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RESEARCH ARTICLE

ANALYSIS OF PROTEIN PROFILE USING PAGE IN CONTROL TISSUES OF HOUSE CRICKET *GRYLLODES SIGILLATUS* WALKER (GRYLLIDAE)

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ABSTRACT

The electrophoretic protein profile of various tissues namely fatbody, haemolymph and ovary were analysed with reference to interrelationship among the tissues of control female insects during their first reproductive cycle of House cricket Gryllodes sigillatus.The reproductive cycle of insects were divided into three phases based on the accumulation of vitellogenin namely previtellogenic, vitellogenic and postvitellogenic phases. During the first reproductive cycle of adult insects the two fractions viz.,2 and 8 could be detected only in control female insects. Of the two fractions, fractions 2 was recorded in all the tissues of the control insects, which indicates the appearance of an extra ovarian fraction that could be synthesized in fatbody, released into haemolymph and sequestered in the ovary. Fraction 8 was detected only in the ovary of control insects and was absent in the fatbody, and haemolymph indicating the intraovarian synthesis. Further, certain fraction were recorded in the fat body and haemolymph of control insects. Since, these fractions were not seen in ovary, they might be involved in the general metabolism of the insect.

Key words: *Gryllodes sigillatus,* Fat body, Haemolymph, Ovary, Vitellogenin.

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INTRODUCTION

The insect *Gryllodes sigillatus,* a house hold pest living under the stones and in kitchen, was selected for testing the interference of pesticide in the metabolism in general and the reproduction in particular. The insect under study was subjected for 8 days during its first reproductive cycle i.e., from the day of adult emergence to the day of egg laying. Further, the protein was taken as the biological constituent towards the maturation of oocytes during different stages of its reproductive cycle, *viz.,* previtellogenic, post-previtellogenic, vitellogenic and post vitellogenic stages. Therefore, the protein profile as well as the protein concentration of different tissues namely fat body, haemolymph and ovary regarding the synthesis, release and sequestration of proteins respectively were studied in control insects. . Protein, an essential compound of all cells and many special secretions, is required for adult maintenance and to supply energy nutrients for provisioning the eggs and egg production in insects (Dadd and Kleinjan, 1979). A lot of information is available on the electrophoretic studies of proteins present in the haemolymph, ovary and fat body etc., of various insects (Gliuski and Jarosz, 1985; Yadav and Kumar, 1986).The purification and properties of proteins that accumulate in the fat body of *D. grandiosella* has been studied by Dillwith and Chippendale (1984).

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The various haemolymph proteins are identified and studied during different stages in various insects by William (1970), Chrysanthis *et al.* (1981) and Sabita *et al.* (1986). The protein pattern of haemolymph and various tissues during last larval and pupal stages of *B.mori* were investigated by Seo Eul Won *et al.* (1985).

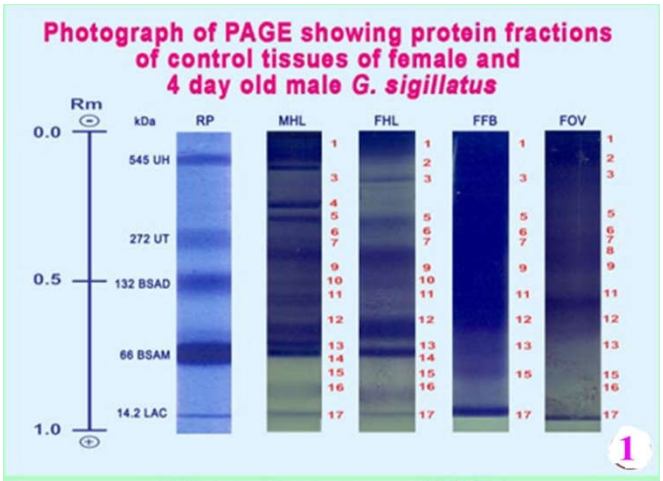
MARERIALS AND METHODS

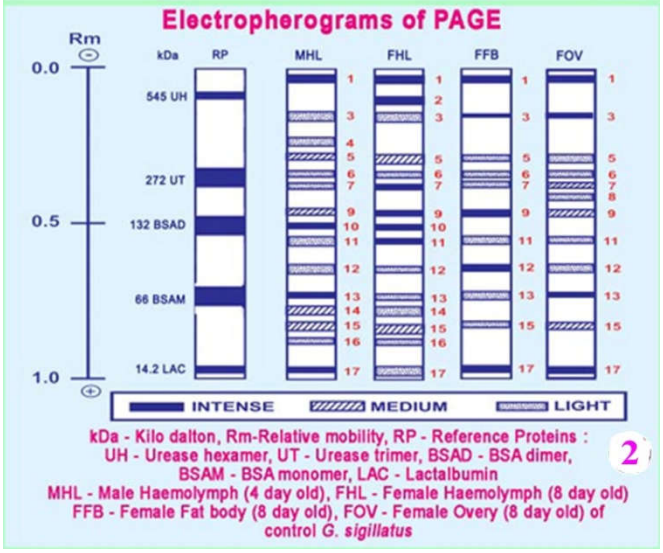
Adult males and females were collected from the storehouses and maintained in the laboratory in glass containers. They were fed *ad libitum* with moist dog biscuits. In *G. sigillatus,* the first instar nymphs hatched out from the eggs on the day 13 or 14 after incubation. The nymphs were also reared with moist dog biscuits. The wing buds appeared in the nymphs, destined to develop into males, around day 40 after hatching and the ovipositor in the female nymph around day 50 after hatching. The gross anatomy of the female reproductive system was studied from the dissections of the system made in Insect Ringer solution (Ephrussi and Beadle, 1936).The entire fat body obtained from the abdominal regions of the insect was pooled in a cavity block and washed thoroughly with Insect Ringer solution. Prior to anaesthetizing insects, the haemolymph was collected with the help of graduated capillary tubes, by cutting the prothoracic leg at the coxal joint. The non-dissociating system of electrophoresis was done by adopting the method of Hames and Rickwood (1981). Protein extracted from fat body, haemolymph and ovary of previtellogenic,

vitellogenic and post-vitellogenic phases of control female insects and the haemolymph of 4 day old male insects were analysed.

Characterization of proteins

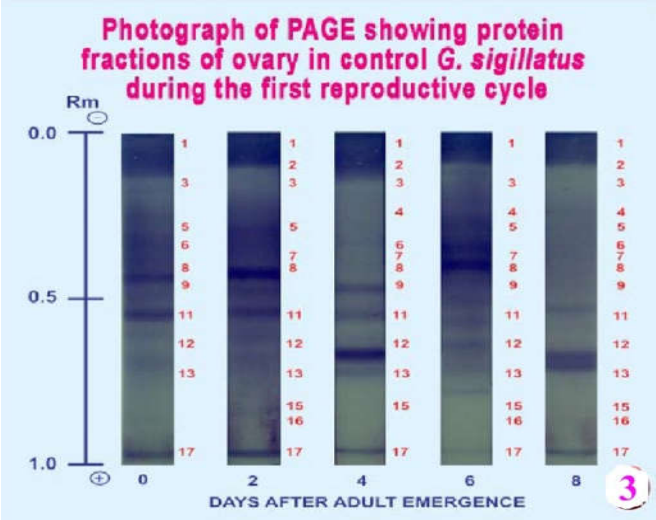
Fat body or ovary extract or 10 µl of female or male haemolymph was mix individually with an equal volume of 40% sucrose (w/v) solution prepared in 0.0625 M Tris-HCl buffer (pH 6.8), containing 0.002% bromophenol blue (w/v) The gel tubes were fixed to the upper buffer reservior and the samples along with equal volume of 40% sucrose (w/v) and 0.025% bromophenol blue (w/v) as the tracking dye were loaded on to the gel tubes. The lower and upper reservoirs of the electrophoretic apparatus were each filled with 250 ml of reservior buffer. The apparatus was connected to a constant power supply of 3 mA per gel tube. The run lasted 75 to 90 min at 15o C. As the tracking dye reached the bottom of the gel tubes, the tubes were removed and the gels were separated and stained. The gels were stained in the staining solution for 60 min in dark and destained for several hours by washing them extensively in a solution of methanol, GDW and acetic acid in a ratio of 5:5:1 (v/v). The gels were stored in 7% acetic acid (v/v) and photographed using reflected light.

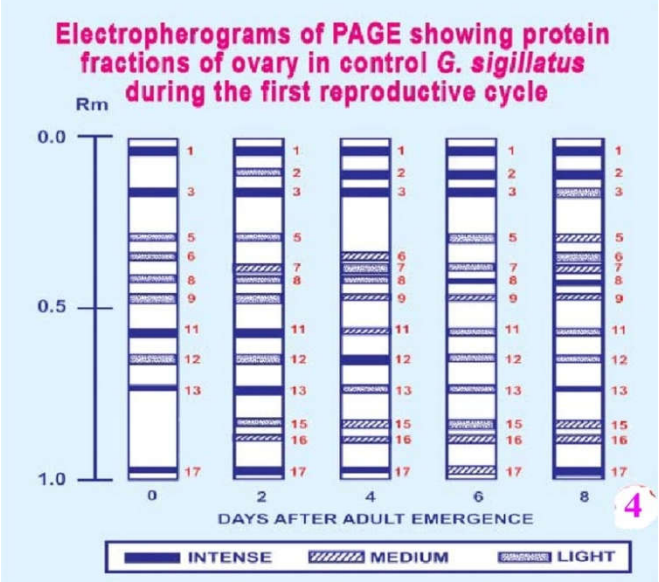




RESULTS

The electrophoretic protein profile of the various tissues namely, fat body, haemolymph and ovary were analysed with reference to the interrelationship among the tissues of control female insects during their first reproductive cycle. During the frist reproductive cycle of adult insects the two fractions *viz.,* 2 and 8 could be detected. Of the two fractions, fraction 2 was recorded in all the tissues of the control insects, which indicates the appearance of an extraovarian fraction that could be synthesized in the fat body, released into the haemolymph and sequestered in the ovary. Fraction 8 was detected only in the ovary of control insect sand was absent in the fat body and haemolymph, indicating the intraovarian synthesis (fig 1- 2 and 3-4). Further, the fractions 10 and 14 were recorded in the fat body and haemolymph of all the adult insects. Since, these fractions were not seen in ovary, they might be involved in the general metabolism of the insect. Fraction 16 could be detected in all the tissues indicating the synthesis, release and sequestration. Of the remaining bands, fractions, 1, 3 and 17 were present throughout the period of study in all the tissues and at different phases. The fractions 5, 6, 7, 9, 11, 12 and 15were utilized as and when they were synthesized (fig 1- 2 and 3-4).





DISCUSSION

A detailed analysis of the fat body, haemolymph and ovary of control *G. sigillatus* revealed the presence of a number of protein fractions in adult. The number of prootein fractionsin various tissues showed greater variations at different stages of development. The electrophoretic studies of female fat body,

haemolymph and ovary showed the presence of certain fractions common to them.The fraction number 2 in the fat body, haemolymph and ovarfy are found only in the female insects. Hence, this fraction is suggested to be the FSP.A similar result has already been presented in the case of *Nauphoeta cinerea* (Imboden *et al.,* 1987). The female specific proteins or vitellogenins are yolk protein precursors synthesized by the fat body of mature females (Wyatt and Pan, 1978) and secreted into the haemolymph. The vitellogenins are selectively taken up by the developing oocyte and deposited as the major yolk proteins, vitellins (Wyatt and Pan, 1978; Engelmann, 1979; Hagedorn and Kunkel, 1979; Veera Raghavan, 1983; Imboden *et al.,* 1987). Based on the above information, it is suggested that femal adult control *G. sigillatus* has a single native protein fraction 2 in the haemolymph which is under the control of JH produced by corpus allatum. Similarly, fraction 2 is also observed in the fat body extracts of adult control *G. sigillatus.* This appeared from the previtellogenic phase of the reproductive cycle. Hence, it is suggested that the fraction 2 which is synthesized by the fat body is secreted into the haemolymph and sequestered by the ovary through the follocular epithelium. So, this fraction would be an extraovarian protein, identified as the FSP, that could be synthesized by the fat body only during the reproductive cycle of female adult insects.

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