and uninjured heads revealed that it could attack injured heads only, *Heliothis* larvae and birds usually cause injury to heads and which, probably, serve as the avenue for pathogen's attack.

This research work was done under the All India Coordinated Research Project on Oilseeds sponsored by the I.C.A.R. Author is thankful to the Project Coordinator (Oilseeds) and Director (Research), Agriculture Experiment Station, University of Udaipur, for facilities.

Agriculture Research Station, J. M. AGRAWAT.
Karkhana Bagh, Kota, *May* 4, 1978.

1. Bekesi, P., Voros, J. and Calvert, C. H., *Plant Disease Reptr.*, 1970, **54**, 286.
2. Kolte, S. J. and Mukhopadhyay, A. N., *Pans*, 1973, **19**, 392.
3 Sackston, W. E., *Proc. Canad. Phytopath. Soc.*, 1959, **26**.
4. Schultz, H., *Phytopath. Z.*, 1950, **17**, 200.
5. Sideris, C. P., *Phytopathology*, 1929, **19**, 11407 (Abstr.).
6. —, *Mycologia* 1932, **24** (1), 14.

STUDIES ON TOTAL, FREE AND ESTERIFIED STEROLS IN THE HEAD PART OF *ANTHERAEA MYLITTA* (LEPIDOPTERA) DURING LARVAL DEVELOPMENT

Introduction

DURING the growth and development, insects periodically shed their exoskeleton and it is the moulting hormone *β*-ecdysone, the crucial regulator that initiates this process. The neurosecretory cells of brain have been known to control the moulting process *via* ecdysiotropic hormone which activates the prothoracic glands[1, 2] to secrete the moulting hormone which in its turn stimulates the moulting process.

Cholesterol is indispensable for insect growth and development and it also participates in the biosynthesis of moulting hormone and several other hormones[3, 4]. Ecdysiotropic hormone is known to be released from the insect brain into the haemolymph. Hence, with a view to investigating if cholesterol played any role in the biosynthesis of ecdysiotropic hormone, the following study on the cholesterol in the head part of *A. mylitta* at various stages of its larval development was undertaken.

Materials and Methods

Larvae of *Antheraea mylitta* were procured from Tasar Seed Supply and Research Centre, Ranchi (Bihar). Head part (1st segment) was cut off from chilled insects, weighed and homogenized in chloroform-methanol mixture (2 : 1, v/v) till complete extraction of lipids was effected. The chloroform layer was evaporated to dryness at 30 40° C *in vacuo* and dissolved in isopropanol (2·0 ml). This was employed for the estimation of total, free and esterified sterols by Leffler's method[5].

All assays were carried out in triplicates in three sets of experiments employing 10 insects in each and the average values were employed for calculation. Estimations were also made on three individual insects. For plotting of graph, the average value was taken. Variations are presented in Fig. 1.

Results and Discussion

Esterified cholesterol constituting about 74–85% of the total sterols is markedly utilized till the 2nd day of the ecdysed fourth instar larva (Fig. 1). A high peak of total and esterified sterols on the 3rd day, after every larval moult indicates the utilization of the accumulated dietary sterols for the hormonal activity required during the moulting process. The high level on the eve of spinning commencement followed by a decline during the process is noteworthy.

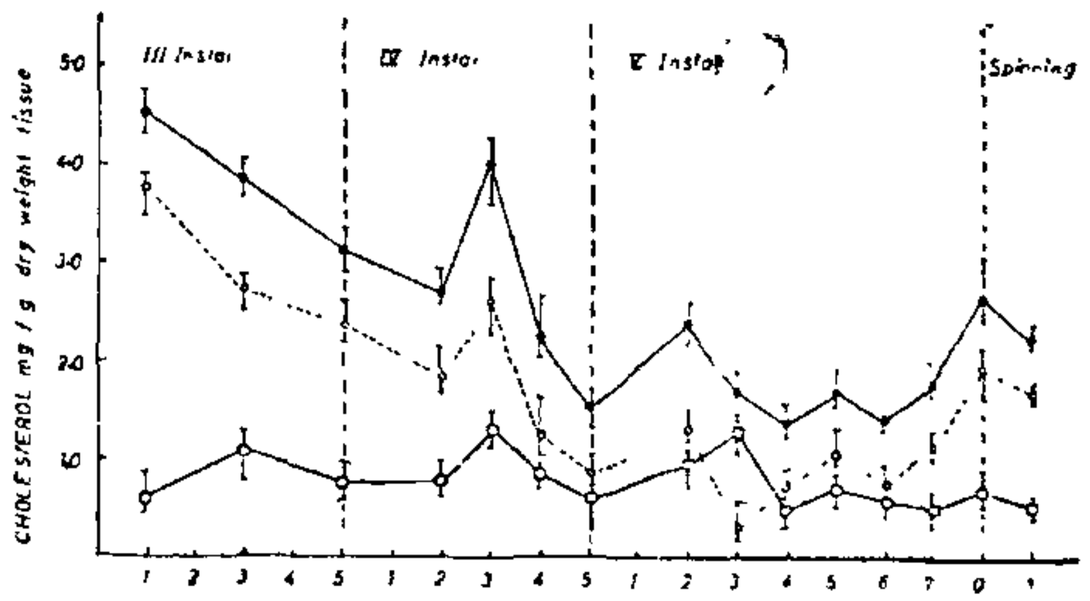

FIG. 1. Variation in Total, □ Free and ○ Esterified cholesterol in the head part of *Antheraea mylitta* during larval and spinning period.

Free cholesterol, although not exhibiting much marked variation, the pattern followed is more or less similar to that of total and esterified cholesterols, evincing its accumulation during feeding period and utilization during moulting.

There appears to be a controversy regarding the chemical nature of insect brain hormone. Kobayashi and Kirimura[6] extracted an oily substance from *B. mori* pupal brain which when injected induced adult development in "Dauer-pupae" produced by the removal of the brain immediately on pupation. On purification, this substance was identified as cholesterol[7]. Similar reports were also made by Williams for *Hyalophora*[8]. Thus, while these authors consider the insect brain hormone as a sterol, Ichikawa and Ishizaki[9] have forwarded evidence to its being a polypeptide. This material was also shown to possess several properties similar to those of a protein.

In the present investigation the significant depletion of cholesterol (both free and esterified) in the head part of *A. mylitta* at each and every moult, emphasizes

the possibility of the brain hormone(s) participating in the moulting process, to be of steroidal nature having cholesterol as the precursor.

This research has been financed, in part, by the United States Department of Agriculture, Agricultural Research Service, under PL 480.

Department of Biochemistry, RADHA PANT.
The University, Allahabad 211 002, SUMAN KUMAR.
May 23, 1978.

1. Gilbert, L. I. and King, D. S., In *The Physiology of Insecta*, (Ed. Rockstein, M.), Acad. Press, New York, 1973, **1**, 249.
2. Doane, W. W., In *Developmental System : Insects* (Eds. Counce, S. J. and Waddington, C. H.), Acad. Press, New York, 1973, **2**, 291.
3. Karlson, P., " Chemistry and Biochemistry of Insect Hormones ", *Angew. Chem.*, 1963, **2**, 175.
4. —, Hoffmeister, H., Hoppe, W. and Huber, R., *Ann. Chem.*, 1963, **662**, 1.
5. Leffler, Harrison, H., *Am. J. Clin. Path.*, 1963, **31**, Abstract 137.
6. Kobayashi, M. and Kirimura, J., *Nature (Lond.)*, *1958*, **181**, *1217.*
7. Kirimura, J., Saito, M. and Kobayashi, M., *Ibid.*, 1962, **195**, 729.
8. Williams, C. M., *Biol. Bull.*, Woods Hole, 1959, **116**, 323.
9. Ichikawa, M. and Ishizaki, H., *Nature (Lond.)*, 1961, **191**, 933.

GLUCOSE-6-PHOSPHATE DEHYDROGENASE ACTIVITY IN DENERVATED AMPHIBIAN GASTROCNEMIUS MUSCLE

PROGRESSIVE decrease of oxidative and glycolytic activity[1–4] and increased proteolytic activity[5,6] are prominent features of hereditary and nutritional muscular dystrophies and atrophy resulting from disuse, denervation, and tenotomy. Succinate[7], malate[3], lactate[9], glutamate[7], isocitrate dehydrogenases[10] of gastrocnemius muscle were found altered in their levels on denervation. Although the relationship between mitochondrial abnormality and the mechanism of muscle atrophy is not immediately evident, the mitochondrial dysfunction may contribute to a loss in muscle structure as a consequence of the decreased amount of energy available for muscular function[11]. Under these circumstances the energy demand for the increased sarcoplasmic or non-contractile protein synthesis may be met by the extramitochondrial oxidations mainly by hexose monophosphate shunt. It was reported that two distinct types of the enzymes having glucose-6-phosphate dehydrogenase activity exist in the tissues of various vertebrates, one located in cytosol relatively specific to NADP and the other located in microsomes which utilizes either NADP or NAD[12–15]. In view of this an attempt was made to study the activity levels of both NAD and NADP-linked glucose-6-phosphate dehydrogenase (G6PDH) on progressive denervation of amphibian gastrocnemius muscle which may provide some insight into the relative importance of co-enzyme specificity in atrophied muscle.

Rana hexadactyla (Lesson) of medium size were denervated by sciatic nerve section about 1 cm from its origin on one side of the leg, while contralateral muscle was considered as control. The frogs were fed *ad libitum* with cockroaches, and water was changed regularly. After 1, 2, 3 and 4 weeks post-operatively, animals were sacrificed and both the denervated and contralateral control gastrocnemius muscles were excised quickly. Homogenates of tissues (10%, wt/vol) were prepared in 0·25 M ice-cold sucrose using Potter-Elvehjem type glass homogenizer and cehtrifuged at 2500 rpm for 15 min. Supernatant (0·5 ml containing 50 mg tissue) was assayed for glucose-6-phosphate dehydrogenase activity by the method of G. D. Lohr and H. D. Waller[16] with the following modifications. The reaction mixture of 2 ml contained 20 μ moles of glucose-6-phosphate, 100 μ moles of trichthanol amine buffer (pH 7·4), 4 μ moles of INT (2-*p*-iodophenyl-3-nitrophenyl tetrazolium chloride), 10 μ moles of $MgCl_2$ and 0·2 μ moles of NAD for NAD linked glucose-6-phosphate dehydrogenase or 0·3 μ moles of NADP for NADP-linked glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of 0·5 ml of supernatant. Controls received 0·5 ml of sucrose in place of enzyme extract. After an incubation of 30 min at 37° C, the reaction was stopped by the addition of 5 ml of glacial acetic acid, and the formazan was extracted into 5 ml of toluene. After overnight stay in cold, the intensity of the colour was measured in UV-spectrophotometer (Hilger and Watts, England) at 495 nm using silica cuvettes of 10 mm path length. Individual zero time controls were maintained for all the samples by the addition of glacial acetic acid to reaction mixture prior to the addition of enzyme. Enzyme activity was expressed as μ moles of formazan/mg protein/hr. Protein levels were determined by the method of Lowry *et al.*[17]. Data were subjected to statistical processing according to standard procedures using students *t* test[18].

Progressive decrease of NAD-linked glucose-6-phosphate dehydrogenase activity level was found in the muscle subjected to denervation, the decrease being 9·81% after 4 weeks (Table I). Contrastingly the NADP-linked glucose-6-phosphate dehydrogenase showed progressive increase, *i.e.*, 26·16% after 4 weeks. Though the NAD-specific G6PDH has shown decreased activity level when compared to normal