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Changes in the Titer of Protein and Cholesterol Content in Non-Diapause, Artificially Diapause Terminated and Diapause Eggs of Silkworm, *Bombyx mori.*

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A differential specific pattern of variation in the metabolism of protein and cholesterol was noticed in non -diapause and diapause eggs due to the significant differences in embryonic development. The rate of metabolism was different due to specific demands of such metabolites during active embryogenesis and maintenance of diapause respectively. In general, the metabolic rate was found to be accelerated in non- diapause eggs just after egg deposition, while it was very slow in diapause eggs. When the diapause eggs were treated with hydrochloric acid within 16-20 hrs, the rate of turnover was found to very similar to non- diapause eggs, though the base level of protein and cholesterol was recorded to be different.

Key words: Diapause egg, Non-diapause egg, Protein and Cholesterol

Introduction

In sericulture contribution of silkworm seed is significantly high. Practically univoltine, and bivoltine silkworms produce superior cocoons in terms of both quantity and quality to those of polyvoltine. In a given strain, silkworms programmed to lay diapause eggs are superior to those lay non-diapause eggs (Yamashita and Hasegawa, 1985). But the resultant diapause eggs are not suitable for rearing throughout year in tropical condition. Diapause is characterized by certain specific conditions, such as depressed metabolism and internal environments differ from other developmental stages (Harvey, 1962). The silkworm, *Bombyx mori* is a typical insect, which enter egg diapause and exhibits diapause during the early embryonic stage. Though silkworm egg belongs to close or cleodic system, embryogenesis needs the best eco-physiological conditions to retain the vigour of newly hatched larvae (Yamashita and Hasegawa, 1985). There are variety of ways for studying the diapause in insects based on physical, physiological and biochemical parameters. Much more information on the physiology and biochemistry of diapause in *B. Mori* are available (Chino, 1958; 1961; Kai and Haga, 1978; Furasawa *et al.*, 1987; Sugimoto *et al.*, 1990; Yaginuma *et al.*, 1990; Moribe *et al.*, 2001). In this study the turnover of some basic physiologically important biomolecules, like protein and cholesterol in the non-diapause, artificially diapause terminated and diapause eggs of *Bombyx mori* was investigated.

Materials and methods

The eggs used in this study were from a bivoltine strain SK-4 and multivoltine strain , Nistari of silkworm *B.mori.* Eggs laid within 1 hour were pooled and used for experiment. Eggs with brown colour (fertilized) were selected and kept for the following treatments.

1. Eggs obtained from bivoltine strain SK-4 were kept at $25\pm1^{\circ}C$ with relative humidity of 70-80% for 90 days.
2. The diapause eggs were artificially terminated by hydrochlorination and then kept at $25\pm1^{\circ}C$ with relative

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humidity of 70-80% till hatching.
3. Non-diapause eggs obtained from Nistari were also maintained at 25±1°C with a RH of 70-80% till hatching.

Estimation of protein and Cholesterol
The diapause eggs with the age of 0 hr to 90 days old (aestivated at 25°C), non-diapause eggs and artificially diapause terminated eggs were sacrificed with different period of intervals to assay the protein and cholesterol. Protein was extracted following the method of Chaudhuri and Medda (1987) and Chaudhuri *et al.* (2001) specified for silkworm tissues. Protein content of egg was determined by the method of Lowry *et al.* (1951).The cholesterol content of egg was determined by the method of Kabara (1962).

Results

Protein turnover
Eggs of non-diapause (ND) breed (Nistari) of silkworm, contains significantly higher ($P<0.05$) protein (10.40%) at 0 hr of egg laying, compared to the eggs of diapause breed (SK4). The total protein titer in ND eggs was not much altered up to day 5 of embryonic development. Then, a sudden increase (16.75%) was recorded on day 6 followed by a gradual fall on day 7 till hatching (day 9). While, protein concentration in diapause eggs remain unaltered up to day 4, when a significant ($P<0.05$) rise (13.53%) was noted on day 5. Such increased level was maintained up to 30 day of diapause development followed by a drastic fall (43.93%) in protein titer on 40 day and gradually reached a minimum level (14.67±1.44 μg/egg) at the age of day 90 (Fig. 1).

It is interesting to note that in acid treated diapause eggs, the protein level suddenly up regulated on day 2, which was preceded by a fall at 24 hrs (day 1). However, such significant ($P<0.01$) elevated level of protein was maintained only up to day 3 followed by a gradual fall which continued till day 7 of embryonic development and then, remained unchanged up to the day of larval hatching (day 10).

Cholesterol turnover
The base level of cholesterol in ND eggs was recorded to be very high (74.38-18.98%) during the entire period of embryogenesis [0hr of egg laying to the day of hatching (9/10)], when compared to the artificially diapause terminated eggs (AT) and diapause eggs as well (Fig. 2). There is a specific pattern of cholesterol turnover in the eggs of ND and diapause breeds. A significant ($P<0.01$) and continuous fall in this biomolecule was recorded in ND eggs on day 7 onwards and reached a minimum level (4.89±0.12 μg/egg) on the day of hatching (9 day). While, a significant ($P<0.01$) and continuous fall in cholesterol in diapause eggs starts on 20 day of its embryonic age and continued till day 90 after oviposition to reach a minimum level of 1.65±0.29 μg/egg. However, diapause breeds exhibited a very slow rate of decrease in cholesterol metabolism in eggs with the advancement of embryonic age. Acid treated diapause eggs showed almost same pattern of cholesterol turnover during embryogenesis as found in ND eggs. Thus, slow rate of cholesterol metabolism (utilization) in diapause eggs suggests the active participation of this biomolecules in maintaining the egg diapause for a longer duration.

Discussion

Diapause, which is characterized by a number of morphological, behavioral, physiological and biochemical features, is a widespread form of dormancy among insects (Beck, 1980). In general, these insects are characterized by a considerably diminished metabolism, low oxygen consumption, preservation of substrates such as lipid and carbohydrate, resistance to desiccation and resistance against the gases like, carbon monoxide, cyanide etc., (Yamashita and Hasegawa, 1985).

Protein
The silkworm, *Bombyx mori* egg contains 20% of protein (Otsuki *et al*., 1997) and is in the form of highly polymerized basic molecular units known as amino acids and covers the major part of the dry weight. During diapause, the protein content increased from 0 hour of oviposition to 5th day. Afterwards, a steady and constant level was maintained. According to Saito *et al.* (1984a,b) in diapause eggs of *Bombyx mori,* the rate of protein synthesis is low, since little or no morphogenetic activity occurs during the dormant period. Grzelak *et al*. (1979) reported that polysomal poly (A) containing RNA from wintering eggs of *B.mori* was completely inert in a protein synthesizing system derived from wheat germ. This result seems to indicate that protein synthesis in egg is blocked at the translation of polysomal mRNA. In the Blowfly, *Lucila seicata*, during larval diapause the protein content was maintained at low and constant levels, reflecting the fact that diapause is a period, when synthetic and mitotic activity are minimal (Ring, 1973;Karnavar and Nair 1969). They concluded that fat and glycogen are the chief sources of energy during diapause and that protein may also be mobilized to underwrite the energy demands during prolonged diapause, when the stored fat and glycogen

are considerably depleted in the absence of food. Diapause associated proteins are synthesized around 2 days after oviposition in silkworm , *Bombyx mori* (Sonobe and Odake, 1986) and our findings corroborates such observations, where it was recorded that protein content increased up to day 5 of diapause.

It was also observed that decline of protein content in non-diapause eggs and artificially diapause terminated eggs during embryonic development was much earlier than diapause eggs. During embryogenesis intensive protein metabolism takes place, which involves mainly breakdown of pre-existing yolk reserves and the conversion of those into tissue and organ specific proteins. In mature eggs of most insects, vitelline is 80-90% of the total protein content and it is considered to be a storage protein, which is utilized during embryogenesis to build up a new organism (Kunkel and Nordin, 1985). In *B. mori*, in addition to vitelline, other proteins make up approximately 60% of total egg proteins and these are indeed degraded throughout embryogenesis (Zhu *et al*., 1986). Like *B. mori*, the aquatic bug, *Laccotrephes griseus* also showed the same trend during embryogenesis the yolk reserves remains essentially intact and only at a later stage, the protein reserve may be used by the embryos observed by decline in protein content after 6 days of post incubation (Premkumar, *et al*., 1991).

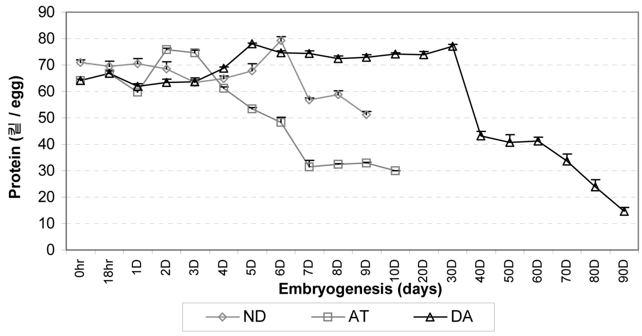

Fig. 1. Protein turnover during normal embryogenesis in non-diapause (ND), diapause terminated eggs by acid treatment (AT) and diapause (DA) eggs. The eggs were incubated at 25±1ºC. Each mean value is the average of 5 replications. Vertical bars represent the standard error of the mean.

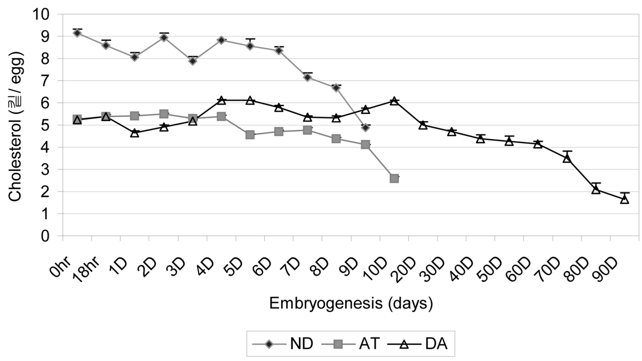

Fig. 2. Cholesterol turnover during normal embryogenesis in non-diapause (ND), diapause terminated eggs by acid treatment (AT) and diapause (DA) eggs. The eggs were incubated at 25±1ºC. Each mean value is the average of 5 replications. Vertical bars represent the standard error of the mean.

Cholesterol

Cholesterol, being a major zoosterol acts as a precursor of ecdysone and is also involved in a variety of functions, such as growth, development, moulting, oogenesis, egg production, hatching etc., (Lavinson and Bergmann, 1957; Monroe, 1959; Robbins and Shortino, 1962). In silkworm *Bombyx mori,* as in other insects, cholesterol is formed from conversion of b-sistosterol in dietary mulberry leaves (Ikekawa *et al*., 1966).

The present investigation showed that non-diapause eggs contain more cholesterol as compared to diapause eggs. This may be due to breed specific demands of cholesterol for embryogenesis in this insect, where non- diapause eggs exhibit an immediate need of such biomolecules for larval hatching. Monroe (1959) reported that feeding of cholesterol accelerates the larval hatching. Thus, in diapause eggs the negative regulation of cholesterol metabolism is indicative of induction and maintenance of diapause. The other cause of such difference in the base level of cholesterol may be due to differential ecdysone metabolism in non-diapause and diapause eggs. Sonobe *et al*. (1999) and Makka and Sonobe (2000) have also reported such differences.

Decline in concentration of cholesterol in diapause eggs after 20 days of aestivation indicates that the cholesterol may be utilized during diapause period. Since it is a source of energy other than glycogen during diapause (Karnavar and Nair 1969). It has been well established that the eggs of insects, including those of silkworm, *Bombyx mori*, contain various ecdysteroids and the amounts of these ecdysteroids fluctuate during embryonic development (Makka *et al*., 2002; Sonobe and Yamada, 2004), which again proves that the present findings on variations in cholesterol during diapause and active embryogenesis is also due to conversion into ecdysone / ecdysteroids.

The diapausing individuals are differ from non-diapausing individual in many aspects including synthesis and accumulation of "diapause associated proteins" (Chippendale, 1988). Thus the pattern of protein turnover in diapause eggs are different from Non-diapause and acid treated diapause eggs suggesting the differential demands of protein utilization to maintain a prolonged state of diapause by slowing the rate of metabolism, when the diapause eggs were kept at 25±1ºC.).

Similarly variations in cholesterol content between non-diapause and diapause eggs and during embryogenesis are supposed to be the corresponding reflections of the increased or decreased formation of cholesterol from sterols deposited in the maturing ovum by the mother moth and / or from the simple ingredient as acetate in egg and increased or decreased demands for tissue differentiation.

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References

Beck, S. D. (1980) Insect photoperiodism, 2nd edition. Academic Press, New York.

Chaudhuri, A. and A.K. Medda (1987) Thyroxine induced alterations in protein and nucleic acid contents of fat body of female silkworms during different developmental stages. *Insect. Sci. Appl*. **8**, 43-48.

Chaudhuri, A., N. Krishnan, G.C. Roy, A.K. Sengupta, S.K. Sen and B. Saratchndra (2001) Starvation induced changes of some biomolecules in eggs and hatched larvae of indigenous strain of *Bombyx mori* (Lepidoptera: Bombycidae). *Int. J. Indust.Entomol.* **2**, 107-110.

Chino, H. (1958) Carbohydrate metabolism in diapause eggs of the silkworm, *Bombyx mori* - II. Conversion of glycogen into sorbitol and glycerol during diapause. *J. Insect. Physiol.* **2**, 1-12.

Chino, H. (1961) Enzymatic pathways in the formation of sorbitol and glycerol in the diapausing eggs of silkworm, *Bombyxmori.*II. On the phosphatases. *J. Insect. Physiol.* **6**, 231-240.

Chippendale, G.M. (1988). Roles of proteins in insect diapause; in *Endocrinological frontiers in physiological insect ecology.* Sehnal, F., A.Zabza., D.L.Denlinger, (eds.), pp. 331-346. Wroclaw Technical university press, Wroclaw.

Furasawa, T. and S.L. Indrasith (1987) Quantitative and Qualitative changes of polypeptides of egg-specific protein and vitellin depending on the diapause state of *Bombyx* eggs exposure to low temperatures. *J. Seri. Sci. Jpn.* 56, 467-473.

Grzelak, K., E. Szczesna and Z. Lassota (1979) Polyadenylated RNA in diapausing and developing embryos of *Bombyx mori*. *Insect. Biochem.* **9**, 125-128.

Harvey, W. R. (1962) Metabolic aspects of insect diapause. *Ann. Rev. Ent.* **7**, 57-80.

Ikekawa, N., S.M. Saito, M. Kobayashi and K. Tsuda (1966) Studies on the sterol of *Bombyx mori* L. IV. Conversion of the sterol in the silkworm. *Chem. Pharm. Bull.* **14**, 834-836.

Kabara, J.J. (1962) Determination of and microscopic localization of cholesterol. *in Methods of biochemical analysis.* Glick, D (eds), pp. 263-318, Wiley-Inter Sciences, New York.

Kai, H. and Y. Haga (1978) Further studies on "Esterase A' in *Bombyx* eggs in relation to diapause development. *J. Seric. Sci. Jpn.* **47**, 125-133.

Karnavar, G.K. and K.S.S. Nair (1969) Changes in body weight, fat, glycogen and protein during diapause of *Trogoderma Granarium. J. Insect. Physiol.* **15**, 95-103.

Kunkel, J.G. and J.H. Nordin (1985) Yolk proteins. *in "Comprehensive insect physiology, bio-chemistry and pharmacology,* Kerkut, G.A. and L.I. Gilbert (eds.), 1. pp. 83-111. Pergamon press, New York.

Lavinson, Z.H. and E.D. Bergmann (1957) Steroid utilization and fatty acid synthesis by the larvae of the house fly, *Musca vicina* Macq. *Biochem.J.* **65**, 254-260.

Lowry, O.H., A.A. Rosebrough and R.J. Randall (1951) Protein measurement with Folin-phenol reagent. *J.Bio.Chem.* **193**, 265-275.

Makka, T. and H. Sonobe (2000) Ecdysone metabolism in diapause eggs and non-diapause eggs of the silkworm, *Bombyx mori. Zool Sci..* **17**, 89-95.

Makka, T., A.Seino, S. Tomita, H. Fujiwara and H. Sonobe (2002) A possible role of 20-hydroxyecdysone in embryonic development of the silkworm *Bombyx mori. Arch Insect Biochem Physiol.* **51**, 111-20

Monroe, R.E. (1959) Role of cholesterol in housefly reproduction. *Nature* **184**, 1513.

Moribe, Y., T. Niimi, O.Yamashita, and T. Yaginuma (2001) *Samui*. a noval cold- inducible gene, encoding a protein with a BAG- domine similar to silencer of death domines (SODD/BAG-4), isolated from *Bombyx* diapause eggs. *Eur. J. Biochem.* **268**, 3432-3442.

Otsuki, R., R. Sato, I. Nagai, N. Horigone and S. Yoshitake (1997) Silkworm egg production. Oxford, IBH & Publishing house, NewDelhi, Calcutta.

Premkumar, D.R.D., P.J. Esther, and S. Mathavan (1991)Biochemical changes during embryonic development in the aquatic hemipteran bug *Laccotrephes Griseus*. *Insect. Biochem.* **21**, 381-388.

Ring, R.A.(1973) Changes in dry weight, protein and nucleic acid content during diapause and normal development of the blowfly *Lucilla sericata*. *J. Insect. Physiol.* 19, 481-494.

Robbins, W.E. and T.J. Shortino (1962) Effect of cholesterol in the larval diet on ovarion development in the adult housefly. *Nature* **194**, 502-503.

Saito, A., Y. Sugimoto, K. Koga and B. Sakaguchi (1984a) Translation of RNA in eggs during post diapause development of *Bombyx mori*. *Dev. Growth. Diff.* **27**, 13.

Saito, A., K. Chikko, S. Yasushi, K. Katsumi and S. Bungo (1984b) Presence of traslatable mRNA in diapause eggs of *Bombyx mori*. *Insect. Biochem*. **14**, 625-629.

Sonobe, H.and Y. Odake (1986) Studies on the embryonic diapause of *Pnd* mutant of the silkworm, *Bombyx mori*. V. Identification of a Pnd's gene-specific protein. *Roux's. Archs. Dev. Biol.* **195**, 229-235.

Sonobe, H., H.Tokushige, T. Makka, N. Hara and Y. Fujimoto (1999) Comparative studies of ecdysteroid metabolism between diapause eggs and non-diapause eggs of the silkworm, *Bombyx mori*. *Zool Sci.* **16**, 935-943.

Sonobe, H. and R. Yamada (2004) Ecdysteroids during early embryonic development in silkworm *Bombyx mori*: metabolism and functions. *Zoolog Sci.* **21**, 503-16.

Sugimoto, Y., A. Saito, E. Kosegawa, K. Koga and H. Fujii (1990) Comparision of egg and embryo proteins and a trial to detect proteolytic activities in eggs of *Bombyx mori*. *Comp. Biochem. Physiol.* **96B**, 253-256.

Yaginuma, T., M. Kobayashi and O. Yamashita (1990) Distinct effects of different low temperature on the induction of NAD -sorbitol dehydrogenase activity in diapausen eggs of the silkworm, *Bombyx mori*. *J. Comp. Physiol.* **160B**, 277-285.

Yamashita, O. and K.Hasegawa (1985) Embryonic diapause. *in Comprehensive insect physiology, bio-chemistry and pharmacology,* Kerkut, G.A. and L.I. Gilbert (eds.), 1, Pergamon press, New York.

Zhu, J., L.S. Indrasith and O. Yamashita (1986) Characterization of vitteline egg specific protein and 30Kd protein from *Bombyx* eggs and their fates during oogenesis & embryogenesis. *Biochem. Biophy. Acta.* **882**, 427-436.