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).