



FIG. 3. Exponential model:  $\ln(v^2\mu)$  vs.  $\ln(r)$  plot for II-IV- $V_2$  crystals.

$\bar{R}$  in force constants and the maximum percentage deviation in the lattice vibration frequency  $M$  from the experimental points. The latter quantity is within 4%, which is of the same order as that in sphalerite crystals. For I-III- $VI_2$  chalcopyrite crystals, the lack of accurate values of interatomic distances does not show good plots. However, least-square analyses of the data, for all these models, show a value of  $M$  as high as 8%. The model constants are presented. Using such constants the sphalerite-like

mode frequency and hence the infrared transmission cut-offs can be calculated. The approximate infrared transmission cut-offs are available for  $CdSiAs_2^8$  and  $AgInSe_2^9$ . These values ( $13\mu m$  and  $20\mu m$ ) can be compared with the predicted values using the model constants  $12.8\mu m$  and  $21.3\mu m$ . In this way the predicted values of infrared transmission cut-offs of  $CdSnAs_2$ ,  $ZnGeAs_2$  and  $ZnSnAs_2$  are 22.2, 17.8 and  $21.6\mu m$  and for  $AgAlS_2$ ,  $AgAlSe_2$  are 11.5 and  $13.7\mu m$  respectively.

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VARIATION IN THE MAJOR LIPID COMPONENTS, TOTAL, FREE AND ESTERIFIED STEROLS. GLYCFROL, GLYCOGEN AND LIPASE ACTIVITY IN THE FAT BODY OF THE DIAPAUSING PUPA OF *ANTHERAEA MYLITA* (LEPILOPTERA)

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ABSTRACT

Lipase, total lipids and their associated components, free fatty acids (FFA), phospholipids (PL), neutral lipids (NL), total cholesterol (TC), free and esterified cholesterol (FC and EC), glycerol and glycogen have been studied in the fat body of the diapausing pupa of *A. mylitta* and their role have been discussed.

INTRODUCTION

**A.** *MYLITA* is an insect of economic importance in tasar silk Industry. It has a feeding larval phase and a quiescent pupal period during which adult formation takes place. The energy required for the differentiation is supplied by oxidation of carbo-

hydrates and lipids which are stored in the fat body which is analogous to the mammalian liver.

Fat body is a major site for lipid metabolism. Lipids are stored in the fat body mostly in the form of triglycerides<sup>1</sup>. Glycerol has been considered to bring about a marked depression in the freezing point of

TABLE I

Variation in the major fat body lipid components total, free and esterified cholesterol in the diapausing pupa of *A. mylitta*.

Values are expressed in g/100 g of fat body (fresh wt.)

Age of pupa in days	Total lipid	Free fatty acid	Phospho- lipids	Neutral lipid	Free cholesterol	Esterified cholesterol	Total cholesterol
16	24.17	0.20	0.02	14.68	0.11	0.76	0.87
23	26.37	1.13	0.71	22.36	0.05	1.01	1.06
30	36.10	0.72	1.096	20.49	0.09	0.80	0.89
37	28.90	1.85	1.08	24.79	0.09	0.55	0.64
45	23.90	0.22	1.28	21.39	0.17	0.62	0.79
55	25.63	0.47	0.24	13.58	0.24	0.57	0.81
65	22.89	0.13	0.05	14.43	0.22	1.20	1.42
76	24.42	0.14	0.76	14.33	0.01	1.30	1.31
86	21.27	0.29	0.87	17.92	0.02	0.02	0.04
97	26.32	0.58	3.64	21.30	0.08	0.53	0.61
102	24.16	0.42	1.04	22.20	0.06	0.37	0.43

insect haemolymph during their winter diapause<sup>2</sup>. Cholesterol, having a basic cellular and sub-cellular structure and a precursor of moulting hormone, plays an important role in insect development, moulting, oogenesis and hatching<sup>3</sup>.

Therefore a detailed study on the changes occurring during lipolytic activity in *A. mylitta* fat body during pupal development was undertaken. In addition, glycerol and glycogen which have a direct bearing on lipid metabolism, were also investigated.

#### MATERIALS AND METHODS

Diapausing pupae of known date of pupation were procured from Jagdalpur Sericulture Institute (M.P.). They were kept in wire-netted cages at  $27^{\circ} \pm 2^{\circ}$  C. Humidity was controlled by placing water soaked pads of cotton in the cage.

Chilled pupae of known age were selected randomly from the colony and were dissected in Bodensteine Insect Ringer solution. The pooled pale yellow fat body was homogenized in chilled glass-distilled water to 10% (w/v) tissue concentration<sup>4</sup>.

Triglyceride lipase was determined according to Cherry and Crandall<sup>5</sup> with slight modification and the free fatty acids liberated were estimated by Novak's<sup>6</sup> method.

Lipids were fractionated by the method described by Pant *et al.*<sup>7</sup>. Total, free and esterified cholesterol were assayed according to Leffler's method<sup>8</sup>. Glycerol was estimated as described by Korn<sup>9</sup>. Glycogen was assayed by the method of Krishnan and Srivastava<sup>10</sup>. All assays were conducted in triplicates and repeated twice over, employing twelve insects each time.

#### RESULTS AND DISCUSSION

Total fat body lipids (Table I) gradually increase during early diapausing period and concentrate maximally on 30th day. Thereafter they maintain almost a steady level except on 97th day when they increase slightly. On 102th day prior to emergence, lipids get depleted. This indicated their accumulation and storage during early pupal days as a reserve energy source for utilization during vigorous tissue transformation and pupal-adult development at a later stage.

The initial low lipase activity in the developing pupa during days 23–37 days suggests lipogenesis and storage thereof in the fat body. These stored triglycerides are utilized during the development of the pupa in carrying out various metabolic activities. This is evinced by the increased lipase activity during days 55–86. The present observation confirms our previous findings<sup>11</sup>. The enhanced activity through days 86–97 once again depicts lipid utilization to provide energy for tissue formation, whereas the low activity through days 97–102 is suggestive of storage thereof, for post-emergence development and various other physiological processes associated with flight, mating and reproduction<sup>11</sup> which demand energy.

Neutral lipids constitute 70–90% of the total lipids. They vary similar to total lipids which accumulate to a maximum and minimum on 37th and 76th days respectively.

Neutral lipids accumulate during days 76–120 suggesting them to be the main energy source stored in the fat body for adult flight. These need to be transported to

the flight muscles before utilization. This is accomplished in the blood as lipoprotein complexes<sup>12</sup>, the lipid part being the diglycerides.

During early pupal period, free fatty acid titre increases (Table I). Through days 45–86, their marked depletion suggests their utilization as an energy fuel for tissue transformation. Their accumulation, prior to emergence during days 97–102 is indicative of their catabolism as evinced by the lipase activity (Table II) and the total lipid content (Table I) during the same period.

TABLE II

Variation in fat body lipase activity and glycerol content in the diapausing pupa of *Antheraea mylitta*

Age of pupa in days	*Lipase	†Glycerol	‡Glycogen
16	4.53	79.5	0.13
23	0.78	110	0.31
30	0.72	52.11	1.39
37	Nil	65.92	3.23
45	4.93	74.32	0.80
55	1.97	87.46	0.43
65	2.85	104.4	0.11
76	3.04	53.27	1.02
86	0.36	28.96	0.10
97	3.09	53.58	0.57
102	1.84	62.53	0.76

\*  $\mu$  mole palmitate liberated/mg protein.

† mg/100 gm fat body (fresh wt.).

‡ g/100 g fat body (fresh wt.).

Phospholipids (Table I) vary similar to free fatty acids. They increase during early pupal period upto day 45, decrease during days 55–65 and again increase near emergence during days 76–102. The rise and fall in both FFA and PL are suggestive of the role of phospholipids in transport of lipids at the time of transformation. The relatively high concentration of phospholipids particularly on the 97th day is in agreement with the earlier observations of Pant *et al.*, in *Philosamia ricini*<sup>7</sup>. This is supported by others<sup>13</sup> confirming that phospholipids play a dual role in insects as a medium for lipid transport and also as structural units in histogenesis during metamorphosis.

Total and esterified cholesterol (Table I) vary almost similarly. The initial low total cholesterol levels registers a high peak on 65th day.

Increase in cholesterol and its esters suggests their storage in the fat body for utilization during late pupal development as evinced by their depletion during days

76–86. Further decrease on day 102th day is suggestive of their utilization for hormonal as well as for structural purpose.

Free cholesterol maintains rather a low level all through diapause except during 37–76 days. During the tail end of diapause it keeps at a remarkably low level.

Esterified cholesterol, hydrolyzed by cholesterol esterase is transported to the body tissue *via* the haemolymph exclusively in the unesterified form<sup>14</sup> and this perhaps accounts for the high concentration observed.

Glycerol (Table II) has been observed to decrease in stages during diapause, the highest concentration being on 23rd day and the lowest on the 86th. Thereafter it exhibits an increase near emergence.

Glycogen in the diapausing egg is quantitatively converted to sorbitol and glycerol<sup>15</sup>. This is supported by the present observation where glycogen more or less follows the pattern of glycerol variation (Table II). Glycerol plays a direct role in "cryoprotection" of insects by depressing the haemolymph melting point and lowering the super-cooling point through a wide range<sup>2</sup>.

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