mostly emerged as abnormal adults with crumpled wings, the frequency was highest in case of 10 µg dosage. These moths of two higher dosages were weak in their mating performance than their counterparts of control set. The weak female moths were unable to lay eggs at all although they had fully developed reproductive system. An average number of 193 unlaid eggs per female were counted after dissection whereas in case of control the average number of total (laid unlaid) eggs per female was 217 (Table -41). Further, there was no significant effect of this hormone on the moth weight (Table-40)

4.5.3 Gonad weight and size

Weights of testes and ovaries along with their lengths and widths were significantly increased over those of control pupae on 150 day with 2,5 and 10 µg doses of 20-HE whereas 1 µg dosage was ineffective. 10 µg dosage of the hormone had the greatest effect. Effect of the hormone was reflected in the morphology of average and testis. Ovarian maturation was promoted progressively with the higher dosages of the hormone . This was evident from the impression of the ovarian follicul inside the ovariole, the follicles quickly became distinct when treated with a higher dose (10 µg) (Table 42 and 43).

4.5.4 Reproductive performance

Fecundity and total egg production by a female were recorded to be significantly higher in 5 and 10 µg doses except. In case of 2 µg dose; the fecundity was higher with comparison to that of control. However, hatching percentage was increased over the control only in case of 5 and 10 µg of the hormone. (Table 41).

4.5.5 Protein concentration in the gonads

An increased concentration of protein was recorded in the individuals who received 2, 5 and 10 µg of 20-HE, but 1 µg of the hormone was ineffective and 10 µg was found to be the most effective dose. Further, female gonads contained more protein than the male gonads (Table 43).

Table 40. Effects of exogenous 20-HE on the diapause development after application to the pre-pupae of *A. mylitta*.

Treatment	No-treated	No died	Time took for pupatioin (day) Mean±SE	No. of pupae which terminated diapause in			Duration of diapause (days) Mean±SE	Moth emergence (%)	Moth weight (g)*		Abnormality out of pupae survived (No)	
				<60 days	<200 days	>200 days			Male Mean±SE	Female Mean±SE	Pupa	Adult
Control	50	13	8.36±0.76	0	7	30	217.5±3.5	74.34	2.80±0.46	7.12±0.29	0	0
1µg/prepupa	50	12	5.88±0.63 a	0	14	24	205±3.0 a	75.65	3.25±0.37	7.39±0.29	0	0
2µg/prepupa	50	12	5.96±0.67 a	0	19	19	198.5±4.0 a	76.66	3.36±0.50	7.22±0.33	1	0
5µg/prepupa	50	11	5.48±0.52 a	0	24	15	194.0±5.0 a	78.20	3.00±0.25	7.36±0.30	3	1
10µg/prepupa	45	11	5.20±0.70 a	2	27	5	172.5±7.0 b	75.43	2.91±0.18	7.81±0.31	7	6

't' - test probability differences (Control vs each of the treatments) :

a = P<0.01, b = P<0.001, *Non-significant.

Table 41. Effect of exogenous 20-HE on the fecundity, total egg production and hatching performances when applied to the diapausing pre-pupae of *A. mylitta* Values represent mean ± SE.

Treatment	Egg production (No.)			Hatching (%)
	Fecundity(laid)	Unlaid	Total	
Control	175 ±14	42 ±13	217 ±17	79.59 ±2.50
1 µg/pre-pupa	197 NS ±25	24 ±4	221 NS ±19	79.88 NS ±2.18
2 µg/pre-pupa	256 b ±25	18 ±2	274 NS ±24	83.49 NS ±1.84
5 µg/pre-pupa	248 b ±20	25 ±10	273 a ±21	87.88 a ±2.23
10 mg/pre-pupa	273 c ±17	23 ±4	296 b ±17	86.87 a ±1.81

't' - test probability differences (Control vs each of the treatments) :

a = P<0.05 , b = P<0.01 , c= P<0.001, NS = Non-significant.

Table 42. Effect of exogenous 20-HE on the measurements of the gonads of the diapausing 150 - day - old pupae following application of the hormone to pre-pupae of *A. mylitta*. Values represent mean ± SE.

Treatment	Testis (mm)		Ovary (mm)	
	Length	Width	Length of ovariole	Width of single ovariole
Control	3.525 ±0.023	2.683 ±0.038	5.816 ±0.083	0.177 ±0.007
1 µg/pre-pupa	3.550 NS ±0.045	2.772 NS ±0.051	5.983 NS ±0.095	0.183 NS ±0.008
2 µg/pre-pupa	3.717 b ±0.028	2.877 a ±0.035	9.767 b ±0.601	0.219 b ±0.006
5 µg/pre-pupa	4.167 b ±0.051	3.455 b ±0.045	19.850 b ±0.126	0.211 b ±0.009
10 µg/pre-pupa	4.900 b ± 0.041	3.789 b ±0.042	22.083 b ±0.206	0.360 b ±0.009

't'- test probability differences (control vs each of the treatments) :

a = P<0.05 ; b = P<0.001 NS = Non-significant.

Table 43. Effect of exogenous 20-HE on the weights and protein contents of gonods of diapausing 150-day-old pupae following administration of the hormone to the pre-pupae of *A. mylitta*. Values represents mean ±SE.

Treatment	Testis		Ovary	
	Weight(mg) (2-testes)	Protein content (mg/100mg)	Weight(mg) (2-Ovaries)	Protein content (mg/100 mg)
Control	19.37 ±0.39	4.56 ±0.07	6.10 ±0.54	10.10 ±0.13
1 µg/pre-pupa	20.02 NS ± 0.46	4.49 NS ±0.08	6.25 NS ±0.21	10.32 NS ±0.24
2 µg/pre-pupa	22.49 a ±0.56	5.11 b ±0.06	35.40 c ±1.34	11.35 b ±0.23
5 µg/pre-pupa	24.55 b ±0.87	5.67 c ±0.07	123.80 c ±2.42	12.58 c ±0.25
10 µg/pre-pupa	25.84 b ±1.01	5.98 c ±0.16	145.69 c ±3.67	12.96 c ±0.32

't' - test probability differences (control vs each of the treatments) :

a = P<0.05, b = P<0.01, c = P<0.001, NS = Non-significant.

5. DISCUSSION

5.1. Comparative Phenology of two Generations

5.1.1. Phenology of larvae, pupae and adults :-

The present investigation demonstrates that the ambient factors not only govern the growth and development pattern of wild *A. mylitta,* but also determine its morphogenetic and behavioural criteria of the diapause and non-diapause generations. Pupal diapause in *A.mylitta* is an evolved character which enables the species to tide over the winter adversities including foliage scarcity, and which is only induced during the autumn months. This finding corroborates almost a similar situation in flesh flies (Fraenkel and Hsiao, 1968; Denlinger, 1971). It is imperative from the observed data that a short daylength of <12 hrs in combination with a low temperature of $<18°C$ trigger the larvae in the field from third instar onwards to orient progressively for the induction of diapause after pupation. This is reflected in the larval developmental pattern; whereas those larvae experienced a long daylength of >12 hrs with a temperature of $>20°C$ have been prevented from undergoing pupal diapause and continued a non-diapause development. In *A.mylitta* voltinism has been claimed to be governed by climatic condition of the altitude (Nayak and Dash, 1991) and it appears that photoperiodic response is strongly affected by the length of scotophase of short days, that decisively influences the diapause behaviour of this species. Diapause inducing effects of short days and the stages sensitive to photoperiod have also been demonstrated in many multivoltine insects (Danilevski, 1961; Saunders, 1976). It has been reported that the insects are able to discriminate between short and long day regimes by their time measurement mechanisms and to store the photoperiodic information in the brain. This information is ultimately summated and the impact is transmitted to the hormonal system for deciding whether the insect should enter diapause or not (Denlinger, 1985). This mechanism may also hold good for *A.mylitta.*

In a number of insects the developmental rates have been found to differ under different daylengths (Beck, 1980) which influences the growth rates. Higher larval weights through longer feeding duration as well as slower RGR from third instar onwards have been recorded in the diapausing brood of *A.mylitta* under the influence of short daylength and low temperature. Low

range of temperature also enhances the duration of latent feeding period of fifth instar larvae of diapause-destined generation and thereby increases the quality indices of *A.mylitta* (Discussed in the next chapter). Tropical insects which experience seasonal temperature changes are thought to rely on temperature as a diapause regulating factor as in the case of flesh fly, *Sarcophaga spilogaster* in which diapause is induced and maintained by cool temperatures (Denlinger, 1974). Bivoltine brood of *A.mylitta* also exhibited similar strategies and the coincident timing of these strategies has led to speculation on the extent of their physiological, biochemical and evolutionary relationship (Pullin and Bale, 1989; Pullin *et.al.,* 1991; Denlinger, 1991).

Larval, pupal and cocoon weights and shell ratio in diapause generation have been significantly increased due to higher amount of food consumption and consequently a higher energy storage covering the longer duration of larval life. However, the moth weight and fecundity have been significantly reduced. This has happened because the pupae have had to overcome the long diapause period at the cost of stored resources. The entire life span of this insect is significantly longer in diapause generation only due to extended larval and pupal durations.

The progressively higher temperatures and longer daylengths from the spring onwards may serve to accelerate in *A.mylitta* the phases of diapause termination after 150 day of pupation. This is what is known as the end of diapause development (Danks, 1987). This adult development may have a relation with changes in the carbohydrates in the haemolymph, fat body and gonads (Chaudhuri *et al.,* 1993). A change in phosphate metabolism has also been reported in *Pieris brassicae* (Pullin, 1992) at the end of diapause development. Ovarian growth index drastically changes onwards after 150 day of diapause development . Such progress in development is further confirmed from the present observation of retraction of wing and associated tracheae from the pupal cuticle. It is reported that availability of water promotes post-diapause development (Chippendale and Reddy, 1973). Emergence of moth of *A. mylitta* in diapausing brood commences on the first week of June with the advent of monsoon. There exists a close relationship between the date of first rain and emergence phenology of tasar moth (Sinha and Chaudhuri, 1992). Thus, in *A.mylitta* natural environmental signals dictate its developmental fate and regulate the whole mechanism by which a somewhat synchrony in

emergence after the diapause is achieved among the pupae. Further, the appearance of suitable food for the establishment of young larvae on host-tree which also reflushes with abundant leaves by this time after a long denudation period during the winter and spring months, also provides optimum life condition for this insect.

5.1.2 GRAINAGE PERFORMANCE :

The emergence rhythm, mating behaviour, oviposition, egg incubation period and hatching percentage are all intimately related to the ambient temperature and humidity. These factors also affect the phenology of other insects; (Messenger and Filters, 1959; King, 1975; Beck, 1980). Normal adult emergence of *A.mylitta* in first brood has taken place from the first week of June onwards after maintaining a prolonged diapause for about 6-7 months. The onset of monsoon almost from the first week of June has an impact on the concomitant emergence phenology of tasar moth. Such a correlation between the time of first monsoon rain and the phenology of the broods has also been recognized in other insects (Fewkes and Buxo, 1969 ; Evans and Buxo, 1973).

The adult eclosion rhythm almost exclusively from the late photophase to the late scotophase in each brood resemble the similar behaviour of the Mediterranean flower moth, *Anagasta kuhniclla* (Beck, 1980). The time of emergence of an individual adult insect is determined by the temporal relationships among the underlying physiological rhythms which are again, governed by the environmental factors. This may be the reason for the males of tasar moth to emerge proportionately in greater frequency earlier during the quite prolonged emergence period than the females. Such protandry along with the greater numerosity of the males in the population do not fit the model for natural selection for optimum reproductive strategy for males as proposed by Wiklund and Fagrostrom (1977). However, it is not possible to explain at this position why the higher number of males occur and why the male development is pre-cocious.

The mating percentage decreases apparently with decreasing humidity and temperature and such variation in mating behaviour has been observed in

natural condition. It may be that *A.mylitta* has a critical temperature for mating in which male responds to female sex pheromone as in case of *Chilo suppressalis* (kanno, 1990). The periodicity of mating behaviour is controlled basically by the endogenous circadian rhythm which is further affected by exogenous factors such as temperature, daylength, humidity and so on (Kanno, 1990). This explanation on *C.suppressalis* seems to hold good for *A.mylitta* .

Oviposition often shows a diurnal rhythm, strongly indicating that there are gating processes which are controlled by the photoperiodic clock and mediated through the neuroendocrine system (Saunders, 1982); Page, 1985; Truman, 1985). Present results indicate that the temperature at which the maximum number of eggs are laid and the extent of the favourable temperature range for egg production are brood specific and depend on other climatic factors. Higher fecundation in second brood demonstrates that the females produced in the combination of thermophase and photophase during September-October were more fecund.This corroborates the similar argument by Ochieng-Odero (1991). The females begin oviposition shortly after mating and this could be caused by either mechanical or chemical stimulation of the oviposition mechanisms. Stimuli associated with copulation and (or) insemination, might have accelerated oviposition significantly (Gerber *et al.,* 1991).

The shorter egg incubation period in the first brood might be due to adaptive influence of photoperiod and thermophotoperiod, although longer photophase might lead to a shorter development time (Philogene, 1982). Fluctuating temperatures have been associated with faster development so long as the temperatures are within the optimal range of the species (Beck, 1986).

Being wild the variations in the hatching of eggs of *A.mylitta* in the two broods are probably due to the suffering from frequent fluctuation of ambient temperature and humidity. Such variations may also be influenced by the diapause and non-diapause state of the insect.

Little information is available regarding the sex ratio of this species. However, sex ratio varies in each generation and does not reflect any impact to potentiate fecundity. The variation may be under genetic control influenced

by external environmental stimuli.

Thus, it emerges that every brood of *A.mylitta* has the optimum range of temperature, from 23.08 to 25.59°C and r.h. 90.46% for the first brood while 25.14 to 27.62°C temperature and 81.89% r.h. for the second brood. Under these conditions the *A.mylitta* exhibits maximum adult emergence, mating, fecundity and hatching, though there may be a season specific variation. The whole phenomenon is obviously dictated by the environmental physiology of the insect.The grainage performance, on the whole, is relatively poor in the diapause generation than in the non-diapause generation.

5.2 Timing of PTTH Release for Larval-Pupal Transformation Through Starvation and Neck Ligation:

A.mylitta is an ideal holometabolan silk producing wild insect. The present experimental race under the geographic location has two successive generations, non-diapausing and diapausing (Kapila *et al* ., 1992; Sinha and Chaudhuri, 1992). The ambient temperature, r.h., photoperiod (L:D) are different for the two generations, the non-diapausing generation (first brood) grows during August-September and the diapause-destined generation (second brood) during October-November.

It is revealed that low rearing temperature and short photoperiod (day) coupled with moderate r.h. cause the prolongation of larval phagoperiod, hence, an increase in Lmw of the 5th stage larvae of diapause -destined generation. This observation in *A.mylitta* is quite consistent with that of Hagazi and Schopf (1984) and Ochieng-Odero (1992) in other lepidopterans. Inspite of slower RGR, the longer latent feeding period contributes to higher Lmw and Pmw in diapause-destined brood. Ochieng-Odero (1990a, b) observed in *Cnephasia jactatana* that the pupal and adult weights are related to the length of latent feeding period, under longer latent period the author has recorded higher Lmw, Pmw and Amw. But in the bimodal generations of *A.mylitta* a different result has been obtained. Inspite of longer latent feeding period the Amw is lower in the diapause generation than in the non-diapause generation. Thus, the duration of latent feeding period may not be the exclusive and universal cause for Amw. The higher Lmw and Pmw of diapausing generation is

an essential requisite for storing a greater abundance of nutrients as the maintenance cost during the forthcoming adverse conditions over the longer period. The depletion of resources as maintenance cost has resulted in the relatively lower Amw and fecundity.

The drastic fall of carbohydrate contents in the tissues after 150 day of pupal life justifies the critical phase for the initiation of diapause termination by way of the onset of relatively faster rate of adult development in *A.mylitta* (Chaudhuri *et al.,* 1993). The present observation on several parameters also justifies that diapause termination has already been initiated at or after 150 day of pupal life.

Higher cocoon shell wt, SR% and relative silk conversion ability are the adaptive strategies of this insect to protect the diapausing pupae from prolonged adversity. Again, the relative silk conversion ability is greater in the males than in the females of both the generations when calculated with relation to the larval wt. after gut purging. This may be due to the greater resource allocation by the female larvae for egg production. Such sexual dimmorphic nature of Lcw, Lmw, Pmw and Amw due to differential resource allocation in the two sexes has also been proposed by Ochieng-Odero (1990a) . Though Lcw and Pcw of the two sexes are different, the values are almost similar for the same sex of the two generations. This explains that these two qualities of *A.mylitta* are independent of environmental factors, hence, are genetically determined.

The Lcw of *A.mylitta* may be used as a stable assessment standard in pupal and adult quality assessment as has been proposed for *C.jactatana by* Ochieng-Odero (1990a) and for *Chilo partellus* also by Ochieng-Odero *et.al.* (1994). The Lmw gradually decreases during the prepupal life to give the Pmw. Pupal wt. decreases further during the pupal life to give the adult wt. The rate of DP (a constant of wt decrease from Lmw to Pmw) and DA(a constant of wt decrease from Lmw to Aw) for both the generations of *A.mylitta* are quite different. Lcw thus, ensures that inspite of weight reduction in the subsequent developmental steps, a functional amount of resource is left for the perpetuation of the race. In the prevailing ecological system the biological performance has contributed well to the survival and reproduction of diapause-destined generation.

Results of starvation and classical neck ligation experiment show that in *A.mylitta* PTTH is released at least thrice during the larval-pupal transformation. The sequential timing of PTTH release is evident from the step-wise prodormal signs for pupation expressed in both the generations. The physiological significance of the gated PTTH release can be interpreted with relation to the steps of development. The first release occurs on the day of attainment of Lcw which may induce only the onset of wandering and gut purging. The second release occurring on the day after 5 hrs of complete gut purge contributes to the partial formation of pupal cuticle and later on causes larval pupal intermoult in a few cases. Finally, the third release possibly takes place on 2-3 days before pupation (during the prepupal stage) and induces only the larval-pupal ecdysis in most of the individuals. However, the expression of all the prodormal signs together with larval-pupal ecdysis are ultimately mediated directly by the action of ecdysone, since PTTH acts on the prothoracic glands to release ecdysone (Bollenbacher and Granger, 1985 ; Riddiford, 1985; Smith, 1985 ; Gelman, 1992 ; Thyagaraja *et. al.,* 1992).

Moreover, it is revealed that the neck ligation after the attainment of Lcw seriously interferred with the gut purging behaviour of this silk worm. The purging is delayed variably without showing apparent correlation with the time of ligation as observed in *Samia cynthia ricini* by Fugishita and Ishizaki (1982). In *S.cynthia ricini* the authors have reported that the endocrine events in the larval pupal development involve a two step process. The first secretion of PTTH and ecdysone induces the prodormal signs of pupation such as the heart exposure, pink pigment formation, gut purging and wandering while the second one induces pupal cuticle formation. Ligation experiments on *Manduca sexta* also yielded the similar result (Truman and Riddiford, 1974; Nijhout and Williams, 1974). Furthermore, the differences in the percentage of the animals that lost the anal proleg mobility, formation of partial pupal cuticle and production of some larval-pupal intermoults have reasonably a relation with the time of ligation and the subsequent release of the PTTH in *A. mylitta.*

It is pertinent to mention here that in *B.mori* 5 times PTTH release have been established (Shirai *et.al.,* 1993) through direct *in vitro* assay of the hormones. The physiological significance of 5 PTTH releases in *B.mori* differs slightly with the 3-step release in *A.mylitta* based on starvation and ligation experiments only.

Thus, it emerges that attainment of Lcw and thereafter expression of all the prodormal signs of pupation are intimately correlated with the 3 sequential steps of the gated release of PTTH. The ambient thermo-photoperiodic regimes have a significant influence on it besides improving the overall quality of the tropical tasar silkworm, *A.mylitta* in non-diapausing and diapause-destined generations.

5.3. Biochemical Status of Cholesterol, Protein, DNA and RNA in the Haemolymph, Fat body and Gonads of Prepupae, Pupae and Adults.

5.3.1. Cholesterol :

The cholesterol contents of haemolymph, fat body and gonads of pre-pupae, pupae and adults of both the generations of *A.mylitta* exhibit almost the same pattern of fluctuation in both the sexes. The fluctuations implicate the variable physiological processes pertaining to the growth and morphogenesis of adult structures. The replenishment or depletion of cholesterol in haemolymph and fat body occur at different stages and in different tissues for their suitable functions and final expression as adult structures. In the pre-pupae the lowest level of cholesterol in the haemolymph and simultaneously considerable amount in the fat body is due to cessation of feeding and storage in the fat body. Immediately after pupation there is a remobilization of cholesterol from fat body to the haemolymph for initiating adult development. This is true for both the sexes and in both the generations of course, at different levels. It is reported that cholesterol requirements in phytophagous insects are satisfied from the ingested phytosterol (Downer, 1978; Rees, 1985). The highest level of cholesterol in haemolymph and fat body has been attained around mid-pupal age i.e. on 14th day in non-diapausing pupae and on 150th day of diapausing pupae. This is possibly due to the transformation of cholesterol from the unconverted food phytosterols and biosynthesis of cholesterol from the simple ingredient such as acetate in fat body as has been advocated in case of *Bombyx miori* and *Philosamia ricini* (Saito *et.al*, 1963; Pant and nautiyal, 1974). The sharp decline in cholesterol content in the haemolymph and fat body in both the sexes from 14 day and 150 day of pupal development in non-diapause and diapause generations respectively upto the adult emergence is the positive indication of

increased and rapid utilization of cholesterol for meeting the developmental demands associated with the pupal - adult transformation. Because, sterols in insects are used as essential components of subcellular membrane, constituents of lipoprotein carrier molecules and surface wax of insect cuticles (Kerlson and Hoffmeister, 1963; Downer, 1978).

In both the generations of *A.mylitta* there is a steady and gradual increase in cholesterol content of testis from pre-pupa to adult indicating the accumulation of the biomolecules for the development of various spermatogenic stages and for the production of ecdysteroids through the augmentation of haemolymph cholesterol. This is consistent with the findings of Loeb *et.al.* (1984) and Hurkadli *et al.* (1989).

In case of ovary, a similar gradual increase of cholesterol from pre-pupa to adult stage in both the generations also signifies the accumulation of cholesterol for ovarian maturation. Higher cholesterol in ovary is due to its higher demand in female for growth and maturation of eggs including hatching as it has been reported that feeding of cholesterol stimulates hatching of eggs (Monroe, 1959) and ovarian development (Robbins and Shortino, 1962).

Results show that cholesterol level in different metabolically important tissues is meant for specific functions of reproduction and development in both the sexes. From this view point, the peak level of adult differentiation of *A.mylitta* takes place around the third week of pupal life of non-diapausing generation. In case of diapause generation the peak occurs after 150 day of pupal life.

5.3.2 Protein :

The total protein contents in the haemolymph of both the sexes of non-diapausing generation gradually decreases from pre-pupal to adult stage. The active tissue differentiation occurs during the pupal stages for pupal-adult metamorphosis. Hence, the reduction in plasma protein level during the pupal and adult stage is due to utilization of plasma proteins to the development of various organs particularly the gonads (Wyatt and Pan, 1978; Bradley, 1983; Fujii and Kawaguchi, 1983; Kim *et.al., 1983).* Further, higher plasma protein level in female may be due to the presence of additional proteins, such as female specific protein and/or vitellogenin which are lacking in male insect (Irie

and Yamashita, 1980; Ogawa and Tojo, 1981; Izumi *et.al.*, 1981). Because of temporary held up or a very slow rate of adult development there is a fall of haemolymph protein of 40 day pupa of diapausing generation. A relatively higher level of protein content is attained after 105 day for rendering a slow rate of differentiation of adult organs during the remaining part of pupal life. Proteins are taken up from the haemolymph and stored in the fat body as has been obtained at the time of pupation in *Pieris* (Chippendale and Kilby, 1969) and *Diatraea* (Chippendale, 1970).

In general, proteins are synthesized by the fat body and released into the haemolymph during the course of larval growth. At the onset of pupation some of the haemolymph proteins are sequestered back into the fat body and stored in large granules until metamorphosis at which they disappear (Chen, 1985). The deposition of such protein granules in male and female fat body together with newly formed yolk precursor protein (Vitellogenin in female) possibly lead to the enhanced fat body protein content in both the sexes during the early pupal life of each generation of *A.mylitta* . However, the gradual decline in fat body protein content in the two sexes during the later part of pupal life and adult emergence in the two generations has been due to the increased utilization of proteins for the development and and maturation of gonads (Wyatt, 1980 ; Bradley, 1983) and morphogenesis of other adult tissues (Walker, 1966 ; Ishizaki, 1965).

The gradual incremental rise in the protein content in both testis and ovary from pre-pupa to adult stage follows the same pattern of variation in the two generatioins and indicates the enhancement of spermatogenesis or spermiogenesis as has been reported in *B.mori* (Chaudhuri and Medda, 1986). In case of ovary the increased protein level is due to the transformation of female specific protein, vitellogenin from the fat body (Pan *et.al.* , 1969; Ono *et.al.*, 1975; Wyatt, 1980; Bradley, 1983) and/or due to increased protein synthesis by the follicular cells of the growing ovary (Anderson and Telfer, 1969; Bast and Telfer, 1976; Glass and Emmerich, 1981). The fat body and gonadal protein profiles also implicate that in the diapausing generation adult development is initiated around or after 105 day and the process is enhanced after 150 day of pupal life.

5.3.3 DNA :

In the non-diapausing generation a little increase in DNA content of male and female fat body from pre-pupa to '0'-day pupa i.e. during larval-pupal transformation may be the result of DNA accumulation from the involuted silk gland and other larval tissues (Matsuura *et.al.*, 1968). In the pupae and in the adults the DNA content in fat body declines steadily but slowly in correlation with the differentiation of adult structures (Lang *et.al.*, 1965).

But in the diapausing generation because of a halt in the developmental activity and delay in the silk gland involution, there is no apparent changes in fat body DNA content during larval-pupal moult in both the sexes. Thereafter, the possible source of increase in DNA level during mid pupal period, with a peak on 150 day may be the histolysis of many of the larval tissues (Chinzei and Tojo, 1972) and/or may be the result of decrease in other cellular components (Wyatt, 1980). From the late pupae to the adult stage the decline in fat body DNA level is possibly due to the involvement of the biomolecule for pupal adult transformation (Brookes and Williams, 1965).

The steady fall in DNA content in the testis and ovary during the later part of the life cycle preceded by a higher level at the early pupal age of non-diapause generation may be the result of accumulation of DNA originated from the histolysis of silk gland or other larval tissues during the early pupal period (Chinzei and Tojo, 1972) and subsequent utilization for active tissue differentiation in the late pupa for adult development. In the diapausing generation the ovarian DNA profile follows the similar pattern of variation as in the non-diapause state. Gradual increase in DNA content in this organ from pre-pupa to mid-pupal stage with peak on 150 day pupa is probably due to endo-polyploidy at early pupal life since there was possibly no cell division at this stage but subsequent decrease from late pupa to adult stage is either due to disintegration of follicular cells and nurse cells of ovary (Chinzei and Tojo, 1972) or huge accumulation of organic substances and thereby increase in organ weight or both (Chaudhuri and Medda, 1985a). But in the testes of diapausing generation the gradual increase in DNA content from pre-pupa to adult stage indicates the sperm formation or sperm maturation in the organ which is reflected in the concomitant increase in testis weight (Chaudhuri and Medda, 1986).

5.3.4 RNA :

The gradual fall in RNA level in the fat body from pre-pupal to adult stage of non-diapausing brood is probably due to progressive low rate of synthetic activity in the fat body with the progress of organogenesis and/or rapid RNA degradation due to less demand for developmental raw materials. This result is quite consistent with the earlier studies (Faulkner and Bheemswar, 1960; Stevenson and Wyatt, 1962 ; Sass and Kovacs, 1974). But, in the diapausing insect the initial fall in RNA level of fat body from pre-pupa to early pupal age may be accounted for the utilization of RNA at a higher rate of pre-pupal turnover needed for larval-pupal moult. A further decline in fat body RNA upto the 40 day of pupal life indicates a deep state of diapause. Thereafter, increase in RNA content during mid-pupal age is supposed to be due to increased synthesis and/or decreased degradation while the subsequent decrease in RNA level during late pupal stage and adult emergence may suggest more utilization of the bimolecules in fat body protein synthesis for organogenesis or vitellogenin synthesis (Stevenson and Wyatt, 1962; Pan *et.al.,* 1969 ; Chinzei and Tojo, 1972).

The increase in RNA content of male and female gonads in non-diapause and diapause generations of this species from pre-pupal to early pupal age suggests a higher rate of synthesis of the macromolecules in these organs, and also may be due to high RNA in follicular cells of ovary for yolk protein synthesis as in case of *H.Cecropia* (Pollock and Telfer, 1969). The reduction in RNA content from mid-pupa to adult emergence is perhaps due to either the disintegration of follicular cells and nurse cells of the ovary (Chinzei and Tojo, 1972) or huge accumulation of organic substances and thereby increase in organ weight or both (Chaudhuri and Medda, 1985a). But in testes the changes in RNA levels and the concomitant increase in testes weights may be the positive indications of enhanced rate of spermatogenesis or sperm maturation as in *B.mori* (Chaudhuri and Medda, 1986). The peak gonadal RNA on 105 day of pupa signifies the highest level of protein synthesis for gametogenesis. The fat body RNA reaches peak amount on 150 day of pupal life and gradually declines in the adult. Thus, the pattern of RNA content in fat body and gonads suggests that gonadal development is initiated prior to 105 day and other adult organs between 105 and 150 day of pupa.

5.3.5 Weight of Gonads :

The gradual increase in weight of testis and ovary in both the generations of *A.mylitta* from pre-pupa to adult stage suggests the accumulation and utilization of cholesterol, protein, DNA and RNA in these organs from other tissues for their maturation.

In non-diapausing individuals the maturation of gonads takes place in about 20-25 days while in diapause-destined generation it is in about 200-220 days of pupal life. This delay in maturation in diapause generation is because of slow rate of metabolism over the long pupal life. This is again, evident from the age-specific variations of cholesterol, protein, DNA and RNA of important tissues physiologically responsible for growth reproductive development and maturation.

5.4 Effect of Vertebrate Insulin on the Termination of Pupal Diapause :

The impact of vertebrate insulin in insects' life processes (Seecof and Dewhurst, 1974; Mosna and Borigozzi, 1976; Davis and Shearns, 1977; Kramer, 1980, 1985 ; LeRoith *et.al.*, 1981, 1988) has created a fascinating chapter in which the present experimental results provide interesting and fundamental informations on various developmental and metabolic changes in *A.mylitta* particularly in respect of the pupal diapause of this saturniid species.

In the diapausing generation the effect of insulin on several physiological and biochemical parameters was studied after injection of the hormone during pre-pupa, early pupa and late pupa stages of *A.mylitta* separately in relation to the physiology of pupal diapause of this species and as such the doses are different depending on the age of the insect. In case of diapausing pre-pupae and pupae insulin has exerted growth promoting effects by way of shortening the pupal life span by about 10 days particularly with 10 µg dose leading to early moth emergence, increase in moth weight, ovarian weight and egg production in most of the cases possibly due to rapid stimulation of macro-molecular synthesis and cellular metabolism. Such induced growth effects are the clear indications of early diapause termination. The growth promoting activity of the peptides of insulin family in varying degrees has been reported in

Manduca sexta (Thorpe and Duve, 1988). Insect peptide hormones are involved in the control of a wide range of physiological, biochemical and developmental functions including water balance, lipid and carbohydrate metabolism, muscle contraction, reproduction, growth and development (Steele, 1985). In *Bombyx mori* homology of prothoracicotropic hormone (PTTH), an insect neuropeptide with vertebrate insulin has been established (Nagasawa *et.al.*, 1984, 1986; Ishizaki and Suzuki, 1988; Kawakami *et.al.*, 1990). Pupal diapause in insects occurs due to failure to release or supply of PTTH from the brain to pgls which in turn becomes inactive to produce ecdysone necessary for growth and metamorphosis in insect. Analysis of the titers of PTTH in the brains of diapausing *Antheraea* suggests that the arrest of development in this species probably occurs at the level of release since the levels of PTTH in short day brains remained high during diapause (Bollenbacher and Granger, 1985). So, there is reason to believe that the secretion of PTTH is inhibited during pupal diapause of *A.mylitta* and it is worthwhile to state that the exogenous insulin possibly induces an action mimicing that of PTTH in the diapausing insect leading to the termination of pupal diapause in this species. The profile of cholesterol, protein, DNA and RNA content in different tissues obtained after insulin application also afirms this relatively earlier termination of diapause by insulin. The growth stimulating effects of vertebrate insulin in *B.mori* (Magdum and Hooli, 1989) and in other insects (Kramer, 1985) and even in protozoans (Lawrence, 1991) have been established. It seems that in *A.mylitta* the exogenous insulin may either induces the inactivated brain-prothoracic gland complex or directly triggers the target tissues showing suppressed metabolic rate during pupal diapause in insect (Beck, 1980; Denlinger, 1985) resulting in diapause termination. It is of particular interest that insulin alone can cause increase in body weight, shortening of the adult emergence period by least ten days and increased number of egg production for which several endogenous insect hormone are required. The precise localization of these hormones is unknown although Thorpe and Duve (1984) and Tager *et.al.* (1976) suggested that these hromones might be located in median neurosecretary cells and in Lepidoptera in the corpora cardiaca/corpora allata complex. However, the mode of action of insulin in *A.mylitta* needs further elucidation whether it acts directly to the target organ or through neuroendocrine systems of the insect.

5.5 Effect of Exogenous 20-hydroxyecdysone on the Termination of Pupal diapause.

The initiation of adult development takes place in the diapausing pupae of *A.mylitta* around 150 days (Chap. 4.1 and 5.1). Biochemical and other evidences also provide clue that this initiation results from the resumption of PTTH release at slow pulses during this period, the action is translated through the synthesis and release of MH from the pgls.

The present results demonstrate that exogenous 20-HE plays a major role in the reproductive development in addition to its regulation of diapause physiology in the diapausing pupae of *A.mylitta.* The efficacy of exogenous ecdysteroids in terminating pupal diapause has been well documented in several lepidopteran species and also in other insect orders (Frankel and Hsiao, 1968; Baird, 1972; Ohtaki and Takahashi, 1972; Sieber and Benz, 1980). A range of developmental responses has been elicited in these studies in a dose-dependent manner. It has also been elaborately reported in the diapausing puape of the saturaiid *Samia cynthia* that a small dose of 20-HE is sufficient for retraction of wing epidermis but not for further development; intermediate doses help to develop a normal adult and in response to a very high dose the early phase of metamorphosis proceeds so rapidly that the sequence of events normally requiring 11 days is reduced to 4 days in this insect (Williams, 1968; Waldabauer *et al.,* 1978). A dose-dependent relationship as observed for almost all the parameters in the present investigation on *A.mylitta* corroborates an almost identical physiological mechanism occurring in other saturniids. Further, range of responded doses of exogenous 20-HE varies from species to species (Bodnaryk, 1975; Zdarek and Denlinger, 1975; Meola and Adkisson, 1977 ; Bradfield and Denlinger, 1980; Browning 1981). Excessive amount of ecdysteroid breaks diapause immediately but produce morphological derangements in some insects (Denlinger, 1985) which too corroborates the present findings of pupal and adult abnormalities.

Involvement of ecdysteroids in the control of reproduction appears to be a primary function of ecdysteroids whereas the control of moulting may be a secondary one (Hagedorn, 1989; 1990).This idea is also corroborative for the increased ovarian weight and protein concentrations using 2-10µg of 20-HE/ individual closely associated with greater fecundity. There is evidence that

exogenous ecdysone or 20-HE stimulates protein synthesis and increases oxygen consumption in insect tissues alongwith fat body growth (Behrens and Hoffmann, 1982). Furthermore, ecdysone - induces both *in vivo* and *in vitro* protein synthesis in several species of insects (Neufeld *et.al.*, 1968; Arking and Shaaya, 1969; Sahota and Mansingh, 1970 ; Thomson *et.al.*, 1971). It is suggestive that 20-HE not only induces protein synthesis in *A.mylitta* but also induces Oogenesis process during ovarian development as in the treated groups the ovarian maturation appeared to occur before 150 day of pupal development. It has been reported that growth and differentiation of new ovarian follicles and meiosis in the primary Ooocytes are induced by this hormone in some insects (Loeb *et.al.*, 1984 ; Hagedorn, 1985; Lafont, 1991). Reports are also available that ecdysone promotes the development of eggs with the capacity of developing to the imaginal stage and addition of ecdysone rapidly induced egg maturation and follicle formation (Hardie and Lees, 1985). Elevated protein content in the ovary on 150 day of diapausing pupae of *A.mylitta* treated with this hormone may be due to an induced effect of the exogenous hromone on vitellogenin synthesis by the fat body which in turn, has uptaken in the ovary during vitellogenesis. It is generally believed that ecdysteroid is essential for vitellogenin synthesis and evidences suggest that the ovarian ecdysteroids are thought to control vitellogenin production in the fat body (Goltzene *et al.*, 1978; Hoffmann *et al.*, 1980; Hagedorn, 1981). Increased rate of egg hatching in *A.mylitta apparently* reflects the involvement of 20-HE in embryogenesis. Such involvement may be only circumstantial (Hagedorn, 1985).

Many lepidopteran species discontinue spermatogenesis during larval and pupal diapause (Cloutier and Beck, 1963; Chippendale and Alexander, 1973). Though the detail state of spermatogenesis is unknown during the diapause of *A.mylitta*, the process may remain in a halt and is resumed after being induced by 20-HE. Such a resumption is indirectly evident from the weight, morphology and protein content of testes on 150 day of pupal development. The fact that exogenous ecdysteroids cause renewal of *in vitro* spermatogenesis in intact testes explanted from diapausing lepidoptera has been repeatedly confirmed (Yagi *et al.;* 1969; Friedlander, 1989). Further, 20-HE is necessary for maximum spermatocytic division and resumption of sperm development after diapause (Dumser, 1980) and also determining the developmental morphology of testis and genital tract of other lepidopterans (Nowock, 1972 ; 1973).

Activation of brain for releasing PTTH is a pre-requisite for initiation of adult development. It appears that the external signal input in brain regulates the secretion of PTTH, the key factors for controlling the pupal diapause. Among the signal input systems some aminergic neurones in the brain may play a key role for transmitting the "off" and "on" signals to PTTH secretory cells in the brain (Evans, 1980; Orchard, 1982; 1984). Recently, it has been established that biogenic amines are particularly implicated to the response to photoperiodic variations and consequently on the regulation of development especially in diapause induction and termination (Puiroux *et al.*, 1990; Fields and Woodring, 1991). Furthermore, the biogenic amines control energy metabolism in insects and acts as releasing factors of other brain hormones (Rauschenbach et.al., 1993).

Regarding the mode of action of exogenous 20-HE in terminating pupal diapause of *A.mylitta* it may be assumed that either the target tissues are directly stimulated by this hormone (Zdarek and Denlinger, 1975) or the hormone exerts a stimulatory effect on the pupal brain in an unknown way (Denlinger, 1985). There is also possibility that biogenic amines which are involved in the diapause regulatory mechanism might be stimulated by 20-HE. However, the mode of action of this exogenous hormone on the target tissues awaits further investigation and clarification.

6. SUMMARY

The dissertation embodies the following five aspects of diapause physiology of the tropical tasar silkworm, *Antheraea mylitta* Drury (Lepidoptera : Saturniidae) :

1. Phenology of different stages of life history of non-diapause and diapause generations,

2. Determination of critical weight of fifth instar larvae and timings of PTTH release during larval-pupal transformation in the two generations,

3. Profile of cholesterol, protein, DNA and RNA contents in some tissues (such as haemolymph, fat body and gonads) of pre-pupae, pupae and adults of both the generations for ascertaining the state of pupal-adult development in the diapausing pupae,

4. Effect of insulin on pre-pupae and pupae of diapause generation for the termination of pupal diapause, and

5. Effect of exogenous 20-hydroxyecdysone on pre-pupae for the termination of pupal diapause.

A considerable variation occurs in the total larval duration, spinning duration, pre-pupal life span and the pupal life span of the two generations. Pupal duration of non-diapause generation is about 20 days and that of the diapause generation is about 208 days. The daily temperature and daylength appear to be crucial for pushing the larvae for diapause orientation. A short daylength of <12 hours in combination with a low temperature of $<18°C$ trigger the larvae from third instar onwards to orient progressively for the induction of diapause after pupation. Because of utilization of stored energy as maintenance cost during the

prolonged pupal life, the grainage performance is quite poor in the diapause generation than in the non-diapause generation.

The larval critical weight (Lcw) is attained on the 9th day and on the 15th day of 5th stage larvae of non-diapause and diapause generations respectively. Lcw has been determined from the starvation experiment.

A three-step sequential release of PTTH occurs in both the generations during larval-pupal transformation. From the neck ligation experiment it is revealed that the first release takes place on the day of attainment of Lcw, the second one at about 5 hours after gut purge and the third one occurs 2-3 days before pupation. This phenomenon is consistent in both the generations.

Biochemical profiles have been assessed for the prepupae, pupae and adults of both the generations in order to have a comparative picture of mobility of building molecules in different tissues pertinent to development. Cholesterol and protein contents have been determined in the haemolymph, fat body and gonads while the DNA and RNA contents have been estimated in the fat body and gonads.

The pattern of biochemical profiles is quite similar in the two generations. The overall mobility of the biochemical contents among the tissues assessed reveals that adult development is initiated around 14th day of pupa in case of non-diapause generation and around 150 day of diapause generation.

The effect of insulin on the diapause physiology has been evaluated after application to the pre-pupae and 40 and 150-day old pupae of diapause generation. A 10-µg dose of insulin/individual when applied to the pre-pupae and 40-day pupae, reduces the pupal life span by at least 10 days. This implies that the function of insulin is analogous to that of the PTTH in the initiation of adult development. This conclusion is based on the life span and weights of pupae and adults, fecundity, hatchability, gonad weights and profile of the biochemical contents.

The 20-hydroxyecdysone when applied at the rate of 1,2,5 and 10 µg/ pre-pupa, induces adult development earlier. The pupal life span is reduced variably according to the doses applied. A dose of 5 µg/pre-pupa has given the best result considering all the parameters studied. The reduction of pupal life at this dose has been always by more than 10 days.

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