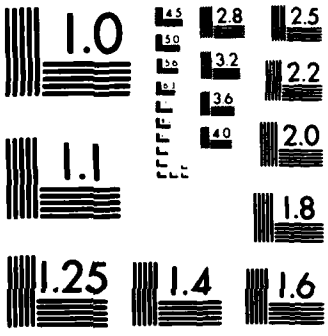
AD-A151 608 HORMONAL CONTROL OF SYSTEMS ASSOCIATED WITH EGG DEVELOPMENT IN PHYCITID MOTHS(U) OREGON STATE UNIV CORVALLIS DEPT OF ENTOMOLOGY V J BROOKES 12 DEC 84 1/1

UNCLASSIFIED ARO-17670.2-LS DAAG29-81-K-0124 F/G 6/3 NL



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS 1963-A

UNCLA
SECURITY CLASS

AD-A151 608

(2)

	READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER: ARO 17670.2-LS	2. GOVT ACCESSION NO.: N/A — 3. RECIPIENT'S CATALOG NUMBER: N/A
4. TITLE (and Subtitle): HORMONAL CONTROL OF SYSTEMS ASSOCIATED WITH EGG DEVELOPMENT IN PHYCITID MOTHS	5. TYPE OF REPORT & PERIOD COVERED: FINAL 7/1/81-9/30/84
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): VICTOR JACK BROOKS	8. CONTRACT OR GRANT NUMBER(s): DAAG 81-K-0124
9. PERFORMING ORGANIZATION NAME AND ADDRESS: DEPARTMENT OF ENTOMOLOGY, OREGON STATE UNIVERSITY, CORVALLIS OREGON 97331	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS
11. CONTROLLING OFFICE NAME AND ADDRESS: U. S. Army Research Office, Post Office Box 12211, Research Triangle Park, NC 27709	12. REPORT DATE: 12/12/84
	13. NUMBER OF PAGES: 5
14. MONITORING AGENCY NAME & ADDRESS (if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited.

DTIC ELECTE MAR 20 1985 S D

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

NA

18. SUPPLEMENTARY NOTES

The view, opinions, and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Plodia interpunctella, oocyte development, yolk proteins, ecdysteroids, tissue culture

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

See Reverse Side of this Form

DTIC FILE COPY

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

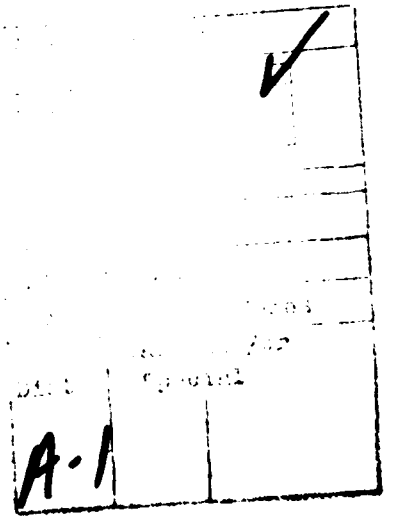
UNCLASSIFIED

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

85 03 07 214

20.

Several proteins associated with oocyte development in Plodia interpunctella were isolated and characterized. Antibodies to these proteins were prepared. Conditions were established for the long term culture of fat body and ovaries, which produce these proteins. The concentration of ecdysteroids, the major hormones associated with metamorphosis and the initiation of ovarian development, was measured by radioimmune assay and HPLC. Ecdysteroids reach a major peak at the beginning of adult development when the oocytes begin to mature. The concentration then declines gradually as development continues to eclosion. The decline may be a means for controlling sequential development of adult structures because elevating hormone titre by injection delays development. Specific effects of elevated titre are complex. Protein synthesis in ovaries is substantially reduced and oocytes do not sequester yolk precursors from the hemolymph. The fat body synthesizes protein at what appears to be a normal rate but elevated titres slow the utilization of what has be identified tentatively as storage protein. Fat body and ovaries can be maintained in culture for periods equal to the time of metamorphosis. Such preparations should be valuable for studies of hormonal controls of developmental processes



ARO 17670.2-LS

HORMONAL CONTROL OF SYSTEMS ASSOCIATED WITH EGG DEVELOPMENT IN PHYCITID MOTHS

FINAL REPORT

VICTOR JACK BROOKES

DECEMBER 12, 1984

U. S. ARMY RESEARCH OFFICE

DAAG 29-81-K-0124

OREGON STATE UNIVERSITY

The view, opinions, and/or findings contained in this report are those of the author and should not be construed as an official Department of the Army position, policy, or decision, unless so designated by other documentation.

OBJECTIVES

The principal long term objective of the research is to understand fundamental processes associated with egg development in insects that damage stored products. Of special interest are the hormonal mechanisms associated with proteins vital to the development of oocytes. An understanding of the mechanisms of hormonal action may lead to the development of hormone-based insecticides, and their use in pest management strategies.

Several species of the lepidopterous family, Pyralidae, are serious pests of stored products. Of these we selected the Indian meal moth, Plodia interpunctella ,to work on. The larvae feed on a large variety of stored products, and Plodia may be the most serious pest of this group of isects.

The immediate objectives of the research conducted over the past three years was to identify and isolate proteins associated with oocyte development and to establish culture conditions for tissues or organs synthesizing these proteins. Among insects in general, vitellogenin (Vg) is the principal protein synthesized during oocyte development. It is synthesized in the fat body and incorporated into the oocyte as yolk. Both juvenile hormone and ecdysone are implicated in devlopment and among lepidopterous insects such as those infesting stored products, ecdysone is believed to be the hormone involved in vitellogenin synthesis. In the course of our work we discovered several other proteins that formed a major part of the yolk, but are synthesized in the oocyte or in the follicle cells. We also discovered that although ecdysone is important in initiating development of the oocytes the normal development depends upon a declining titre of the hormone, and if titres are maintained above a certain level, the development is retarded. Because both of these findings were relevant to our long term objectives we devoted a substantial amount of time to them.

RESULTS

I. Development

Larvae . Larvae hatch from the egg four days after the egg is laid. Larval development proceeds through 5 instars and takes about 14 days under the conditions we impose. The completion of larval development is signalled by the beginning of the 'wandering' stage at which time the larvae cease feeding and leave the culture medium. If a pupation site is provided (we use corrugated cardboard), the wandering period is brief, a few hours, but in the absence of a site the larvae may wander for several days before becoming immobile. The larvae pupate 48 hours after entering the pupation site. About 74 hours after pupation, adult development begins (pharate adult stage)

Pharate adult . Adult development takes about 8 days and can be divided into 8 or more clearly recognized stages based on morphological changes that occur. Vitellogenin appears in the hemolymph about 24 hours after the beginning of adult development and indicates the initiation of the developmen of the oocytes. Eclosion follows a circadian pattern begining on noon of the 8th day and with females emerging about 24 hours ahead of the males. Egg laying begins 24 to 48 hours after emergence and continues for about 12 days.

II. Yolk polypeptides

We have isolated from freshly laid eggs several proteins that are directly associated with oocyte development. One of these is vitellin (Vt, M_r=462,000), which is produced by the fat body as vitellogenin, and made up of multiples of two polypeptides, YP1 and YP3, (M_r=153,000 and 43,000). We have found no difference between Vg and Vt, and assume that Vg from the fat body is deposited in the oocyte as Vt without changes that occur in some other insects. Another protein is one synthesized in the oocyte or in the surrounding follicle cells,(M_r= 264,000) which consists of two polypeptides, YP2 and YP4, (M_r=69,000 and 33,000). The characterization of these proteins is described in the reprint which has been submitted.

Although YP2 and YP4 purify as a single protein we have been able to show that they originate from two separate mRNAs indicating the existence of two genes. We also have evidence that YP1 and YP3 are also discrete proteins.

During embryogenesis the oocyte protein disappears more rapidly than vitellin, and is completely gone when the larva emerges from the egg. Substantial amounts of vitellin are still present at this time.

III. Storage proteins

Patterns of proteins described by electrophoresis on polyacrylamide gels show that larval fat body contains substantial amounts of one or several proteins (M_r=80,000) just before metamorphosis. This protein(s) carries over to the pupal stage and slowly disappears during adult development. The amount, molecular weight, and other characteristics suggest that this is storage protein. Such protein has been identified in a number of insects, and is synthesized by the fat body early in the last instar, secreted into the hemolymph, and then recovered just before the beginning of metamorphosis.

IV. Ecdysteroids

A. Titre

The titre of ecdysteroids was measured by radioimmune assay (RIA,) and by high pressure liquid chromatography (HPLC). Measurements were made at about 12 hour intervals from the end of the wandering stage, at which time the hormone is barely detectable, until the emergence of the adult. The titre begins to rise shortly after the larvae enter the pupation site, and reaches a peak in 24 hours or about 24 hours before pupation. The titre then declines to about one half the peak value and remains at this level until 24 hours after pupation. A second, much higher peak is reached 48 hours after pupation. The titre declines slowly during adult development. At the first peak the tire is about 15 ng 20-OH ecdysone equivalents/individual and about 250 ng/individual at the second peak. During the first peak the principal component is 20-OH ecdysone but the second peak is a mixture of ecdysone, 20-OH ecdysone, and other components.

B. 20-OH ecdysone and adult development

In an early series of experiments we tried to determine the critical period for the release of brain hormone and of 20-OH ecdysone. This was done by ligation to isolate the abdomen from the endocrine centers and injecting hormone into the abdomen. In these experiments we discovered that 20-OH ecdysone could delay development if presented after the critical period for the release of the second hormone peak. In another series of experiments we tried to clarify this observation. Pupae were injected at the beginning of adult development with one of three amounts of 20-OH ecdysone, 10, 50, or 250 ng, with saline, or were not treated. The experiments were terminated when the first adults appeared in untreated controls. The remaining experimental pupae were scored on the basis of extent of development of several structures, but most importantly, of the oocytes. In some experiments labeled methionine was injected three hours before termination and the extent of incorporation into proteins of the hemolymph, fat body, and oocytes was measured. Qualitative differences among labeled proteins were analyzed by SDS-PAGE.

The most obvious effect of injection of hormone was the delay in development which could be scored on the basis of time to eclosion. Even small amounts delayed eclosion for several days, and with large amounts delays were prolonged. The effects on the oocytes were measured in terms of number of mature oocytes per ovariole, and the number of vitellogenic oocytes per ovariole. Even at the lowest dose the number of maturing and matured oocytes was reduced compared to controls but at the highest dose no more than 5 vitellogenic oocytes were found in ovarioles compared with a range of 16 to 40 in the controls.

The effects of elevated hormone on protein synthesis, measured as the incorporation of labeled methionine was complex. In ovaries, protein synthesis in treated insects was only 20-30% of controls with all levels of hormone. The pattern of labeled proteins as displayed by SDS-PAGE was similar in ovaries from treated insects as well as from controls except that vitellin (YP1 & 3) was not present. Hemolymph from treated insects contained less labeled protein than controls although the amount of protein was not significantly different. The pattern of proteins was the same and both YP1 and YP3 were present. The amount of protein was much higher in treated insects even though the rate of synthesis of protein was the same as controls.

Our interpretation of these results is that an elevated titre of hormone retards development of the oocytes, possibly by a general inhibition of protein synthesis, which also prevents recovery of proteins from the hemolymph. The accumulation of protein in the fat body suggests that storage protein is not being utilized in treated insects.

V. Tissue Culture

We have been able to maintain fat body in culture media for five days. Longer periods may also be possible but this was not tried. We used Grace's insect tissue culture medium (without hormones) and incubated in an atmosphere of pure oxygen. We have cultured fat body from wandering stage larvae and from 7 stages between pupation and adult emergence and measured the incorporation of labeled methionine into protein secreted into the medium at the beginning of culture and 5 days later. After 5 days, tissue proteins were also measured. The patterns of

polypeptides synthesized were analyzed by SDS-PAGE. Many polypeptides were synthesized but we could identify YP1 and YP3, and what we believe to be the polypeptides of storage protein and a lipid carrying protein called lipophorin. In general, the rate of incorporation diminishes somewhat during the 5 day incubation period, but there were no obvious changes in the pattern of polypeptides synthesized by fat body of all stages except larvae. During the 5 day incubation of larval fat body the rate of synthesis of some polypeptides increased while others diminished.

Participating Scientific Personnel

Victor J. Brookes Ph.D.	Project Leader
Paul D. Shirk Ph.D.	Assistant Professor
Daniel Bean Ph.D.	Research Associate
Audrey M. Milleman M.S.	Technical Assistant
M. McSwain	Student Assistant
G. Stormberg	"
N. Olsen	"
M. Brookes	"

Papers Published

Identification, synthesis, and characterization of the yolk polypeptides of Plodia interpunctella. P. D. Shirk, D. Bean A. M. Milleman, V. J. Brookes, J. Exp. Zool., 232, 87-98 (1984)

Ovarioles contain mRNA for yolk polypeptides in Plodia interpunctella. P. D. Shirk, A. M. Milleman, V. J. Brookes, J. Exp. Zool., in press

END

FILMED

4-85

DTIC