A study on the postembryonic ovarian development and vitellogenesis of Aulocara elliotti (Thomas) (Orthoptera, Acrididae)
by Roger Allen Leopold

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Entomology
Montana State University
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Abstract:
The morphogenesis and histogenesis of ovarian tissue of Aulocara elliotti (Thomas) were examined throughout the nymphal and prereproductive stages® Differentiation; of the rudimentary ovariole into two functional zones, the germanium and vitellarium, occurs during the second instar. In those oocytes which are to he oviposited first, there is a considerable amount of cytoplasmic augmentation while they reside in the nymphal vitellarium. During the fifth instar a cuboidal epithelium differentiates from prefollieular tissue surrounding oocytes at the proximal end of the ovarioles. In the adult, two layers of muscle tissue are formed between the outer connective tissue sheath and the epithelial lining of the oviducts before the first clutch of eggs are to be laid. Between 8 and 10 days are required after adult emergence to complete the first reproductive cycle. After vitellogenesis, follicle cells around the posterior end of each proximal oocyte enlarge considerably before secreting a specialized chorionic cap overlaying the site of the future hydropyle. Presence of residual pigmentation is one of the gross differentiating factors between a resorbing oocyte and a normal degenerating follicle following ovulation, A cytological and cytochemical study of adult ovarioles was made with special emphasis on the process of vitellogenesis. During oocyte maturation the presence of Feulgen-positive material within oocyte nuclei diminishes, A heavy concentration of RHA is present in the portion of cytoplasm of the follicle cells closest to oocytes experiencing yolk deposition or chorion formation, A major portion of the yolk material deposited within oocytes appears to be a complex of protein and carbohydrate substances. In addition to the protein-carbohydrate fraction, there are significant amounts of acid and neutral mucopolysaccharides within the yolk granules. Although the deutoplasm of the oocytes is highly PAS-positive, the cytoplasm of surrounding follicle cells is PAS-negative.

It could not be determined whether rate of vitellogenesis or oocyte resorption was a function of the amount of light received per day when 127 females were examined seven days After emergence. There does appear to be trend for ovipositing females to lay fewer eggs upon exposure to shorter daylengths. Oviposition was not observed when females were maintained under constant light or three hours of light per day.

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A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Entomology

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Bozeman, Montana

March, 1967

ACKNOWLEDGMENT

I wish to extend my gratitude to my major professor, Dr. James H. Pepper, for the suggestions and encouragement given to me during the course of this investigation and thesis preparation. I would like to offer special acknowledgments to Drs. George Roemhild and Saralee Van Horn for their contributions to the development of this study and their constructive critism of this manuscript. To Drs. Robert Moore and Kenneth Goering I am indebted for the time and effort spent in the critical reading of this manuscript. I wish also to thank Professor Ellsworth Hastings for his assistance in procuring the experimental material and Mr. D. H. Fritts for preparing the photomicrographs. I am grateful to Mr. John Henry and to the USDA Entomology Research Laboratory for allowing me to use their freezing microtome.

This investigation was supported by the U. S. Department of Health, Education and Welfare through a Title IV, NDEA Fellowship and by the Montana State University Agricultural Experiment Station.

TABLE OF CONTENTS

	Page
VITA	ii
ACKNOWLEDGMENT	iii
LIST OF TABLES	v
LIST OF FIGURES	vi
ABSTRACT	ix
INTRODUCTION	1
MATERIALS AND METHODS	8
General Morphology	8
Cytology and Cytochemistry	10
Photoperiodism	15
RESULTS	21
Morphological and Histological Observations	21
Ovarian development	22
Cytological and Cytochemical Observations	46
Nucleic acids	46
Protein	47
Lipids	48
Carbohydrates	52
Vital staining	57
Photoperiodic Study	57
DISCUSSION AND CONCLUSIONS	64
Ovarian Development	64
Cytology and Cytochemistry	68
Photoperiodism	79
APPENDIX	84
LITERATURE CITED	89

LIST OF TABLES

Table		Page
I.	Effects on various pretreatments on PAS-reactive polysaccharides in ovarioles of *A. elliotti*.....	56
II.	An analysis of variance concerning the effect of varied daylengths upon rate of vitellogenesis.....	62
III.	An analysis of variance concerning the effect of varied daylengths upon oocyte resorption.....	62
IV.	Raw data concerning the mean proximal oocyte length measured in millimeters, total number of resorbed oocytes per female, and total number of ovarioles for each female obtained from the photoperiod study.....	85

LIST OF FIGURES

		Page
Fig. 1.	Schematic drawing of an apparatus used to rear *A. elliotti* under different photoperiods.....	17
Fig. 2.	Ovaries dissected from a gravid female.....	24
Fig. 3.	An ovariole dissected from a gravid female.....	24
Fig. 4.	A longitudinal section of an ovary from a newly hatched female.....	24
Fig. 5.	A longitudinal section of an ovariole from a newly hatched female.....	24
Fig. 6.	The terminal filament of a mid-first instar ovariole...	28
Fig. 7.	A first instar ovariole showing the connection to the ventral strand.....	28
Fig. 8.	The ensheathing membranes of a first instar ovariole...	28
Fig. 9.	The terminal filament of a first instar ovariole showing the extension of the outer ovariole membrane...	28
Fig. 10.	A longitudinal section of a mid-first instar oviduct in the gonial region.....	28
Fig. 11.	A longitudinal section of a mid-first instar oviduct posterior to the gonial region.....	28
Fig. 12.	A sagittal section of two germaria from a mid-second instar female.....	28
Fig. 13.	The rudimentary vitellarium of a mid-second instar female.....	28
Fig. 14.	A proximal oocyte of a late second instar ovariole.....	33
Fig. 15.	A nucleus of a fifth instar proximal oocyte.....	33
Fig. 16.	A transverse section of second instar ovaries.....	33
Fig. 17.	A section of a second instar oviducal wall.....	33

Fig. 18. A longitudinal section of a second instar oviduct and pedicel..... 33

Fig. 19. A longitudinal section of a mid-third instar vitellarium..... 33

Fig. 20. A proximal oocyte of a late third instar ovariole..... 33

Fig. 21. A longitudinal section of the distal end of a mid-fourth instar ovariole..... 33

Fig. 22. The proximal end of an early fourth instar ovariole... 39

Fig. 23. Two layers of mid-fifth instar prefollicular tissue... 39

Fig. 24. The posterior end of a late fifth instar proximal oocyte..... 39

Fig. 25. The interfollicular zone between two fifth instar oocytes..... 39

Fig. 26. A longitudinal section of the proximal oocyte, pedicel and lateral oviduct of a late fifth instar female..... 39

Fig. 27. The columnar follicular epithelium of an adult ovariole..... 39

Fig. 28. Two elongate follicle cells during the latter stages of yolk deposition..... 39

Fig. 29. The posterior end of an adult proximal follicle..... 39

Fig. 30. An early adult ovariole..... 45

Fig. 31. A degenerating follicle after ovulation..... 45

Fig. 32. The wrinkled ensheathing membranes surrounding a degenerating follicle..... 45

Fig. 33. An oocyte in an early state of resorption..... 45

Fig. 34. An oocyte in a late stage of resorption..... 45

Fig. 35. A longitudinal section of an adult oviduct..... 45

Fig. 36. A section of an adult oviducal wall..... 45

Fig. 37. A longitudinal section of a portion of an adult ovariole.. 45

Fig. 38. A Feulgen-stained adult penultimate and antepenultimate oocytes..... 50

Fig. 39. Unhydrolized, Feulgen-stained adult penultimate oocyte.... 50

Fig. 40. A longitudinal section of a portion of an adult ovariole stained with gallocyanin-chrome alum..... 50

Fig. 41. Adult follicle cells displaying presence of RNA..... 50

Fig. 42. Adult follicle cells after digestion in ribonuclease..... 50

Fig. 43. An adult penultimate oocyte displaying presence of proteinaceous material..... 50

Fig. 44. Adult follicle cells showing presence of proteinaceous material..... 50

Fig. 45. A nucleus of an adult primary oocyte..... 50

Fig. 46. A portion of an adult penultimate oocyte illustrating presence of sudanophilic lipids..... 59

Fig. 47. A portion of an adult penultimate oocyte showing presence of osmiophilic lipids..... 59

Fig. 48. A longitudinal section of an adult ovariole displaying presence of PAS-positive material..... 59

Fig. 49. A longitudinal section of an adult penultimate oocyte in the early stages of yolk deposition..... 59

Fig. 50. Adult penultimate and antepenultimate oocytes showing presence of PAS-positive material..... 59

Fig. 51. Yolk deposition in an adult penultimate oocyte..... 59

Fig. 52. Adult follicle cells displaying presence of acid mucopolysaccharides..... 59

Fig. 53. Adult follicle cells after digestion in hyaluronidase..... 59

Fig. 54. Total number of eggs laid by female A. elliotti exposed to difference photoperiods..... 63

ABSTRACT

The morphogenesis and histogenesis of ovarian tissue of Aulocara elliotti (Thomas) were examined throughout the nymphal and prereproductive stages. Differentiation of the rudimentary ovariole into two functional zones, the germarium and vitellarium, occurs during the second instar. In those oocytes which are to be oviposited first, there is a considerable amount of cytoplasmic augmentation while they reside in the nymphal vitellarium. During the fifth instar a cuboidal epithelium differentiates from prefollicular tissue surrounding oocytes at the proximal end of the ovarioles. In the adult, two layers of muscle tissue are formed between the outer connective tissue sheath and the epithelial lining of the oviducts before the first clutch of eggs are to be laid. Between 8 and 10 days are required after adult emergence to complete the first reproductive cycle. After vitellogenesis, follicle cells around the posterior end of each proximal oocyte enlarge considerably before secreting a specialized chorionic cap overlaying the site of the future hydropyle. Presence of residual pigmentation is one of the gross differentiating factors between a resorbing oocyte and a normal degenerating follicle following ovulation.

A cytological and cytochemical study of adult ovarioles was made with special emphasis on the process of vitellogenesis. During oocyte maturation the presence of Feulgen-positive material within oocyte nuclei diminishes. A heavy concentration of RNA is present in the portion of cytoplasm of the follicle cells closest to oocytes experiencing yolk deposition or chorion formation. A major portion of the yolk material deposited within oocytes appears to be a complex of protein and carbohydrate substances. In addition to the protein-carbohydrate fraction, there are significant amounts of acid and neutral mucopolysaccharides within the yolk granules. Although the deutoplasm of the oocytes is highly PAS-positive, the cytoplasm of surrounding follicle cells is PAS-negative.

It could not be determined whether rate of vitellogenesis or oocyte resorption was a function of the amount of light received per day when 127 females were examined seven days after emergence. There does appear to be trend for ovipositing females to lay fewer eggs upon exposure to shorter daylengths. Oviposition was not observed when females were maintained under constant light or three hours of light per day.

INTRODUCTION

The economic importance of the rangeland grasshopper, Aulocara elliotti (Thos.), has been verified by Pfadt (1949) and Anderson (1964). This insect is widely distributed over rangelands of the Great Plains region and has been observed to fluctuate greatly in numbers in certain areas of Montana.

In the last decade extensive studies have been conducted concerning the postovulatory physiology of developing A. elliotti eggs (Roemhild, 1961, 1965 a and b; Van Horn, 1963, 1964, 1966 a and b; Svoboda, 1964; Bunde, 1965; Laine, 1966). Only limited information is available, however, on the mechanisms by which eggs of this species are formed prior to ovulation. In order that these developmental processes indigenous to preovulatory eggs may be better understood, the present investigation of postembryonic ovarian development and vitellogenesis was undertaken.

Although embryonic development of female acridid gonads has been described by several workers (e.g. Graber, 1891; Roonwal, 1937; Nelsen, 1931, 1934b), ovarian development following hatching has received little attention. Heymons (1895) was probably one of the first to study post-embryonic development in an Orthopteran. Nelsen (1934b) followed ovarian development throughout embryogenesis and the nymphal instars in the Acridid, Melanoplus differentialis (Thos.). Both investigators noted that newly hatched female nymphs had relatively undeveloped gonads and that considerable morphogenesis and histogenesis of ovarian tissue occurred during the nymphal stages.

Phipps (1949) studied the gross structure of maturing adult ovaries

in seven Acrididae. He noted that several atypical conditions were displayed by adult ovaries during maturation, one of which appeared to be oocyte resorption. Singh (1958) made a histological investigation of degenerating ovarian follicles of Schistocerca gregaria (Forsk.). A comparison of postovulatory follicles and resorbed follicles was made. It was concluded that typical postovulatory follicles upon degeneration contained no pigmentation, whereas an orange-red pigment was present in degenerating follicles where oocyte resorption had occurred. Further study of resorbing oocytes revealed that the residual orange-red pigment within resorbed follicles was B-carotene (Lusis, 1963). Lusis also discovered that follicle cells acted as lecitholytic cells during the early stages of oocyte resorption, first breaking down the deutoplasm before they themselves degenerate.

The origin and composition of yolk has been studied in numerous insect species. However, the conclusions derived from these studies are diverse and, in some cases, contradictory. Because of the advancements made in the field of histochemistry during recent years, these techniques are now widely used in the study of insect vitellogenesis. Although yolk deposition in acridid insects has received limited attention, vitellogenesis in many other insect species has been studied using histochemical methods.

In Perplaneta orientalis Linn., Gresson (1931) detected no Feulgen-positive material within oocyte nuclei either before or during vitellogenesis. This does not appear to be the usual condition displayed by oocytes in

many of the other insect species studied. Instead, the nuclei of oogonia and young oocytes generally exhibit a strong Feulgen-reaction during the early stages of oogenesis and then the reaction gradually diminishes before yolk deposition is completed (Bonhag, 1959; Radecka, 1962; Weglarska, 1962). Nuclei of follicle cells ordinarily show no variation in the high level of DNA present throughout the cytoplasmic growth and yolk deposition in the primary oocytes.

Nucleolar extrusions from the primary oocyte nucleaus of the Oriental roach and the American roach have been reported to give rise to proteid yolk in the peripheral ooplasm (Hogben, 1920; Nath and Mohan, 1929; Gresson, 1931).

Hegner (1914) found that mitochondria at the periphery of developing oocytes of Leptinotarsa decemlineata (Say) increased rapidly in size and number and then formed large yolk bodies. Ranade (1933) studied vitellogenesis in Periplaneta americana (Linn.) and concluded that protein was produced by mitochondria and not by nucleolar emissions.

Yet another origin of proteid yolk was assessed in oocytes of Nepa cinera Linn. (Steopoe, 1926). Golgi bodies were thought to be responsible for the production of proteid yolk bodies in this species. In an earlier study of the same species of bug, Spaul (1922) proposed a nucleolar origin for the yolk bodies.

Bonhag (1959) and Radecka (1962) maintain that nucleolar emissions only serve to transfer material from the nucleus to the cytoplasms of the oocyte and do not give rise directly to proteid yolk. This view appears

to be consistent with the more recent understanding of protein synthesis.

In studying oogenesis in Tachycines asynamorus Adel., Radecka (1962) suggested that the follicle epithelium may contribute portions of the deutoplasmic substances to developing oocytes.

Other workers maintain yolk protein is synthesized elsewhere in an insect's body and is taken into developing oocytes by the process of micropinocytosis (Kessel and Beams, 1963; Roth and Porter, 1964; Anderson, 1964; King and Aggarwal, 1964; Stay, 1965). Telfer (1961) and Ramamurty (1963) suggested that yolk protein enters oocytes during vitellogenesis via the intercellular spaces of the follicular epithelium. It was inferred that there was little participation of the follicle cells in yolk deposition.

Lipoid yolk has also been thought to originate from a variety of sources. Numerous investigators have concluded that Golgi bodies within the oocytes develop into lipoid yolk (Nath and Mohan, 1929; Nath and Metha, 1929; Gresson, 1931; Gupta, 1958a). Most of these authors agree that lipoid yolk first appears in Golgi vacuoles, which swell and change into fat droplets. The fat droplets may remain surrounded for some time by an osmiophilic sheath.

Hsu (1952) observed a direct transformation of mitochondria into fat droplets in oocytes of Drosophilia melanogaster Meigen. Fatty yolk globules were suggested to arise in the ooplasm without relation to any of the formed elements within the oocyte (Steopoe, 1929).

Bonhag (1955b) stated that sudanophilic lipids of both non-phospholipid and phospholipid types appeared to have been contributed to the oocytes of

Oncopeltus fasciatus (Dallas) by the follicular epithelium.

Several workers have suggested that carbohydrate compounds in yolk exist as a protein-carbohydrate complex. Bonhag (1955a) postulated that yolk material, which could not be extracted with a methanol-chloroform solution, consisted of a mucoprotein or a glycoprotein. A similar conclusion was reached by Lusis (1963) when he studied oocyte resorption in Schistocerca.

Yolk material reacting positively to the periodic-acid Schiff test in oocytes of Gerris remigis Say was conjectured to be a mucoprotein, glycoprotein, or a neutral mucopolysaccharide in complex with protein (Eschenberg, 1965).

Although glycogen is one of the main storage polysaccharides of insects, it was not found in developing oocytes of Oncopeltus, Tachycines, or Gerris (Bonhag, 1955a; Radecka, 1962; Eschenberg, 1965). Bonhag (1956), however, found glycogen to be present in the older oocytes of Anisolabis maritima (Géné).

Insect vitellogenesis and oviposition have been found to be sensitive to a variety of extrinsic factors. Many insect species have been discovered to react in some manner to a changing photoperiod since Marcovitch (1924) first demonstrated the importance of daylength upon the appearance of sexual forms in aphids. Reproduction is acknowledged to be one of the physiological processes responsive to changing daylengths in numerous insect species.

In the laboratory, Schistocerca gregaria has been observed to enter

a reproductive diapause upon a lengthening of the daylight periods, whereas Nomadacris septemfasciata Serv. experiences a cessation of ovarian development when the photoperiod becomes shorter (Norris, 1957, 1962, 1965). Middlekauff (1964) has shown that rate of oocyte maturation increases with decreasing daylengths in adult Melanoplus devastator Scud. females.

Oocytes in Dytiscus and Leptinotarsa were observed to form and resorb continually when these insects were maintained under short daylengths comparable to those during winter months (Joly, 1945; De Wilde, 1954).

The mechanism for phtoperiodic control of insect reproduction appears to act through the neuroendocrine system. De Wilde and De Boer (1961) have demonstrated that oogenesis in Leptinotarsa is controlled by way of the brain through the corpora allata. It has been further observed that protein haemolymph concentration rises in adult females before oocyte yolk deposition and is depleted during each reproductive cycle (Hill, 1962; Highnam, Lusis and Hill, 1963; Orr, 1964; Engelmann, 1965). Thomsen and Möller (1959) have indicated that the neurosecretory substance is directly involved in stimulation of protein synthesis.

Highnam et al., (1963) have shown that the fewer the number of ovarioles in Schistocerca the fewer the number of resorbed oocytes. These workers contend that developing oocytes are in competition with each other for available haemolymph protein. Developing oocytes which do not acquire adequate material from the haemolymph are ultimately resorbed.

Using the aforementioned investigations as a background, the present

study on postembryonic ovarian development and vitellogenesis was initiated.

MATERIALS AND METHODS

General Morphology

Grasshoppers used for the morphological study of postembryonic ovarian development, with the exception of first instar nymphs, were collected from wild populations during the summer of 1965. First instar females were obtained from laboratory hatched eggs which had been collected from wild population sites during the fall of 1964.

In order that females with known ages could be obtained for each instar, only females which had hatched or had moulted in the laboratory were used. Newly hatched or moulted grasshoppers were placed into cages containing individuals of the same instar. Vials filled with Western Wheatgrass (Agropyron smithii Rydb.), which is the predominant plant in their natural diet (Anderson, 1964; Pfadt, 1949) were provided daily. Females were taken from these groups every other day and fixed. In this manner the sequential order of development could be followed during the nymphal instars to the adult stage. This study did not include developmental observations on adult females which had completed over seven days of imaginal life as their ovaries were arbitrarily considered to be mature.

Because ovaries of A. elliotti nymphs are small and rather difficult to remove from the younger grasshoppers, a transverse cut was made through the area between the first and second thoracic segments and that portion of the body caudad to the cut was immediately immersed in fixative in toto. Ovaries from teneral through seven-day adults were obtained by vivisection in a 0.65% Ringer's solution. Vivisection was accomplished by pinning the adult female specimen to a wax surface, submerging it with Ringer's

solution, and making a dorsal-longitudinal incision from the epiproct to the cervicum. The exposed ovaries were excised from the grasshoppers by cutting the lateral oviducts and removing the attached fat body and tracheae with the aid of a jeweler's forceps, microdissecting scissors, and a dissecting microscope.

Boiun's, Carnoy's (3:1), Sinha's, and Ammerman's fixatives were used in this investigation. Sinhas's fixative gave the best results for study of the first through fifth instar nymphs. This fixative enhanced sectioning the portions of the body containing the ovaries due to the softening effect it had on the exoskeleton. When ovariectomies were performed and only the excised ovaries fixed, Carnoy's and Ammerman's fixatives yielded the best structural detail. Fixation time for Sinha's Carnoy's and Ammerman's fixatives was 24, 3 and 2 hours respectively.

The fixed material was dehydrated and double embedded according to a modified version of the procedure by Humanson (1962) using a solution of methyl benzoate and celloidin. Due to the high alcohol content of both Sinha's and Carnoy's fluids, dehydration was begun with 95% instead of 50% ethanol. Paraplast (manufactured by Biological Research Inc.) was substituted for paraffin. Infiltration with three changes of Paraplast was executed in a vacuum in an oven at 55-57° C for one hour. The tissue was then embedded in Paraplast and stored until it could be cut with a rotary microtome. Serial sections of all developmental stages were cut at 6 μ and mounted on slides for this study.

The following stains were used: Delafield's hematoxylin and eosin,

Heidenhain's iron hematoxylin, and Mallory's triple connective tissue stain. For study of all stages of ovarian development, Delafield's hematoxylin and eosin proved to be the most helpful as a general stain for all ovarian structures. Mallory's triple connective tissue stain was useful in the examination of the adult ovaries in which yolk was being deposited.

Supravital observations were made with the aid of a dissecting microscope upon adult ovaries exposed by vivisection. Additional observations were made on adult ovaries which had been dissected out and immersed in the 0.65% Ringer's solution.

Cytology and Cytochemistry

For cytological and cytochemical investigations, ovarian tissue was obtained by dissection of females reared in the laboratory or collected from wild populations. Observations were made on ovaries from adult grasshoppers ranging from teneral to senescent forms. The dissection technique was the same as that described previously for adult forms.

Immediately after being dissected, the ovarian tissue was fixed according to the requirements of the staining methods to be used. All material was sectioned on a rotary microtome at 6 μ except for the study concerning the distribution of lipids in the ovarioles. This tissue was cut at 15 μ with a freezing microtome.

For demonstration of nucleic acids the following methods were used: 1) methyl green and pyronin Y for DNA and RNA as outlined by Barka and Anderson (1963); 2) gallocyamin-chrome alum for DNA and RNA according to

Einarson (1951) with the modification of Beswick (1958); 3) Feulgen's reaction by Barka and Anderson (1963) using Lillie's preparation of Schiff's reagent (1951b). For this procedure the hydrolysis time was ten minutes using 1.0 N HCl at 60°C. Alternate sections were counterstained in a 0.05% alcoholic fast green stain after treatment with Schiff's reagent.

Carnoy's solution and cold acetone were used as fixatives for the methyl green-pyronin Y method. Two hours in Carnoy's solution resulted in some loss of RNA. This decrease in staining intensity was not observed when the ovaries were fixed in three changes of cold acetone for 12 hours. To prevent loss of RNA which may occur after prolonged storage in alcohol (Brachet, 1953), material for this technique was embedded as soon as possible following fixation. Carnoy's solution was used exclusively as the fixative for the gallocyanin-chrome alum method. Susa's solution was used as the fixative for the Feulgen reaction. Fixation time for Susa's solution was 24 hours for all methods in which it was used as the fixative.

Control sections for the study of RNA were digested in ribonuclease for four hours at 37°C. Ribonuclease from bovine pancreas which had been recrystallized five times was used at a concentration of 0.3 mg/ml in deionized, glass distilled water adjusted to pH 6.8 with 0.1 N HCl. Unhydrolyzed sections served as the control for the Feulgen reaction.

For study of the general distribution of proteins, ovarian tissue was fixed in Susa's solution and stained with mercuric bromphenol blue (Mazia, Brewer, Alfert, 1953). There was a considerable loss of dye from the sections when the suggested phosphate buffer and succeeding dehydration in

ethanol was used. Consequently, the refinement of Bonhag (1955a) was used in which the buffer is omitted and dehydration is in tertiary butyl alcohol.

The localization of lipids in general was accomplished by using the sudan black B technique of Chiffelle and Putt (1951). The tissue was fixed with buffered 10% formalin for three hours and embedded in gelatin according to Culling (1957). Infiltration of the tissue with the 20% gelatin was increased to 24 hours at 45°C instead of the suggested 12 hours at 37°C. This modification was necessary in order that acceptable sections might be obtained. No alteration in staining intensity or locality was noted when this variation in Culling's procedure was employed.

For demonstration of unsaturated lipids, the osmic acid technique of Mallory (1944) was used. Even though osmic acid is one of the oldest lipid "stains", Bahr (1954) has questioned the specificity of this method because reducing groups of proteins and carbohydrates may yield a positive reaction. However, Adams (1960) has shown that preliminary elimination or blocking of these reducing groups does not diminish osmiophilia but the saturation of lipid -CH=CH- bonds causes its complete extinction. The tissue for this study was handled prior to staining in the same manner as that used with the Sudan black B procedure. The stained sections for both techniques were mounted in glycerin jelly.

Three staining techniques were employed in an effort to demonstrate the presence of glycogen in the ovaries. These staining techniques were: 1) Best's carmine after Humanson (1962); 2) The Bauer-Feulgen reaction of Bauer (1933); and 3) the PAS reaction with the dimedone (5, 5-dimethyl-1,

3 cyclohexadione) blockade as control (Bulmer, 1959).

Absolute alcohol and formalin (9:1) were used as the fixative for the first two methods and Carnoy's solution was used for the third method. Fixation time for all three methods was three hours.

Control sections for Best's carmine and the Bauer-Feulgen reaction were digested in Lillie's (1949) solution of malt diastase for one hour prior to staining. Additional sections were digested in saliva for one hour before treatment according to Best's carmine method. The control sections for the PAS reaction were placed in 5% acetic acid saturated with dimedone for six hours at 60°C after oxidation in periodic acid.

The PAS technique used in the attempt to demonstrate presence of glycogen and other carbohydrates having a positive reaction to this test is as follows: 1) the Paraplast embedding material was removed from the sections with benzene; 2) after immersion in absolute ethanol, the sections were coated with a solution containing 0.5% celloidin and then progressively hydrated to distilled water; 3) the sections were oxidized in 0.75% aqueous periodic acid for ten minutes; 4) the sections were washed in running tap water and placed in Schiff's reagent for 30 minutes; 5) the sections were bleached in a solution consisting of 10 ml of 10% sodium metabisulfite, 10ml N HCl, and 200 ml of distilled water; 6) after a ten minute wash in running tap water the sections were dehydrated in ethanol, cleared and mounted. Ovaries preserved with Carnoy's and Susa's fixatives were used for all carbohydrate analyses employing the PAS technique. No differences in staining intensity or locality could be

ascertained when one fixative was substituted for the other. Lillie's (1951b) formula for Schiff's reagent was used throughout the analysis for reactive carbohydrates.

Several controls were used in the effort to characterize the carbohydrates found to react positively using the PAS technique. These controls were: 1) sections stained without previous oxidation in periodic acid; 2) sections acetylated for two hours in a 2:3 mixture of acetic anhydride pyridine succeeded by deacetylation for 24 hours in a 1:4 mixture of NH_4OH (28%) and ethanol; 3) sections treated with a solution of 0.1 mg/ml pepsin in 0.01 N HCl at 37°C for two hours; 4) sections treated with a solution of 0.3 mg/ml bovine hyaluronidase in 0.1 phosphate buffer, pH 5.9, at 37°C for five hours; and 5) sections placed in a 1:1 mixture of methanol and chloroform at 60°C for 24 hours (Bonhag, 1955a). The coating of the sections with celloidin was omitted when enzyme digestion was utilized. Because sections were lost from the slides when they were treated with the pepsin solution, the Paraplast embedding material was not removed from the sections until immediately before they were mounted (Bonhag, 1955a).

Because protein and carbohydrate yolk granules appear to have similar locations within the more mature oocytes, a modification of the technique of Himes and Moriber (1956) was used to investigate this situation more closely. This technique is designed to display DNA, polysaccharides, and protein when present. The method was modified by omitting the azure A-Schiff reagent in order that only the presence of protein and carbohydrate

could be demonstrated. Ovarian tissue preserved with Susa's fixative was used in this study.

The alcian blue staining method of Steedman (1950), revised by Mowry (1956), was used to demonstrate the presence of acid mucopolysaccharides in ovarian tissue preserved with Carnoy's fixative. A 0.1% aqueous solution of toluidine blue was used for the metachromasia analysis. The sections were stained in this solution for one hour. Control sections were digested in hyaluronidase using the same procedure as described previously for PAS reactive carbohydrates. Some of the sections were examined under water immediately after staining, whereas others were examined following the dehydration, clearing and mounting procedure.

To observe intra-vital staining of the ovarioles, one µl of a 0.5% solution of trypan blue in 0.2 M phosphate buffer, pH 6.9, was injected into the haemoceles of A. elliotti females reared in the laboratory. Grasshoppers from this group were sacrificed at 8, 16, 24 and 33 hour intervals so that ovaries could be dissected and fixed in Susa's solution. The fixed material was then embedded in Paraplast, sectioned, affixed to slides, immersed in toluene to remove the Paraplast, and mounted. No further staining was employed subsequent to examination of these sections.

Photomicrographs were taken of stained sections resulting from the previous studies with a Leitz 4 X 5 camera through a Zeiss microscope equipped with Planapochromat and Neofluar objectives.

Photoperiodism

To examine the possibility that photoperiod may have some control

over the rate of vitellogenesis, frequency of oocyte resorption and frequency of oviposition, grasshoppers were collected from a wild population near Decker, Montana, and reared in the laboratory under a regime of 13 different photoperiods. Twelve of the 13 photoperiods were based upon a 24 hour day. The thirteenth photoperiod was used as a control and grasshoppers reared under it had constant light.

A battery of 24 cages was constructed in such a manner so that each of the 12 pairs of cages would have a different daylength (Fig. 1). A sliding plywood shield was designed so that on hourly intervals it would cover one pair of cages. When this shield had covered each of the 12 pairs of cages, it would then proceed to uncover one pair of cages every hour on the hour until all had been uncovered. The time required for the shield to traverse the distance from one end of the battery of cages to the other and back to its starting position was 24 hours. The 12 photoperiods resulting from this arrangement differed from each other by two hour intervals and ranged from 23 hours of light to one hour of light.

The sliding shield was powered by a 1/8 horsepower, split-phase electric motor. A relay system consisting of two relays governed the direction of movement for the shield. This relay system was wired in such a manner that one relay was in series with the motor windings causing it to go forward and the other relay was in series with the motor windings causing it to go backward. A toggle switch in series to the relay system was tripped by the shield at either end of the battery of cages. This action energized the corresponding relay allowing the shield to reverse

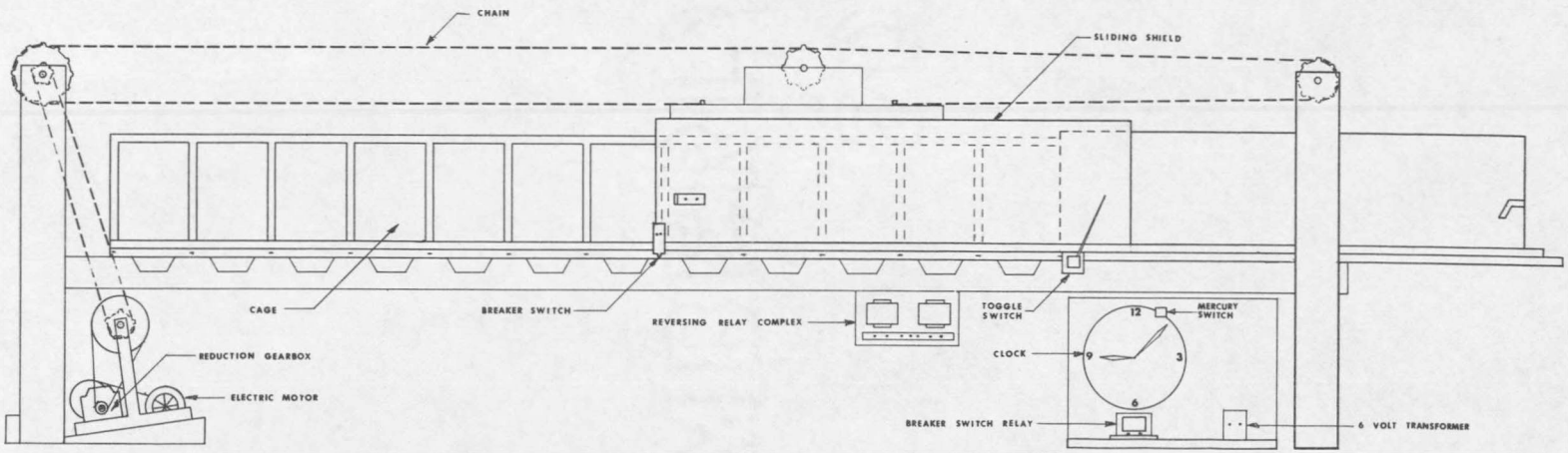


Figure 1. Schematic representation of the apparatus used to rear A. elliotti females under different photoperiods.

direction. It was necessary to connect a thermal time delay tube between the toggle switch and each of the relays which allowed the shield to remain at either end of the cage complex for one hour. If this was not done, the relay system would reverse the direction of the shield before it had come to a complete stop causing breakage of the chains or other moving parts. The relays were activated by a clock that tripped a mercury switch every hour sending a 20 second impulse of current to the motor causing the shield to cover or uncover a pair of cages depending on which direction it was moving.

The dimensions of each cage were 8" X 16" X 12". The top and one side of the cages were covered with fine screen. The pair of cages exposed to constant light was of the same dimensions and design but was not connected to the battery of cages with the sliding cover. A 5" X 9" hole was cut in the floor of each cage under which a baking pan of a similar size was attached. These pans were filled with soil taken from the same area where the grasshopper had been collected. Vials filled with fresh Western Wheatgrass were provided every other day.

Light for the experiment was provided by 16 Sylvania Gro-Lux fluorescent bulbs 96" in length. The fluorescent lights were supplemented with three 200 watt unfrosted incandescent bulbs. The light intensity derived from this system was found to be 52 candlepower when measured at the floor of the cages. The cages were placed in a room with no windows. Ventilation was provided by an exhaust fan. Due to the heat produced by the lights, the ambient diurnal temperature fluctuated only 2-3 degrees

from 84°F. The relative humidity averaged 17± 2% for the duration of the experiment.

Fifteen third and fourth instar nymphs were placed in each cage. Of these 15 grasshoppers ten were females and five were males. Those grasshoppers, males and females, which died during the first week of rearing were replaced. After the first week only males were replaced. Mortality for all grasshoppers after the first week was moderate. In only three cages did more than two grasshoppers per cage die during the experiment.

When the females emerged as adults they were marked with India ink and dated. Ovariectomies were performed on 127 females seven days after their imaginal moult. In most cases ten females were removed from each pair of cages having the same photoperiod. Two pairs of cages had a female mortality which did not permit a sample of this size. A total of nine females were removed from cages six and seven, and eight females were removed from cages 23 and 24.

The criterion used to determine the effect of a varied daylength on the rate of vitellogenesis was the length of the proximal oocyte. The length of the oocyte closest to the lateral oviduct for each ovariole was measured immediately after the ovaries were removed from the female. Those oocytes in which yolk was being resorbed were not measured, however, the number of oocyte resorptions was recorded for each female.

To examine the possible effect varied daylengths have upon frequency of oviposition, two pairs of adult grasshoppers were maintained in each of

the 26 cages for 28 days. Two females were picked at random from those females remaining in each cage after removing the necessary specimens for the previous experiment. In all cases the females used in this study were newly emerged and had not completed the first reproductive cycle.

After a period of four weeks the pans filled with soil were removed from the bottom of each cage and egg pods were separated from the soil by sifting through a 3/16 inch screen. The number of egg pods laid by the females in each cage was recorded as was the number of eggs within each pod.

Male grasshoppers were replaced in any cage in which there was mortality, whereas females were not. Upon death of a female the number of egg pods laid by both females was recorded immediately and then the remaining female was maintained until the end of the 28 day period.

RESULTS

Morphological and Histological Observations

The gross anatomy of the ovaries of adult females will be briefly described before entering into a detailed account of their postembryonic development and the process of vitellogenesis.

Each of the paired ovaries of A. elliotti consists of four to six elongate egg tubes or ovarioles (fig. 2). The typical number is five and only rarely are six ovarioles present. Distally, the ovarioles are drawn out into fine terminal filaments (fig. 3). The terminal filaments of the ovarioles from both ovaries unite to form a single suspensory ligament. The suspensory ligament attaches to the dorsal diaphragm in the mesothoracic region. Proximal to the terminal filament is the portion of the ovariole known as the germarium. The germarium contains oogonia, which are direct descendants of the primordial germ cells and prefollicular tissue. The prefollicular tissue later differentiates into the follicular epithelium that surrounds developing primary oocytes and also into the interfollicular tissue separating each follicle. Proximal to the germarium is the vitellarium. This region of the ovariole contains 15-17 successive egg compartments or follicles within which primary oocytes develop. The vitellarium of each ovariole contains an array of maturing follicles which represent a succession of physiological stages. It is in the vitellarium where primary oocytes undergo the greatest cytoplasmic growth and also where yolk deposition occurs. The proximal ends of the ovarioles are joined to the lateral oviduct in a serial fashion by means of structures known as stalks or pedicels (fig. 2).

The walls of the lateral oviducts are very elastic and are capable of considerable distension while holding mature eggs prior to fertilization and oviposition. An accessory gland is located at the apex of each lateral oviduct which provides materials used in the construction of the egg pod. Posteriorly, the lateral oviducts parallel either side of the gut and then turn ventrally to unite beneath the ventral nerve cord to form a single duct commonly referred to as the vagina.

The ovary of this species is of the panoistic type. Panoistic ovarioles, in contract to meroistic ovarioles, have no "nurse cells" which provide nutrients to the developing oocyte. Panoistic ovarioles are characteristic of most of the older orders such as Thysanura, Ephemeroptera, Odonata, Orthoptera, Isoptera and Plecoptera.

Ovarian development. Ovaries of newly hatched A. elliotti females are small paired structures lying dorsal to the midgut in the first to fourth abdominal segments. Each ovary is approximately 550 μ in length and 95 μ thick. The rudimentary ovarioles, when viewed in longitudinal section, appear as cellular columns, tapered at either end, and arranged in a single plane (figs. 4 and 5). Each ovariole is approximately 140 μ long and 50 μ thick. Unless otherwise indicated, ovariole length measurements do not include the terminal filament or the pedicel. Width measurements were taken at the widest portion of the ovariole. The morphogenesis of only one ovariole will be discussed for each nymphal stadium because all ovarioles of a specimen appear to develop in the same manner and rate.

PLATE I
(Figures 2-5)

Fig. 2. Freshly dissected ovaries from a gravid A. elliotti female. circa 15X.

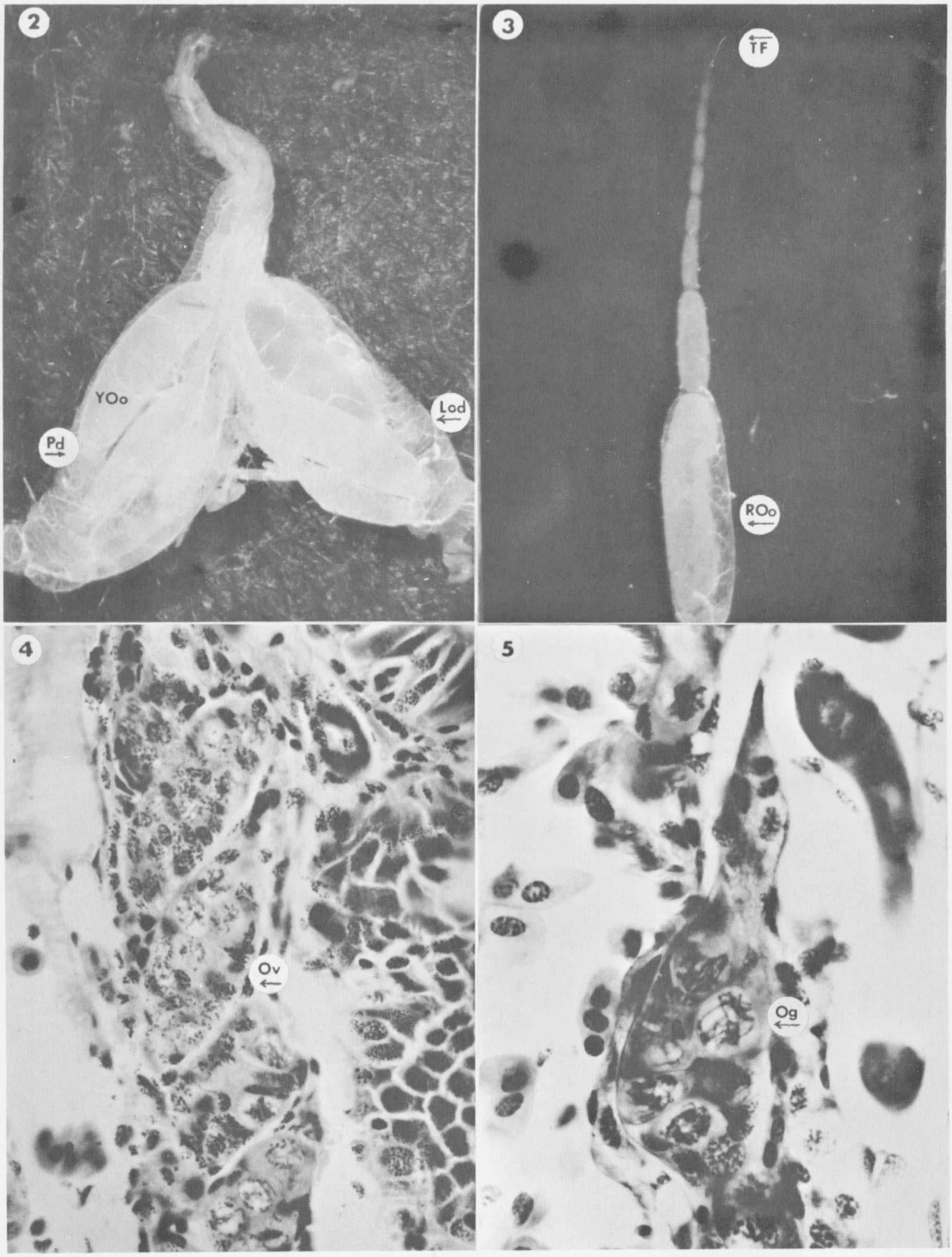
Fig. 3. Freshly dissected ovariole showing the succession of follicles. There is a resorbing oocyte in the proximal follicle. circa 15X.

Fig. 4. A longitudinal section of a first instar ovary from a newly hatched female which shows the serial arrangement of the four ovarioles. Sinha, Delafield's hematoxylin, 320X.

Fig. 5. Similar to the preceeding section but showing a single ovariole at a higher magnification. There are meiotic figures in the oogonia. 500X.

List of Abreviations

BD., basophilic droplets
Ch., chorion
DF., degenerating follicle
FE., follicular epithelium
Gg., gregarine
Gr., germarium
If., interfollicular tissue
IfZ., interfollicular zone
Lod., lateral oviduct
Oo., oocyte
Og., oogonium
Ov., ovariole
OS., ovariole sheath
Pd., pedicel
Pf., prefollicular tissue
PM., primordial muscle
ROo., resorbing oocyte
TF., terminal filament
Vc., vacuole
VCS., ventral cell strand
Yg., yolk granule
YOo., yolk-filled oocyte


2
YOo
Pd
Lod
3
TF
ROo
4
Ov
5
Og

The ovarioles of first instar nymphs are easily recognized. A large portion of the ovariole appears to be of a syncytial nature, wherein the nuclei of cells destined to form the various structural elements share a common cytoplasm. These nuclei, which are of mesodermal origin, are interspersed between the oogonia and form cone-shaped aggregations at either end of the ovariole. The distal group of nuclei tapers to a single strand, the terminal filament, which remains a syncytium for the life of the grasshopper (fig. 6). The proximal group of nuclei is part of the tissue which connects the ovariole to a ventral strand of cells (fig. 7) that is destined to become the lateral oviduct. This proximal aggregation of nuclei and the surrounding cytoplasm later loses its syncytial form and contributes to the cell structure of the pedicel. The follicular epithelium and interfollicular tissue of the more advanced instars are derived from the epithelial elements dispersed between the oogonia.

The nuclei of the oogonia are round, average 15 μ in diameter, and exhibit stages of the first meiotic prophase. Occasionally a mitotic figure is observed in the most distal of these cells. All germ cells within first instar ovarioles tend to have little cytoplasm and are closely packed.

Each ovariole is ensheathed by an outer membrane containing widely dispersed flattened nuclei and by a relatively thick non-cellular inner membrane, the tunica propria (fig. 8). Distally, the outer ovariole sheath becomes continuous with the terminal filament (fig. 9). Apposed

to the outer membrane the length of the ovariole are numerous tracheal endings.

The rudimentary lateral oviducts of newly hatched nymphs lie ventral and slightly lateral to each of the ovaries. The oviducal strand is appreciably thicker in the vicinity of the ovary than in the area paralleling the alimentary tract (figs. 10 and 11). The ovarioles abut directly onto this horizontal strand of cells (fig. 7) as the pedicel has not yet formed at this stage. Toward the end of the first instar the oviducal lumen begins to differentiate in the thicker portion of the strand in the region beneath the ovariole attachment, and progresses anteriorly and posteriorly from this point.

The ovarioles of second instar nymphs increase both in length and width. By the middle of the instar the average size of an ovariole is 820 μ long and 85 μ wide. The increase in length is due to growth and differentiation of the ovariole into two major zones, the germarium and the rudimentary vitellarium (figs. 12 and 13). The germarium of this stage makes up almost one-half of the total length of the ovariole. The germ cells have become more numerous due to cell division. A gradual increase in gonial size from the distal to proximal end of the germarium is apparent.

The division between the germarium and the rudimentary vitellarium is marked by a large increase in the size of the germ cells. The nuclei of these cells average 33 μ in diameter and the cytoplasm has augmented to such an extent that a single germ cell spans the width of the

PLATE II
(Figures 6-13)

Fig. 6. Distal end of mid-first instar ovariole showing the terminal filament. Sinha, Delafield's hematoxylin, 400X.

Fig. 7. Longitudinal section of first instar ovariole illustrating the posterior connection to the ventral cell strand at the time of hatching. Sinha, Delafield's hematoxylin, 400X.

Fig. 8. First instar ovariole showing tunica propria, outer ovariole sheath, and the meiotic figures within the oogonia. Sinha, Delafield's hematoxylin, 1280X.

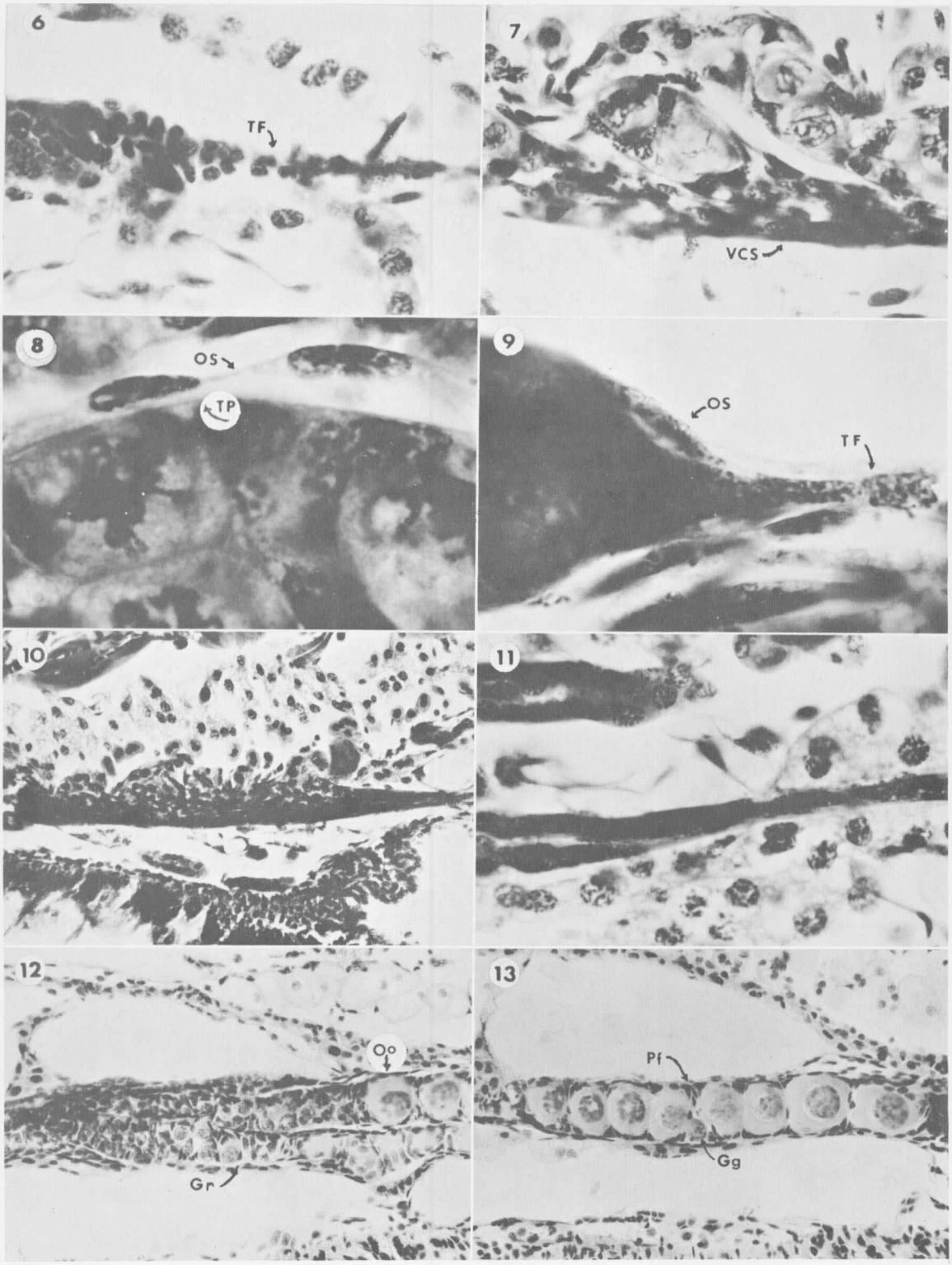
Fig. 9. Similar to above but showing a more anterior region of the ovariole. This photograph shows the extension of the outer ovariole sheath over the terminal filament. 1000X.

Fig. 10. Longitudinal section of a mid-first instar oviduct demonstrating the thick gonial region where the oviducal lumen begins to differentiate. Sinha, Delafield's hematoxylin, 140X.

Fig. 11. Similar to the above but this section shows a narrower portion of the oviduct posterior to the gonial region. The oviducal lumen is starting to form on the left. 430X.

Fig. 12. Sagittal section of two germaria from a mid-second instar female. The large primary oocyte marks the division between the germarium and rudimentary vitellarium. Sinha, Delafield's hematoxylin. 135X.

Fig. 13. Similar to above but shows the rudimentary vitellarium posterior to the region illustrated in the preceeding photograph. There is an encysted gregarine in the middle of this structure. 140X.


6
TF
7
VCS
8
OS
TP
9
OS
TF
10
11
12
Oo
Gr
13
Pf
Gg

rudimentary vitellarium.

All of the germ cells contained in the rudimentary vitellarium and a few of the most proximal germ cells of the germarium have nuclei in which the chromatin is in a diffuse condition (fig. 14). Individual chromosomes cannot be recognized in the nuclear reticulum of these cells. This nuclear condition appears to be a post-pachytene stage of the first maturation division. Thus, these cells are no longer oogonia, by definition, but are considered to be primary oocytes. Further meiotic division is not experienced by the primary oocytes until after they leave the vitellarium. During later instars and in the adult stage the nuclear network frequently becomes more concentrated in certain areas which yields a conformation similar to the "lampbrush chromosomes" of other animals (fig. 15). This concentration of chromatin material is usually more conspicuous during periods of extensive cytoplasmic growth.

The prefollicular nuclei of this stage are considerably more numerous in the germarium than in the rudimentary vitellarium. Mitotic activity is exhibited by many of the prefollicular nuclei throughout the length of the ovariole. Most of these nuclei in the rudimentary vitellarium are located at its periphery or are starting to form a layer between the primary oocytes and the ovariole sheaths (fig. 14). Toward the end of this instar the prefollicular tissue invades the area between one or two of the most proximal oocytes and completely surrounds them (fig. 13). This layer is not yet definitive follicular epithelium as no cellular membranes can be discerned at this time.

As the length of the ovarioles increases, the typical slanted serial arrangement present in the first instar ovary is lost. Instead, the ovarioles now lie parallel to the longitudinal plane of the grasshopper and are depressed between the dorsal diaphragm and the midgut. As shown in Figure 16 this lengthening is accompanied by a change in the position of the ovarioles in relation to each other. Generally, the trend is for the ovarioles to spread laterally and also, for some of them to become situated dorsal to their counterparts. This increase in length is likewise associated with a lateral movement of the proximal ends of the ovarioles which moves the oviduct in the gonadal region from its mostly dorsal position to the definitive lateral position.

By the end of the second instar a lumen is present from the distal end of the lateral oviduct to where it joins the vaginal duct. The oviducal wall of this stage has three layers, one of which is rather indistinct (fig. 17). There is an inner lining of columnar epithelium and an outer connective tissue sheath which appears to be continuous with the outer ovariole sheath. Located between the epithelial layer and the connective tissue sheath are some widely distributed elongate cells. These cells are presumptive muscle cells which later differentiate into muscle tissue in the young adult.

The pedicel differentiates during the second instar as an evagination of the lateral oviduct at the site where it connects to the ovariole. This evagination later lengthens to form a short tube which is continuous with the lumen of the oviduct (fig. 18).

About the middle of the third instar the ovarioles average 1000 μ (1 millimeter) in length and 100 μ in width. There has been little change from the morphology of second instar ovarioles except for the increase in size.

Cell division has increased the number of oogonia over that of the preceeding instar. By division and an increase in cytoplasm of the cells forming the outer ovariole sheath and an increase in mass for the tunica propria, the enveloping tissues are able to keep pace with the expansion of the ovarioles.

The more proximal primary oocytes continue to increase in cytoplasmic volume to where length exceeds the width of these cells (fig. 19.). The average dimensions of the most proximal primary oocyte are 125 μ long and 25 μ in diameter. The nucleus of this cell has an ovoid shape in contrast to the rounded nuclei of the more distal oocytes. Because the pre-follicular tissue surrounding the proximal primary oocytes does not increase in mass in proportion to the growth of the oocytes, the pre-follicular nuclei become quite flattened in appearance (fig. 20).

The lateral oviducts and pedicels continue to increase both in length and width during the third instar.

The fourth instar ovariole has increased about 200 μ in length by the middle of the instar, whereas the width remains near that of the preceeding stage. The augmentation in length appears to arise from the addition of primary oocytes at the distal end of the rudimentary vitellarium. These cells become quite crowded and compact to the point where cell width

PLATE III
(Figures 14-21)

Fig. 14. Proximal primary oocyte of a late second instar ovariole. The nuclear material within the oocyte nucleus is in a diffuse condition. Prefollicular tissue is beginning to surround the oocyte. Sinha, Delafield's hematoxylin, 650X.

Fig. 15. Oocyte nucleus of a fifth instar proximal oocyte illustrating isolated concentrations of chromatin material. Ammerman, Mallory's triple stain, 575X.

Fig. 16. Transverse section of a second instar female showing the tiered arrangement of the ovarioles. Sinha, Delafield's hematoxylin, 135X.

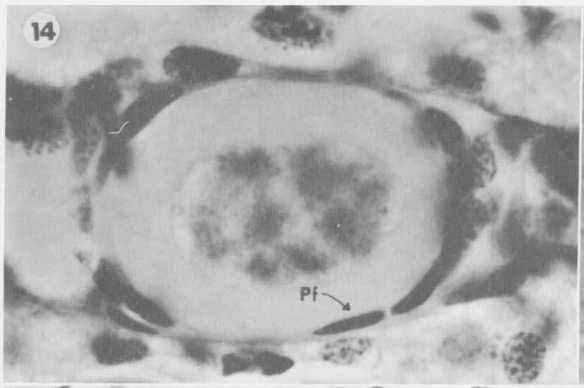
Fig. 17. Section of a second instar oviduct displaying the three cell layers making up the oviducal wall. Sinha, Delafield's hematoxylin, 1000X.

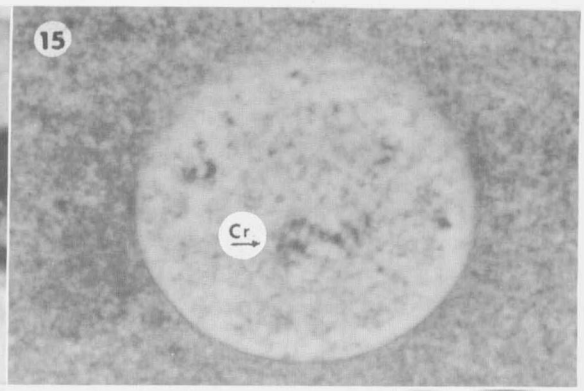
Fig. 18. Similar to the above but at a lower magnification showing pedicel formation by evagination of the oviducal wall. 200X.

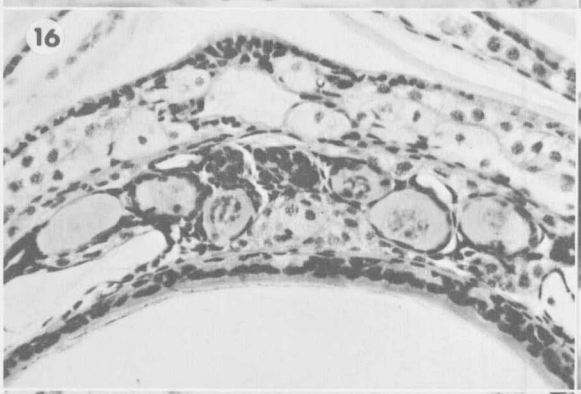
Fig. 19. Longitudinal section of a portion of a mid-third instar vitellarium illustrating the lengthening of the primary oocytes. Sinha, Delafield's hematoxylin, 120X.

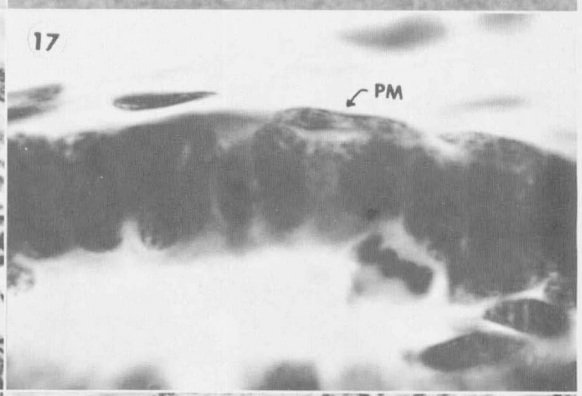
Fig. 20. Proximal oocyte of a late third instar ovariole. The nuclei in the surrounding prefollicular tissue are flattened. Sinha, Delafield's hematoxylin, 450X.

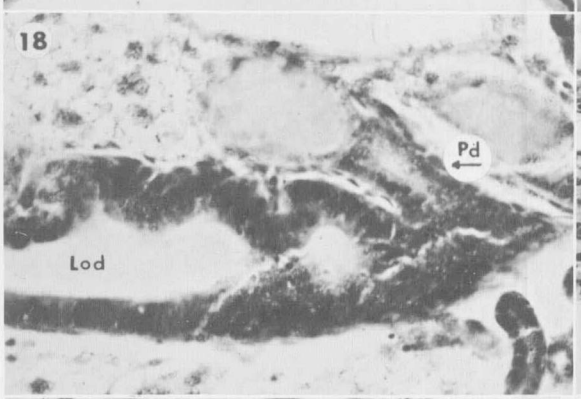
Fig. 21. Longitudinal section of a mid-fourth instar ovariole showing the compacting of primary oocytes near the distal end of the vitellarium. Sinha, Delafield's hematoxylin, 165X.


14
Pf


15
Cr

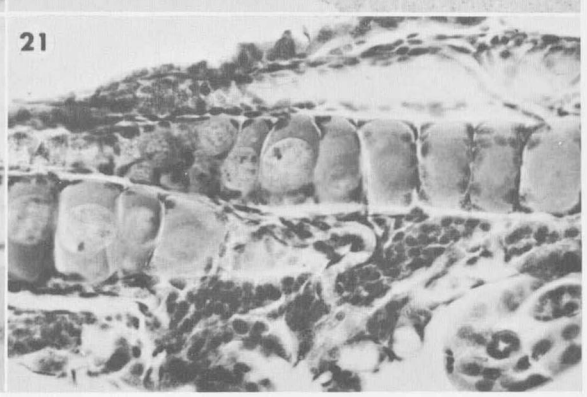

16


17
PM


18
Pd
Lod


19


20


21

exceeds length (fig. 21). This is in direct contrast to the dimensions of those primary oocytes at the proximal end of the rudimentary vitellarium.

During this instar the regions between the last two or three primary oocytes at the proximal end of the rudimentary vitellarium differentiate into interfollicular zones. The proximal primary oocyte of each ovariole and its surrounding layer of prefollicular tissue becomes separated from the oviducal cells forming the pedicel. This separation is due to a proliferation of transversely arranged prefollicular tissue between the pedicel and the oocyte (fig. 22). Similar zones also form between the primary oocytes immediately distal to the proximal oocyte.

By the middle of the fifth instar the ovarioles are almost three times as long as those midway in the preceeding stage. The ovarioles now average 3200 μ (3.2 millimeters) in length and 135 μ in width. The increase in length is accompanied by a considerable gain of cytoplasm by the primary oocytes. For example, the proximal primary oocyte at this stage of development averages 365 μ long and 110 μ in diameter.

It is during the fifth instar that the prefollicular tissue surrounding primary oocytes, in which yolk is to be deposited first, begins to assume its cellular form. An intense amount of mitotic activity results in the encasement of the older primary oocytes by a layer of tissue with many nuclei and little cytoplasm (fig. 23). During the latter portion of this instar this tissue loses its syncytial condition to become a single layer of cuboidal cells. Although by definition this layer is not a true epithelium, it is generally referred to as the follicular epithelium.

There has been little noticeable histological change in the enveloping membranes except for an increase in the number of nuclei present per unit surface area in the outer ovariole sheath.

At the posterior end of the proximal oocytes a number of basophilic droplets were observed (fig. 24). These droplets were noticed only in fifth instar oocytes. Neither the origin nor the significance of these structures could be determined.

The interfollicular tissue has reached its definitive state in the interfollicular zones which serially separate the last three primary oocytes at the proximal end of the ovariole. There is little mitotic activity within these zones after the midpoint of the instar. Anterior to the fully formed interfollicular zones one can still observe the formation of similar separating structures between the younger primary oocytes. The nuclei of interfollicular tissue retain an appearance like that of prefollicular tissue from which it was derived. It is readily noticeable that interfollicular tissue is cytologically distinct from definitive follicular epithelium (fig. 25).

There is a prodigious amount of mitotic activity occurring in the epithelial lining of the oviducts during this instar. The cells become compacted and assume a stratified appearance (fig. 26), although this stratification is not evident in the mature oviducts. The pedicel again increases in length as in the preceeding instars. Its length is approximately 3/4 the length of the adjacent primary oocyte and the cell structure is very similar to that of the lateral oviduct.

The ovarioles of newly moulted adult females are not significantly different from those of the latter part of the fifth instar. However, 2-3 days after the moult the ovarioles begin a period of rapid growth resulting in great increase in size. This tremendous increase in size usually occurs in a span of 7-9 days and appears to be largely due to deposition of yolk in the last and penultimate oocytes. A smaller portion of ovariole enlargement is associated with cytoplasmic augmentation in the other primary oocytes.

The ovariole averages 15.5 mm long and 1.4 mm wide by the time yolk deposition has been completed in the proximal primary oocyte. Of the total mean length of 15.5 mm, the last follicle contributes an average of 6.0 mm and the penultimate, 2.9 mm.

The cuboidal epithelium surrounding the proximal primary oocyte in the fifth instar has so increased in cellular number that it has become a columnar epithelium preliminary to yolk deposition in the early adult stage (fig. 27). The cuboidal cells have divided and grown with such rapidity that they have become compressed into the columnar form. It is only toward the end of vitellogenesis for this particular follicle that most of the epithelial cells lose their columnar shape. Cellular division in the epithelial layer terminates but oocyte expansion does not, consequently, the cells are stretched to such an extent that cell width now exceeds cell height (fig. 28). It is when the cells have this form that they produce a non-chitinous covering, the chorion, around the oocyte after yolk deposition is completed.

It is also during the early adult stage that prefollicular tissue surrounding 4-5 primary oocytes immediately distal to the proximal oocyte differentiates into a cuboidal epithelium. All during the period that the female is reproductively active there are 4-5 primary oocytes encased by a layer of follicle cells besides the proximal oocyte. As soon as a proximal primary oocyte leaves the ovariole or is resorbed, the prefollicular tissue differentiates around the primary oocyte immediately distal to the four or five formed follicles.

As indicated above, not all cells of a follicular epithelium encasing an oocyte at the end of vitellogenesis have the same form. There is further differentiation of a relatively small number of epithelial cells forming a cap at the posterior end of the follicle (fig. 29). These cells are larger than the other follicle cells and are mostly of a columnar form. This aggregation of cells is responsible for secreting the specialized layers of the chorion which is part of the hydropyle apparatus. The hydropyle functions in the uptake of water in the mature egg.

Although movement of the nucleus from its central position is noticeable in the proximal oocyte of a fifth instar ovariole, it is during the early adult stage that additional oocytes at the proximal end of the ovariole acquire a definite polarity (fig. 30). The oocyte nucleus becomes located at the proximal end of the cell and only rarely does it become situated at the distal end of the cell. The nuclei are spherical and average 80 μ in diameter. The chromatin material remains in the

PLATE IV
(Figures 22-29)

Fig. 22. Proximal end of an early fourth instar ovariole illustrating the invasion of interfollicular tissue between the pedicel and the proximal follicle. Sinha, Delafield's hematoxylin, 1000X.

Fig. 23. Two layers of prefollicular tissue surrounding adjacent mid-fifth instar oocytes. This tissue is beginning to form cellular divisions. Sinha, Delafield's hematoxylin, 1000X.

Fig. 24. Posterior end of a late fifth instar proximal oocyte displaying basophilic droplets and peripheral vacuoles. Sinha, Delafield's hematoxylin, 400X.

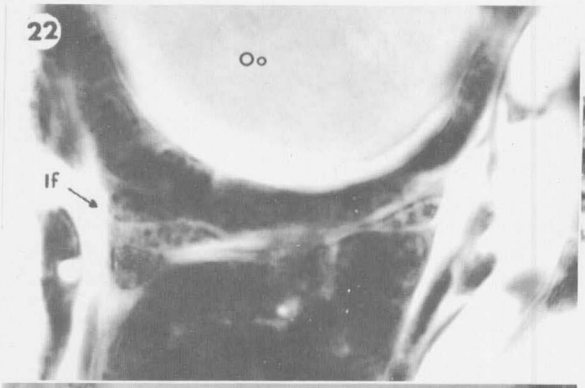
Fig. 25. Interfollicular zone between two successive fifth instar primary oocytes. Sinha, Delafield's hematoxylin, 510X.

Fig. 26. Proximal oocyte pedicel and lateral oviduct of a late fifth instar female. Sinha, Delafield's hematoxylin, 100X.

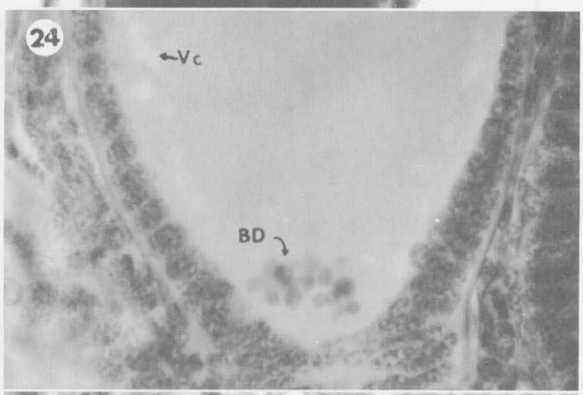
Fig. 27. Columnar follicular epithelium surrounding a pre-vitellogenic adult primary oocyte. Ammerman, Mallory's triple stain, 400X.

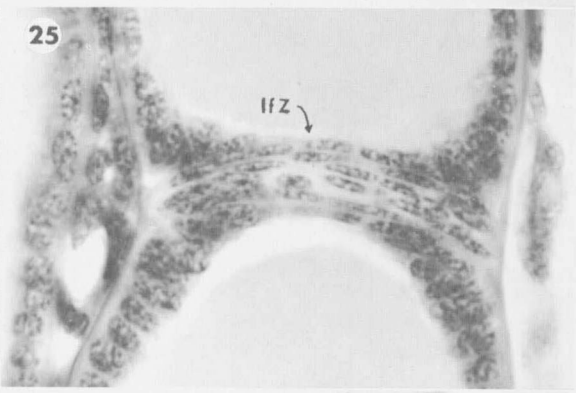
Fig. 28. Section of the follicle epithelium showing two elongate follicle cells during the latter stages of yolk deposition in the proximal oocyte. Carnoy, Gallocyanin-chrome alum, 1000X.

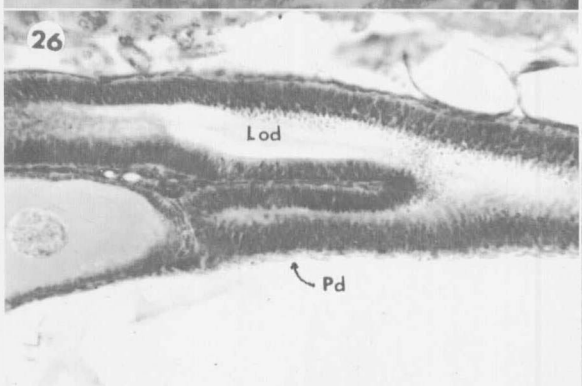
Fig. 29. Posterior end of a proximal follicle illustrating the specialized follicle cells that secrete the chorion above the hydropyle. Carnoy, Gallocyanin-chrome alum, 100X.

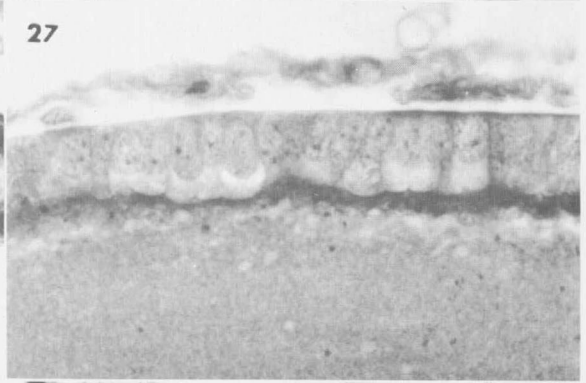

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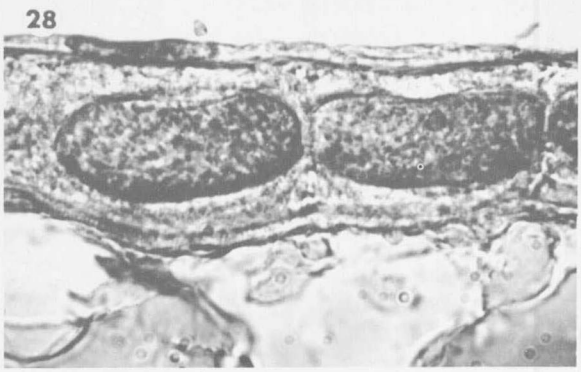

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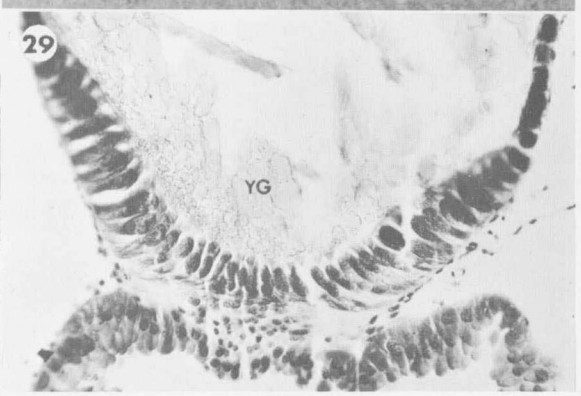

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Vc
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Lod
Pd


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YG

diffuse condition during the deposition of yolk. The resumption of meiosis apparently takes place after the oocyte leaves the ovariole.

During the latter stages of yolk deposition in the proximal ovarian follicle, cellular division greatly increases among cells of the follicular epithelium surrounding the penultimate oocyte. Also, when the most proximal follicle reaches a length of approximately 5.0 mm the penultimate oocyte usually begins to accumulate yolk. It is evident that the developmental events which have occurred earlier in the proximal oocyte are repeated in the same sequence in the penultimate oocyte.

The chorion is produced by the follicle cells immediately after yolk deposition has terminated and just before the yolk-filled oocyte is expelled from the ovariole. The tissue separating the oocyte from the lumen of the pedicel degenerates and finally disappears. The oocyte is then able to pass through the pedicel into the lateral oviduct.

The cells of the follicular epithelium which had recently encased the expelled oocyte also begin to degenerate. The degenerating follicle (fig. 31) shrinks considerably in size after expulsion of the oocyte and has been likened to the corpus luteum of higher animals. This comparison is somewhat ambiguous as no hormonal function can be attributed to this structure. The tunica propria which had recently ensheathed the now empty follicle becomes concentrated as many folds of tissue at the junction between the degenerating follicle and the follicle of the last (previously the penultimate) primary oocyte (fig. 32). The outer ovariole sheath becomes considerably thicker due to the shrinking of the degenerating

follicle. Eventually the follicular cells disappear and the accumulation of enveloping membranes become quite compacted at the base of the ovariole. Usually when a female has experienced successive ovulations, more than one accumulation of follicular membranes can be observed between the pedicel and the proximal oocyte.

The differentiation of follicular epithelium, cytoplasmic augmentation, yolk deposition, chorion formation, oocyte expulsion and follicle degeneration occurs again and again within each ovariole as long as the female is reproductively active.

Although each ovariole is capable of producing one egg each reproductive cycle, there is usually resorption of one or more of the germ cells before yolk deposition is completed. Once resorption begins, yolk deposition for the follicle concerned is terminated. Figures 3, 33, and 34 show oocytes in various stages of resorption.

Resorption may occur at any time in the last or penultimate oocytes after yolk has started to accumulate. It was found however, that the oocyte in the penultimate position was resorbed much less frequently than the oocyte posterior to it. In one case it was noted that the last primary oocyte was completing vitellogenesis whereas the penultimate and antepenultimate oocytes were in the process of resorbing. This condition appears to be quite rare as it was observed to occur only once upon examination of over 300 females.

When egg resorption occurs in the proximal primary oocyte the antepenultimate oocyte begins an accelerated pace of development. The

follicular epithelium differentiates prematurely and the oocyte begins to accumulate yolk while resorption is still in progress in the penultimate oocyte.

It appears that the follicle cells surrounding the degenerating oocyte participate in the early stages of resorption. The cells do not become rhexic until after most of the yolk material has been reduced to a viscous liquid. A relatively small portion of the contents of the follicle is not resorbed. In other investigations this unresorbed portion of the follicle has been referred to as a "resorption body". It consists of a bright orange substance, possibly a carotenoid. This orange substance remains in the ovariole at the proximal end until the oocyte next in line is ready to be expelled. It is not uncommon to observe more than one resorption body at the base of the ovariole which bears testimony that there has been consecutive egg resorption. Such degeneration in an ovariole apparently is not a permanent condition. The ovariole may later form a normal egg after more than one resorption.

The contents of a resorption body is either pushed into the lateral oviduct by the advancing oocyte next in line or is transported by some unknown process. Usually it is possible to see the pigmentation of the resorption body within the lateral oviduct before it has diffused throughout the abundance of mucoid material present.

It is apparent in females ovulating for the first time and have the eggs still in their oviducts, that the number of uncolored degenerating follicles coincides with the number of eggs about to be laid. Ovarioles

from which no egg was produced have follicles displaying various stages of oocyte resorption. These follicles are recognized by either the disintegrating yolk material or by the presence of distinct patches of yellow pigment.

Adult oviducts undergo certain changes in morphology and histology before the first reproductive cycle is completed. Along with the rapid elongation of the gonial portion of the oviduct, the oviducal walls develop epithelial folds or villi (fig. 35). The rapid oviducal elongation is associated with lengthening of the ovarioles, whereas the folding of the oviducal walls exists only when eggs are not present in the duct. The folds in the oviducal walls, upon distention, provide the extra space necessary when the oviduct acts as an ovisac in the temporary storage of eggs.

The oviducal wall becomes more complex as the intermediate layer of cells between the epithelial lining and the thin outer connective tissue sheath differentiates into muscle tissue. There are two layers of striated muscle in the adult oviduct, an outer longitudinal layer and an inner circular layer (fig. 36). The inner lining of the oviducts has returned to the simple columnar form and is bound on the outside by a basement membrane. Between the muscular layers and the basement membrane there is a very thin distribution of connective tissue. This connective tissue, which appears to be of an elastic nature, becomes more concentrated around the insertion of the tracheal endings.

PLATE V
(Figures 30-37)

Fig. 30. Early adult ovariole illustrating the movement of the oocyte nucleus to the posterior end of the older oocytes. Ammerman, Mallory's triple stain, 15X.

Fig. 31. Proximal end of an adult ovariole displaying a degenerating follicle after expulsion of the mature egg. The proximal oocyte immediately behind this structure is being resorbed. Ammerman, Mallory's triple stain, 120X.

Fig. 32. Similar to the preceeding section but showing at a higher magnification the folding of the ensheathing membranes around the degenerating follicle. 400X.

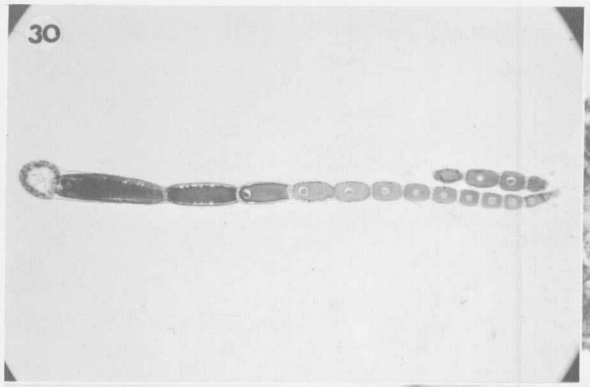
Fig. 33. An adult primary oocyte in the early stages of resorption. Ammerman, Mallory's triple stain, 43X.

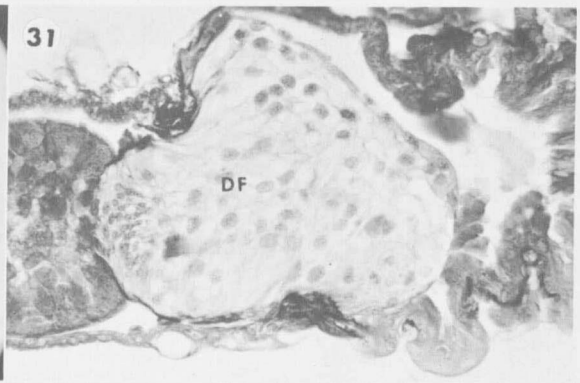
Fig. 34. An adult primary oocyte in a late stage of resorption. The follicle cells have almost all degenerated. Carnoy, Toluidine Blue, 65X.

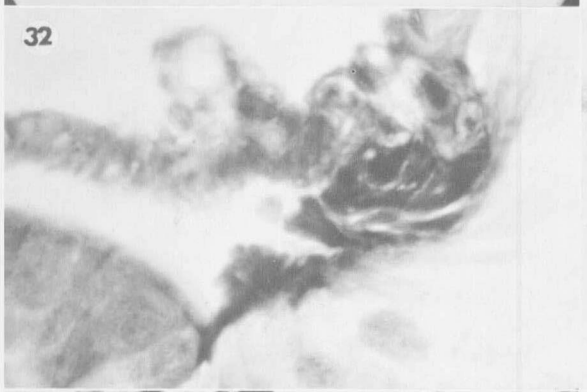
Fig. 35. A longitudinal section of an adult oviduct showing the folding of the oviducal walls. Ammerman, Mallory's triple stain, 40X.

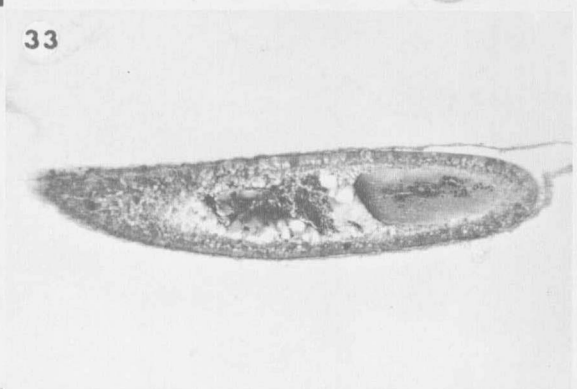
Fig. 36. Similar to the preceeding section but at a higher magnification showing the two layers of muscle tissue perpendicular to each other. 400X.

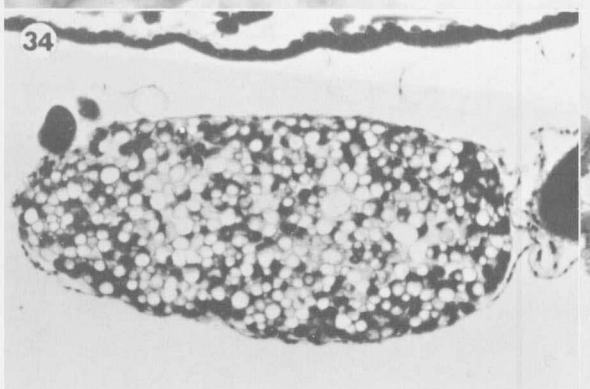
Fig. 37. A longitudinal section of a portion of an adult ovariole. There is a difference in staining intensity between the nuclei of the oocytes and those of the follicular epithelium. Susa, Feulgen, 40X.


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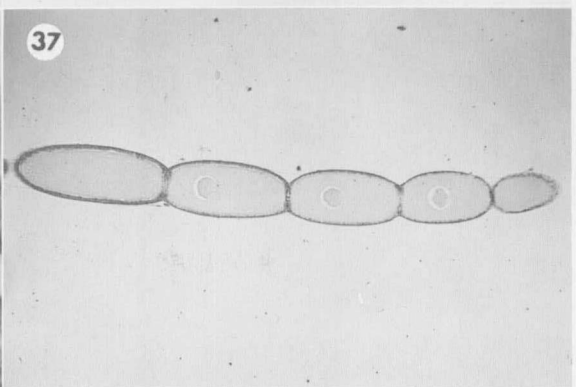

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Cytological and Cytochemical Observations

Observations recorded here were made on the components of ovarian structure which appear to be directly concerned with the process of vitellogenesis, namely the ovarian sheaths, follicular epithelium and primary oocytes.

Nucleic acids. All the methods used to show the presence of nucleic acids gave similar results. Nuclei of prefollicular tissue, of follicle cells and of the outer ovariole sheath all yield a Feulgen-positive reaction (figs. 37, 38 and 39). Nuclei of primary oocytes contained in the approximate upper quarter of the vitellarium react faintly with the Feulgen stain, whereas nuclei of primary oocytes in the remainder of the ovariole are Feulgen-negative. It is interesting to note that when fast green is used as a counterstain, the nuclei of all primary oocytes exhibit the green stain.

When ovarioles are treated with the gallocyanin-chrome alum staining technique, it is found that the nuclei and the cytoplasm of follicle cells and the nuclei of the outer ovariole sheath give a positive reaction (fig. 40). The nuclei and cytoplasm of prefollicular tissue also give a positive reaction. A more intense coloration is displayed by the cytoplasm of follicle cells encasing an oocyte during yolk deposition than cytoplasm of follicle cells surrounding an oocyte undergoing cytoplasmic growth. The cytoplasm of primary oocytes and their nuclei stain considerably lighter than the surrounding follicle cells. However, there are small amounts of darkly staining material within the nuclear network

of those oocytes experiencing cytoplasmic growth. This material appears to be the isolated concentrations of chromatin as described earlier. The presence of ribonucleic acid in the cytoplasm of follicle cells in which yolk deposition is occurring was confirmed by using the methyl green-pyronin Y technique followed by digestion in ribonuclease (fig. 41 cf. fig. 42). The amount of RNA in the cap-like region of the follicle cell that is directed toward the oocyte increases considerably just before vitellogenesis and remains at a high level throughout yolk deposition and chorion formation. Nuclei of the primary oocytes stain only lightly as opposed to the bright blue-green color exhibited by the follicular and ovariole sheath nuclei.

Protein. The mercuric bromphenol blue (Hg-BPB) technique used in studying ovarian tissue is useful in demonstrating concentrations of protein material above the general content found in all animal tissues. It is readily evident that many of the yolk granules making up the deutoplasm are of a proteinaceous nature (fig. 43). These intensely staining granules are relatively small at the periphery of the oocyte and become larger as they progress towards the center of the cell. The cytoplasm of primary oocytes experiencing cytoplasmic growth as opposed to deutoplasmic growth does not stain as darkly as the yolk granules. Nevertheless, the protein concentration is greater than that of the non-germ cells which contribute to ovarian structure.

The tunica propria stains intensely when the Hg-BPB method is employed. The outer ovariole sheath is also rich in proteinaceous

material, but apparently not in as large a quantity as is contained in the inner membrane. Nuclei of the outer ovariole sheath do not stain appreciably, nor does the cytoplasm or nuclei of the follicle cells. It is only when the follicular epithelium begins to produce the chorion that the component cells show recognizable concentrations of protein material in the cap-like regions (fig. 44). The nuclei of follicle cells take the Hg-BPB stain more readily during chorion formation than any time prior to this process.

There appears to be a large amount of nuclear protein present in nuclei of all primary oocytes during the periods of cytoplasmic and deutoplasmic growth. The nuclear network stains more intensely when stained with Hg-BPB than with any of the techniques used previously to display nucleic acids. The nuclear membrane is also protein-rich and appears to have an inner and an outer layer of protein material (fig. 45).

Lipids. The use of Sudan black B as a cytochemical stain specific for certain lipids demonstrates that lipids are abundant and widely distributed in the ovarioles of A. elliotti. Sudanophilia is displayed by all primary oocytes in the vitellarium. However, the cytoplasm of oocytes in which yolk deposition has not yet begun displays much less sudanophilia than those oocytes in which vitellogenesis is in progress.

One of the most conspicuous locations of sudanophilic lipids is at the periphery of the penultimate oocyte and adjacent follicle cells (fig. 46). Here the coloration is so intense that it is difficult to determine the division between the oocyte and follicular epithelium.

PLATE VI
(Figures 38-45)

Fig. 38. Adult penultimate and antepenultimate primary oocytes. The darkly staining nuclei of the follicular epithelium depicts presence of DNA. Susa, Feulgen, 62X.

Fig. 39. Adult penultimate primary oocyte using same technique but with no hydrolysis. The follicle cell nuclei do not stain. 53X (cf. fig. 38).

Fig. 40. A longitudinal section showing a portion of an adult ovariole along side a large proximal oocyte in which yolk deposition has occurred. The oocyte nuclei are lightly stained and the follicle cell and ovariole sheath nuclei are darkly stained. Carnoy, Gallocyanin-chrome alum, 53X.

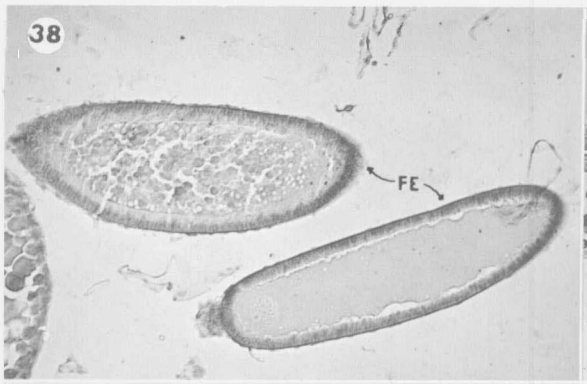
Fig. 41. Follicle cells displaying an abundance of RNA in the cytoplasm during vitellogenesis. Cold acetone, Methyl green-pyronin Y, 1000X.

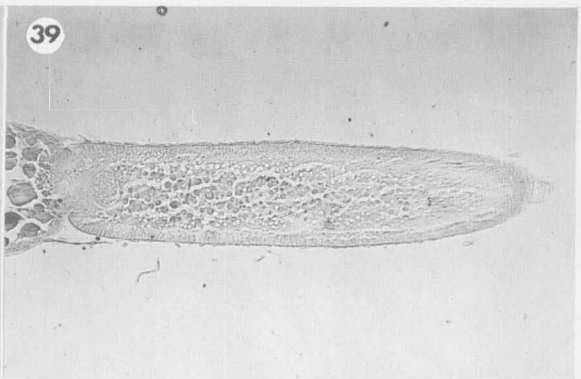
Fig. 42. Similar to the above but this section has been previously digested in ribonuclease. 1000X.

Fig. 43. A portion of an adult penultimate primary oocyte displaying yolk granules giving a positive reaction for protein. Carnoy, Hg-BPB. 400X.

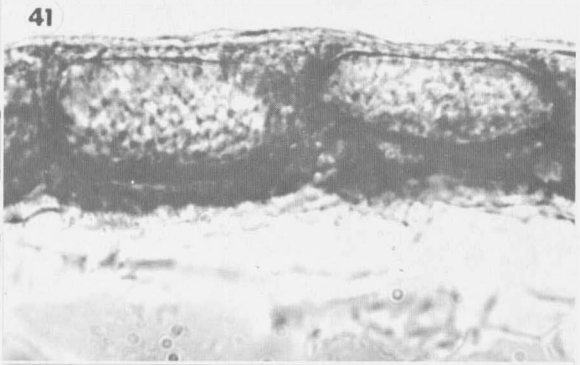
Fig. 44. This section received the same treatment as above but the follicle cells are surrounding an older primary oocyte. The cytoplasm of the follicle cells shows a higher concentration of proteinaceous material than in the preceeding photograph. 790X.

Fig. 45. A nucleus of an adult penultimate primary oocyte showing a noticeable amount of proteinaceous material in the nuclear membrane. Carnoy, Hg-BPB. 710X.

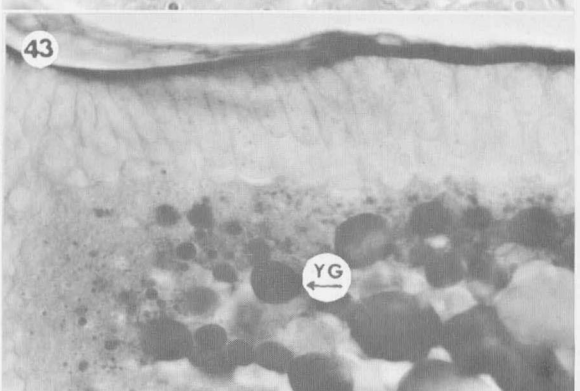

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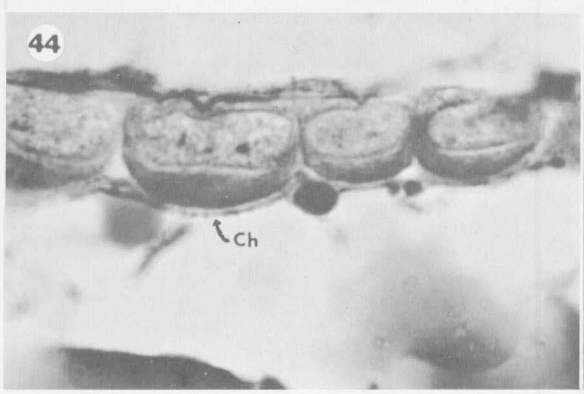

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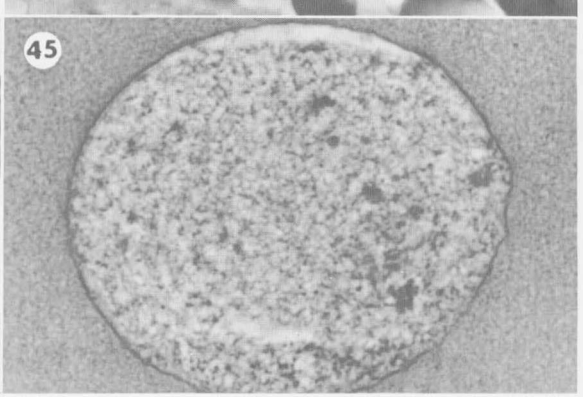

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43
YG


44
Ch


45

When viewed under a microscope at 1250X magnification it is found that the sudanophilic lipid in this region appears as a dense concentration of very fine particles. These lipoid particles are so minute that the portion of the follicle cell directed toward the oocyte has a dark smoky appearance.

Around the non-sudanophilic yolk granules is a layer of sudanophilic material. There are also sudanophilic particles concentrated between the non-lipid yolk granules. The lipoid material found between and around the non-lipoid yolk granules consists of many particles that are considerably larger than those located at the periphery of the oocyte.

Prefollicular tissue surrounding oocytes at the distal end of the vitellarium is intensely colored by the Sudan black B stain. At first it appeared that nuclei of prefollicular tissue and of follicle cells displayed a certain amount of sudanophilia. However, with more careful focusing it became apparent that the coloration came from the surrounding cytoplasm and not from the nuclei.

The outer ovariole sheath shows presence of sudanophilic lipids of the fine particulate type. The nuclei of this tissue do not stain with this method.

Use of osmic acid reduction for demonstration of unsaturated lipids reveals that there are two types of cellular inclusions within certain primary oocytes. In oocytes of the proximal and penultimate position, one recognizes many relatively large globules of yolk material which reduce osmic acid (fig. 47). These osmiophilic globules are as large or larger than the non-osmiophilic granules also present in the deutoplasm of

the penultimate oocyte. This condition was not observed in the proximal oocyte.

Evenly distributed within the cytoplasm of the antepenultimate and the next 4-5 primary oocytes immediately distal, there are some smaller bodies of a granular nature that also display osmiophilia. There is a gradual increase in the number of these osmiophilic inclusions as the oocytes become older. These bodies increase in size either through clumping or individual growth as the primary oocyte gains the antepenultimate position for they are larger than those contained in the cytoplasm of the more distal oocytes.

The nuclear material present in nuclei of all primary oocytes does not stain with the osmic acid technique. The follicle cells and the ensheathing ovariole membranes do not display the black coloration typical of unsaturated lipids. They do, however, exhibit an overall brown color that is darker than tissue showing no affinity for osmic acid.

Although the presence of lipids in ovarioles of the nymphal instars was not ascertained, it was noted that in the proximal oocyte of the fifth instar ovariole a number of peripheral vacuoles were present (fig. 24). When such vacuoles are seen it is often interpreted that material of a lipoid nature may have been extracted by solvents used in the tissue preparation and staining procedures.

Carbohydrates. The periodic acid-Schiff reaction demonstrates the presence of polysaccharides in a large portion of the ovarioles of adult A. elliotti. The ground substance of the cytoplasm in those oocytes which

have no yolk inclusions gives a light colored, but positive reaction (fig. 48). The cytoplasm of all but the two most posterior oocytes is homogeneous and evenly stained. In the peripheral cytoplasm of the penultimate oocyte there are small deutoplasmic inclusions that stain intensely with the PAS technique (fig. 49). As this oocyte becomes older the peripheral yolk bodies appear to coalesce to form the larger yolk granules located toward the center of the oocyte (fig. 50). The granular configuration of the deutoplasm present in the penultimate oocytes is not evident in those oocytes having the proximal position in the ovariole. This is thought to be due to the inability of the infiltrating medium to penetrate an oocyte with its full apportionment of yolk surrounded by the chorion. Nevertheless, yolk material within the proximal oocyte is also strongly PAS-positive.

Nuclei within all the primary oocytes do not yield a PAS-positive reaction. This PAS-negative condition was also exhibited by the nuclei and surrounding cytoplasm of the follicle cells. In contrast to the absence of PAS reactive materials in the follicle cells, the ovariole sheaths react intensely with this staining procedure.

It is well documented that glycogen reacts strongly with the PAS technique. Conflicting results were obtained when the Best's carmine and Bauer-Feulgen procedures were used to determine if all or part of the PAS-positive yolk material was glycogen. Each of the above methods gave a positive reactive on a diffuse granular substance located between the larger yolk granules. However, upon digestion in malt diastase or in

saliva it was found that the staining intensity of this material was not diminished.

Additional studies were made in an attempt to clarify the conflicting results obtained by the above methods. It was observed that treatment of sections of ovarian tissue with dimedone followed by the PAS technique gave no indication of glycogen presence. This procedure is recommended by Bulmer (1959) for demonstration of glycogen in the vicinity of non-glycogenic PAS-positive material.

To further characterize the chemical nature of the PAS-reactive material, a series of separate pretreatments was made before staining the ovarian tissue with the PAS technique. The data are presented in Table I. The section on materials and methods gives the concentrations, lengths of exposure, and other pertinent information relative to the various pre-treatments used. Discussion of the data is given in the next section of this paper.

A modification of the technique of Himes and Moriber (1956) was used to investigate the association of carbohydrate to protein present in adult ovarioles. Cytoplasm of primary oocytes without yolk inclusions has a reticular appearance when stained with this method. The overall coloration is slightly darker than the cytoplasm of the follicle cells which only display affinity for the naphthol yellow S dye. Upon close examination under high power magnification the oocyte cytoplasm exhibits a mosaic appearance in which there is a subtle blending of yellow and red staining material.

Yolk inclusions of the penultimate oocyte give the PAS-positive reaction as when the PAS test was used as the only staining technique. However, the smaller yolk bodies at the periphery of the oocyte do not stain as darkly (fig. 51) as compared with those treated with only PAS reagent. This also appears to be due to a blending of the yellow and red staining material yielding an overall coloration that is lighter than when the peripheral yolk bodies are stained only by the PAS reagent. All yolk contained in the proximal oocyte when stained with the modified Himes and Moriber method exhibits only the rich magenta coloration typical of strongly PAS-positive substances.

Ovariole sheath membranes also display a decrease in the strongly PAS-positive condition observed when treated with PAS reagent. Both membranes have an orange coloration suggesting presence of materials that react positively to both dyes used in this method.

At first it was thought that the orange coloration present in portions of the ovarian tissue was due to diffusion of the two staining solutions producing an artifact. However, upon close examination it was discovered that there are sharply defined borders between structures staining only with the PAS reagent and those staining only with the naphthol yellow S dye.

Although the pretreatment of ovarian tissue sections by the enzyme hyaluonidase did not reduce the intensity of the PAS reaction (Table I), it cannot be concluded that certain mucopolysaccharides do not contribute to the carbohydrate yolk fraction. Conflicting results as to whether hyaluronic acid gives a positive PAS reaction have been obtained by

TABLE I

Effects on various pretreatments on PAS-reactive polysaccharides in ovarioles of A. elliotti

REAGENT	OOCYTE CYTOPLASM	YOLK GRANULES	FOLLICLE CELL CYTOPLASM	OVARIOLE SHEATHS
Periodic acid omitted	Negative	Negative	Negative	Negative
Acetic anhydride and pyridine	Negative	Negative	Negative	Negative
Acetic anhydride and pyridine followed by NH_4OH	Positive	Positive	Negative	Positive
Dimedone	Negative	Negative	Negative	Negative
Chloroform and methanol	Positive	Positive	Negative	Positive
Hyaluronidase	Positive	Positive	Negative	Positive
Pepsin	Positive	Reduced	Negative	Positive

workers in the field of histochemistry (Hotchkiss, 1948 cf. Jeanloz, 1950). For this reason additional studies were made in an effort to determine whether acid mucopolysaccharides might be present in ovarian tissue.

The test for acid mucopolysaccharides using the alcian blue staining technique revealed that yolk inclusions do not give a significant reaction. Nuclei of follicle cells yield a strongly positive reaction to this staining procedure. The cytoplasm of follicle cells also gives a positive reaction as do the ensheathing ovariole membranes. The primary oocyte cytoplasm displays a faint reaction and is considerably lighter in color than tissue forming the follicular epithelium.

The cytoplasm of primary oocytes when stained with toluidine blue is weakly metachromatic which disappears upon alcoholic dehydration. In contrast, cytoplasm of the follicle cells is strongly metachromatic and not alcohol-labile. However, the metachromasia is diminished by treatment with hyaluronidase (fig. 52 cf. fig. 53). The nuclei of primary oocytes, follicle cells, and the outer ovariole sheaths are all orthochromatic. Yolk inclusions are also orthochromatic.

Vital staining. The findings of Ramamurty (1963) on intravital passage of trypan blue into the deutoplasm of Panorpa communis oocytes could not be duplicated with A. elliotti. It was noted, however, that the pericardial cells of A. elliotti take up the dye very readily and hold it indefinitely.

Photoperiodic Study

Most of the observations and results reported here were obtained from

PLATE VII
(Figures 46-53)

Fig. 46. A portion of an adult primary oocyte displaying sudanophilic lipids. There is intense sudanophilia at the boundary between the follicular epithelium and the oocyte. 10% formalin, Sudan black B, 250X.

Fig. 47. A portion of an adult primary oocyte illustrating the presence of osmiophilic fat droplets. 10% formalin, osmic acid, 250X.

Fig. 48. A longitudinal section of an adult ovariole illustrating presence of PAS-positive material. Carnoy, PAS, 17X.

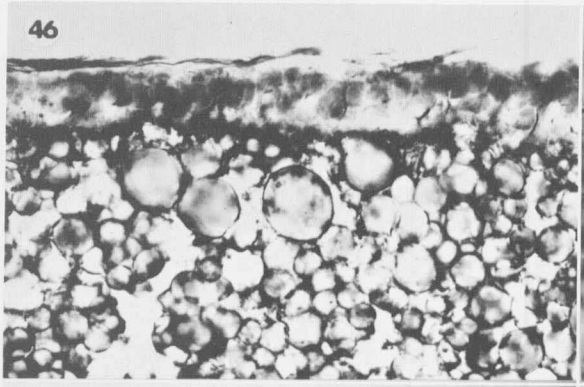
Fig. 49. A longitudinal section of an adult penultimate primary oocyte in the early stages of yolk deposition. There are small granules of PAS-positive material contained in the peripheral ooplasm. Susa, PAS, 77X.

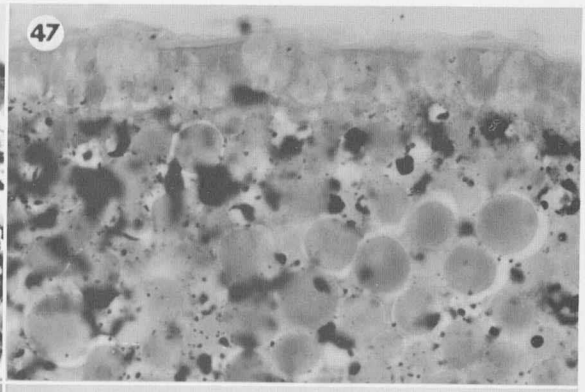
Fig. 50. Adult penultimate and antepenultimate oocytes in which the penultimate oocyte is in a later stage of yolk deposition than in the preceeding photograph. Susa, PAS, 40X.

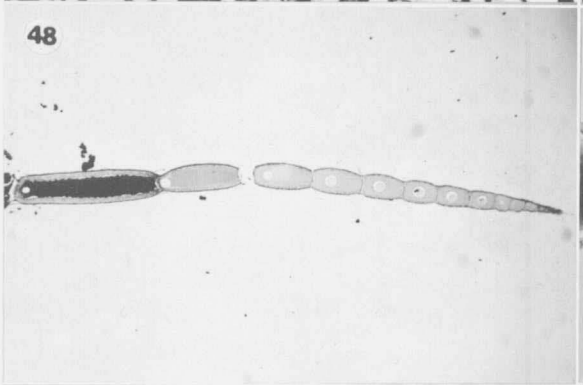
Fig. 51. The proximal end of a penultimate primary oocyte in which yolk is being deposited. The tunica propria is considerably lighter staining than in preceeding sections and the yolk granules become larger as they approach the center of the cell. Susa, PAS-naphthol yellow S, 250X (cf. Figs. 49 and 50).

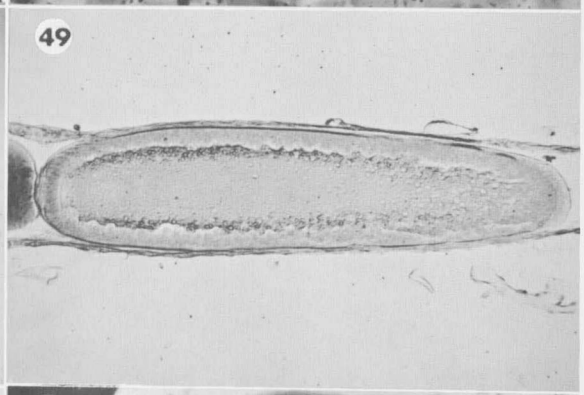
Fig. 52. Follicle cells displaying presence of acid mucopolysaccharides during vitellogenesis. Carnoy, Toluidine Blue, 400X.

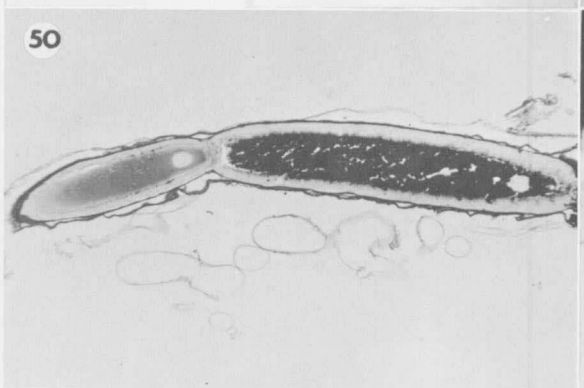
Fig. 53. Similar to the above section but digestion in hyaluronidase preceeded the staining technique. The darkly staining material in the cytoplasm of the follicle cells has disappeared. 400X.

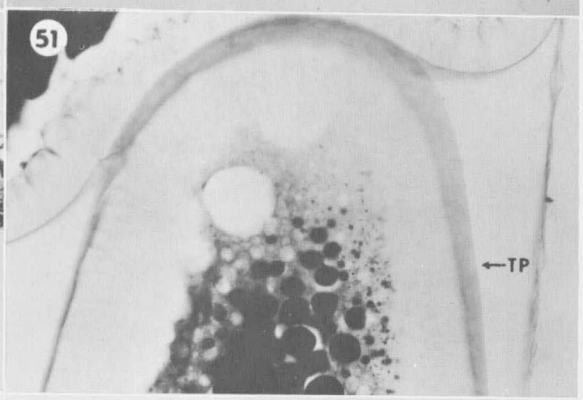

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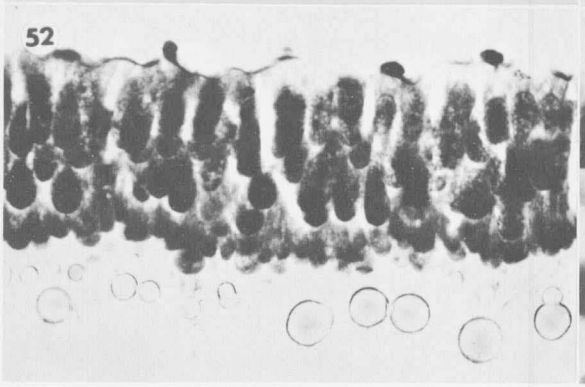

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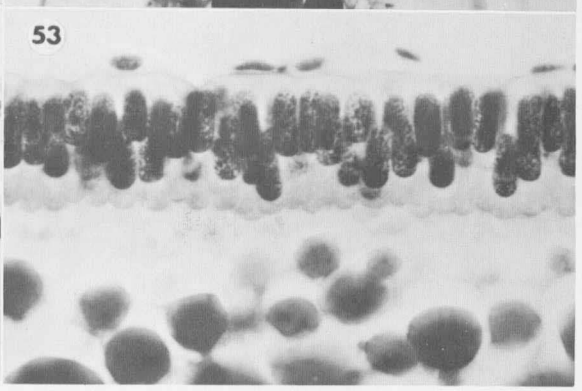

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←TP


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examination of ovaries from 127 females reared in the laboratory under a regime of 13 different photoperiods. The data concerning the effect of daylength upon yolk deposition and oocyte resorption are presented in an appendix at the end of this paper.

In a preliminary investigation it was found that of 15 females examined ten days after adult moult all had either laid their first clutch of eggs or had mature eggs present in their oviducts. Of the 127 females examined seven days after the adult moult only two had mature eggs present in their oviducts and no females had laid eggs. Both groups of females had been maintained under similar conditions.

Three of the above 127 females examined, each of which occupied different cages receiving 9, 3, and 0 hours of light respectively, displayed no noticeable yolk deposition although their ovaries outwardly appeared to be normal. One of the females receiving 15 hours of light per day experience resorption of all first cycle proximal oocytes in both ovaries. It was also noted that one of the females examined in this study had an anomolous condition whereby the oviducts and pedicels were present but ovarioles were not.

A statistical analysis was made of the data obtained from the study investigating the possible effect of varied daylengths upon the rate of vitellogenesis. The experiment was constructed as an unbalanced nested design and analysis of variance was computed accordingly. From this investigation it was determined that the rate of yolk deposition among all females examined was highly variable. Differences among females within

cages were significant at the 1% level with a F value 42.29. No other significant differences could be determined from the data obtained in this study (Table II).

A similar analysis of variance was made on data obtained from a study of the effect of varied daylengths upon oocyte resorption. Here it was also found that the variation among females within cages was far greater than any variation between the cages or groups. The within-sample variance was significant at the 1% level with a F value of 3.02. No other significant differences were found to be present (Table III).

The mean number of ovarioles present per female examined in this study was 9.9 and the mean number of oocyte resorptions per female was 3.4. The average length of a proximal oocyte seven days after a female becomes an adult is 3.98 millimeters.

Results of the 28-day oviposition study from six pairs of cages are recorded in Figure 54. These cages experienced no female mortality during the 28-day laying period. The array of photoperiods represented by the six pairs of cages are 24, 23, 15, 13, 11, 3 and 1 hours respectively. Notice that eggs were not laid by females maintained under constant light or the 3 hour photoperiod.

TABLE II

An analysis of variance concerning the effect of varied daylengths upon rate of vitellogenesis

Source of variation	Degrees of freedom	Sum of squares	Mean of squares
Groups	12	79.155	6.596
Cages within groups	13	99.131	7.625
Females within cages	99	418.732	4.229*
Ovaries within females	118	11.833	.100
Observations within ovaries	549	64.895	.118
Total	791	673.749	

TABLE III

An analysis of variance concerning the effect of varied daylengths upon oocyte resorption

Source of variation	Degrees of freedom	Sum of squares	Mean of squares
Groups	12	34.024	2.833
Cages within groups	13	40.238	3.095
Females within cages	100	226.423	2.264**
Resorptions within females	126	94.500	.750
Total	251	395.186	

* F = 42.229 significant at the 1% level.
** F = 3.02 significant at the 1% level.

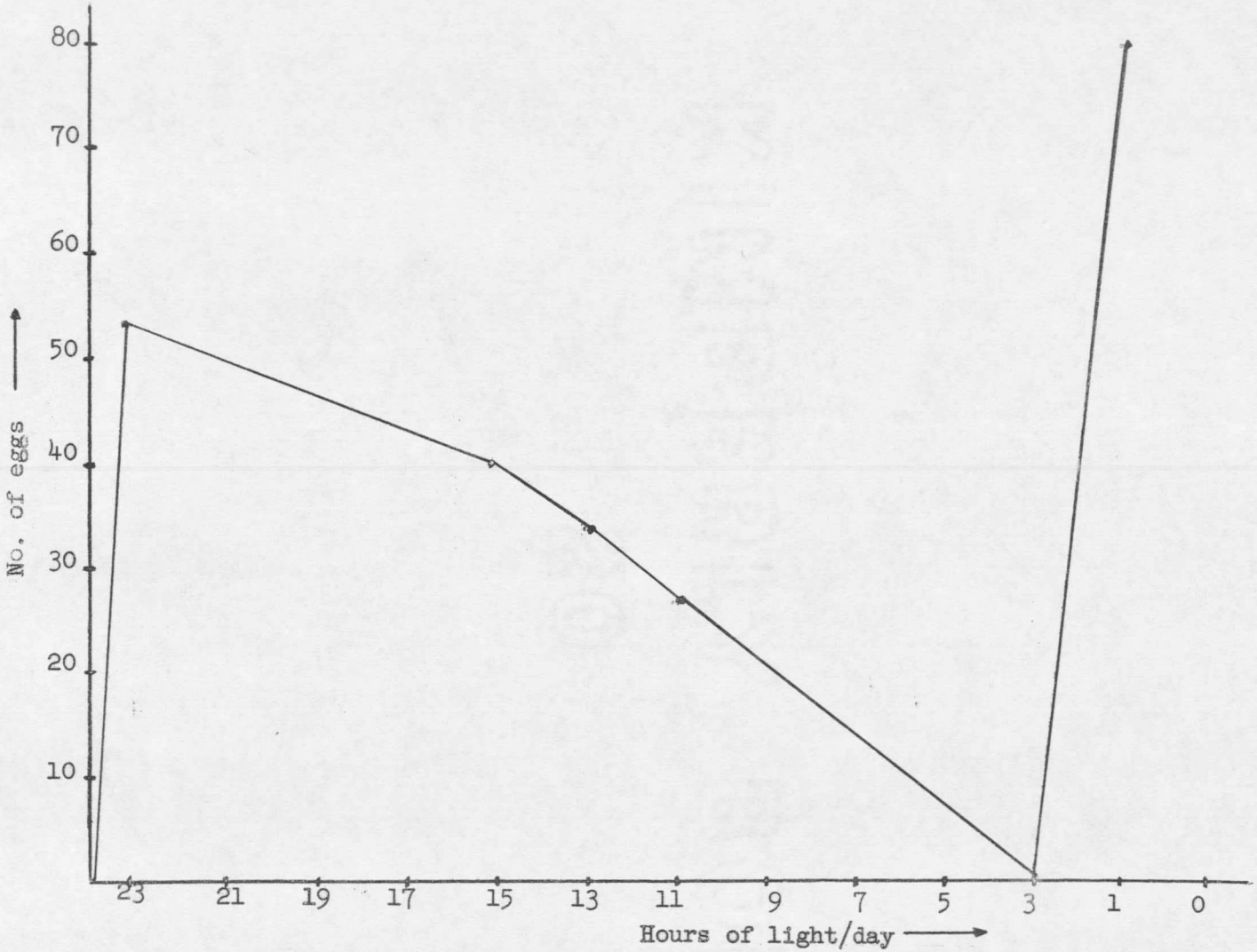


Figure 54. Total number of eggs laid by female A. elliotti exposed to different photoperiods.

DISCUSSION AND CONCLUSIONS

Ovarian Development

The state of development of ovarian rudiments in newly hatched A. elliotti nymphs is in advance of those in newly hatched Melanoplus differentialis nymphs according to the description given by Nelsen (1934b). Ovariole rudiments of A. elliotti at hatching are recognizable as cellular columns, separate from each other, completely enveloped by an outer ovariole sheath, and individually attached to the ventral cell strand. Similar developmental status is not exhibited by M. differentialis nymphs until after the first moult.

A difference in rate of development was also noted by Van Horn (1963) who compared the genitalia of M. differentialis and A. elliotti during embryogenesis. The development of the genitalia of prerevolution M. differentialis embryos lagged behind that of A. elliotti embryos before blastokinesis. It was also observed that this difference in external morphology appeared to be resolved by the time of hatching.

According to the study made by Nelsen (1934b) differentiation of the rudimentary ovariole into a germarium and vitellarium does not become evident in M. differentialis nymphs until the beginning of the fourth instar. Figure 15 in Nelsen's paper shows the proximal end of a fourth instar ovariole in which two oocytes still span the width of the ovariole. This level of ovariole differentiation is surpassed in A. elliotti nymphs during the first part of the second instar.

The postembryonic development of ovarioles, pedicels and lateral oviducts in A. elliotti is essentially the same as found by Nelsen in

his study. However, the overall rate of ovarian development of the grasshopper in this study appears to preceed that of M. differentialis by at least two instars for a major portion of their nymphal lives.

If the adult stage follwed the fifth instar for M. differentialis, it would appear that females of the species would begin adulthood equipped with comparatively underdeveloped reproductive organs. However, M. differentialis nymphs experience a sixth instar in which ovaries and oviducts gain a developmental status coincident with that found in A. elliotti in its preadult (5th) instar. In some respects the ovarian complex of M. differentialis undergoes differentiation in its sixth instar which only occurs in the early adult stage of A. elliotti. For example, Nelsen mentions that there is differentiation of muscle tissue around the lateral oviduct accompanied by formation of epithelial folds or villi during the sixth instar. Similar oviducal morphogenesis in A. elliotti occurs only during the early pre-reproductive adult stage.

The origin of prefollicular tissue present in the germarium and vitellarium of the nymphal and adult ovarioles has been attributed to various sources. Hogben (1920) claims that prefollicular tissue in P. americana is derived from oogonia although Heymons (1892) maintained that only sex cells originated from the germ cell primordium. In more recent studies Bonhag (1958) with P. americana and Krainska (1961) with Cynips folli Linn. have shown that prefollicular tissue and the resulting follicular epithelium are of a somatic, mesodermal origin. Although the germ cell line of A. elliotti was not followed through embryogenesis in

this study, there is no evidence to show that oogonia give rise to prefollicular tissue during the postembryonic ovarian development.

The difficulty encountered by this investigator in attempting to display cell boundaries within prefollicular tissue was also experienced by Bonhag (1959) in a study of adult P. americana ovaries. In attempting to demonstrate cell membranes, special techniques were used by Bonhag with no success. In an earlier study of ovarian development in O. fasciatus, Wick and Bonhag (1955) found that the cellular integrity of prefollicular tissue is maintained. The cell boundaries were observed to appear when the prefollicular nuclei were engaged in mitotic activity.

The development of cellular polarity by the proximal primary oocytes was observed by Nelsen (1934b) to occur in M. differentialis during the sixth instar, whereas this phenomenon occurs in the fifth instar for A. elliotti. It was suggested by Netzel (1965) that movement of the oocyte nucleus to the posterior end of the cell in Gryllus domesticus Linn. may be due to external orienting factors. Netzel found in G. domesticus that the oocyte nucleus exhibited a marked polarity. Nuclear polarity, according to Netzel, is the result of an eccentric positioning of the nucleolus and a cresent granular material along the dorso-caudal quarter of the nucleus. The eccentric positioning of nuclear material was presumed to be the site of the future poles and axes of the coming embryo. Polarization of nuclear material within oocytes of A. elliotti was not observed, although it cannot be said with certainty that it does not occur. Due to the difficulty encountered when sectioning oocytes with their full complement of yolk,

only a few oocyte nuclei were observed in these cells.

The deposition of yolk in penultimate oocytes as found in ovarioles of A. elliotti apprently is not universal to all Acrididae. Highnam, Lusis and Hill (1963) maintain that yolk is not normally deposited in penultimate oocytes of S. gregaria until after the first reproductive cycle. However, in a study of seven British Acrididae, Phipps (1949) found that the penultimate oocyte averaged 3.0 mm in length when the proximal oocyte was ready to be ovulated.

Follicle degeneration and oocyte resorption in insects have long been subjects of study and controversy. Ahrens (1935) observed colorless follicle degeneration in Termes redemani as did Wigglesworth (1936) in Rhodnius prolixus Stal. Vel'tischev (1941) noted pigmentation at the base of ovarioles in Locusta and concluded that it was an indication ovulation had occurred. Bonhag and Wick (1953) observed unpigmented degenerating follicles after eggs had been discharged. The contents of the follicle were later expelled and the remainder of the follicle decreased in size to where it was no longer a conspicuous structure.

Singh (1959), in an attempt to differentiate between pigmented and unpigmented "corpora lutea", concluded that pigmentation in a degenerating follicle was a sign of partial or total oocyte resorption. The uncolored degenerate follicle or "corpus luteum" was a result of the normal degeneration of an empty follicle after a mature egg had been discharged.

Proximal follicle contraction and folding of the tunica propria in A. elliotti following ovulation paralleled the findings of Singh in his

study of follicle degeneration in Locusta migratoria migratorioides (R. & F.) and S. gregaria. Singh maintains that it is the mature egg above the resorption body which pushes the contents of the degenerating follicle into the oviduct during ovulation.

Lusis (1963) objects to the use of the term corpus luteum for the description of a degenerating follicle. He argues, "the term corpus luteum should not be used because at no stage is there any progressive formation of new tissue". He noted that the lipid fraction of the deutoplasm was colored by a B-carotene. Upon concentration or reabsorption of lipid yolk, the carotene crystallizes and forms the pigmented resorption body characteristic of resorbed oocytes.

Lusis observed participation of the follicle cells in the resorption of yolk material before they themselves degenerated. He suggested that all oocytes have an inherent capacity for resorption and the motivating force for resorption depends upon various factors within the insect's body.

Cytology and Cytochemistry

The decrease in Feulgen-positive material within oocyte nuclei during maturation as observed in A. elliotti has been reported by several investigators. Bonhag (1959) noted that the nuclei of P. americana oocytes, when in the diffuse condition, do not yield a Feulgen-positive reaction. Similar results were found by Gresson (1931) in P. orientalis and by Radecka (1962) in T. asynamorus. Although Gresson did not find oogonia in his study sections, Radecka in T. Asynamorus and Kugler, Frankenstein and Hafferty (1956) in P. americana demonstrated the presence of DNA in

nuclei of oogonia contained in the adult germaria.

Observance of Feulgen-negative oocyte nuclei does not necessarily mean DNA is not present. Although some interpret this as a chemical breakdown of DNA during the greater part of oogenesis, most investigators explain Feulgen-negativeness in oocyte nuclei as the result of a dilution effect (Raven, 1961). With the swelling of the oocyte nucleus during maturation, the DNA contained in the chromosomes is dispersed throughout the whole area of this large nucleus. The decrease in intensity of the Feulgen reaction appears to accompany the dilution of positive staining material with greater amounts of non-reacting substances.

Brachet (1947) tested the dilution theory by centrifuging amphibian oocytes before treating with the Feulgen technique. The oocyte nuclei, which are usually Feulgen-negative, were found to have an accumulation of Feulgen-positive material at one side.

Relatively high concentrations of RNA in the ooplasm of developing primary oocytes is not surprising. It is to be expected that RNA would be present in quantity at sites of rapid cellular synthesis. More intriguing is the proportionately higher concentration of RNA within the cytoplasm of the follicle cells. Although these cells are increasing in size, the largely supranuclear location of the pyranophilic material would not seem likely if these cells were experiencing strictly cytoplasmic growth.

Telfer (1961) and Ramamurty (1963) suggest that the follicular epithelium is but a mediating tissue through which substances pass

intercellularly from the haemolymph to the oocyte. In A. elliotti it was noted that the pyranophilia increases among the follicle cells surrounding oocytes experiencing yolk deposition and chorion formation. Taking this into account, it appears that follicle cells may actively participate in the metabolic processes which contribute to the development of oocytes.

Although the technique used to demonstrate proteins in this study has been criticized as relatively non-specific (Baker, 1958), the intensity of staining leaves little doubt as to the high concentration of proteinaceous material present within the deutoplasm. Similar observations were made by Radecka (1962) and Bonhag (1955a) when using the same technique.

The appearance of small proteid yolk granules or platelets around the periphery of the oocyte during vitellogenesis has been well documented in numerous studies (Raven, 1961). Even though the sites of protein synthesis in oocytes of A. elliotti could not be definitely located, it would appear that at least certain precursors of proteid yolk come from the follicular epithelium. The presence of high concentrations of RNA in the follicle cell cytoplasm adds a certain amount of circumstantial evidence to this supposition. In Tachycines, von Kraft (1960) concluded that proteid yolk granules are formed in the follicle cells and are passed on as such to the oocyte. Granules of proteinaceous material, similar in size to those found at the periphery of the oocyte, were not found in the follicle cells of A. elliotti. In this insect it appears that the granular

structure of yolk material is produced within the oocyte. Yolk granules become progressively larger after making their first appearance in the peripheral ooplasm.

The nucleolar emission bodies thought to give rise to proteid yolk by many of the earlier workers were not observed in the primary oocytes of A. elliotti during deposition. This may have been the result of the biased selection of staining techniques used, although many of the methods that were employed are specific for nucleic acids.

Proteid yolk granules may be complexed with polysaccharides or lipids. Bonhag (1955a) in Oncopeltus, von Kraft (1960) in Tachycines, and Bonhag (1956) in Anisolabis found carbohydrates admixed with protein in yolk granules. Evidence accumulated in the study on A. elliotti oocytes shows that a protein-carbohydrate complex in the non-extractable yolk fraction is credible. The reduction in staining intensity of the PAS-positive yolk after digestion with pepsin, the similar location of PAS and bromphenol treated yolk granules, and results received by concurrent staining with the PAS and naphthol yellow S method all indicate a protein-carbohydrate yolk complex.

Results obtained by staining A. elliotti ovarioles with the Sudan black B method are in accordance with those found by Bonhag (1955b) in ovarioles of Oncopeltus. Nath, Gupta and Lal (1958a) in Periplaneta and Mulnard (1954) in Acanthoscelides also found lipoid material appearing first in the peripheral ooplasm at the onset of vitellogenesis. That follicle cells in A. elliotti supply at least a portion of the lipoid

yolk is substantiated by presence of similar appearing sudanophilic material in the peripheral ooplasm and in the cytoplasm of adjacent follicle cells.

The appearance of vacuoles in the peripheral cytoplasm of proximal oocytes of fifth instar A. elliotti nymphs is not conclusive evidence for presence of lipids. However, it is reasonable to suppose that substances of a lipoid nature may have been extracted by solvents used with the paraffin method. Further study on nymphal ovarian tissue is required to verify presence or absence of lipid concentrations in oocytes of preadult stages.

The large globules of apparently unsaturated lipid present in oocytes treated with osmic acid were not visible in oocytes treated with the Sudan black B method. It would seem probable that these globules, if lipid, should be rendered visible with a general staining technique such as the Sudan black B method. There are two possible explanations for the apparent disagreement of results obtained using these two methods:

1. Staining of lipid by the Sudan black B method depends upon the lipid solubility of the azo dye (Barka & Anderson, 1963). The staining effect is determined by the partition of the dye between the solvent and the lipid substance. Partition of the Sudan dye between the solvent and the lipid is such that usually a large amount of dye is dissolved by the lipid. Consequently, if the Sudan black B is not dissolved by the unsaturated lipids making up the large globules, no staining will result. On the other hand, osmic acid will only be complexed at the sites of

unsaturated lipids to the exclusion of saturated ones.

2. In oocytes of Chrotogonus Gupta (1958a) found that by treating the ovarian tissue with 1% phenol before staining with Sudan black B dye, he could unmask certain large globules of lipoid yolk. Previously, without the phenol treatment only an outer sheath around the yolk spheres could be demonstrated. Although a sheath of sudanophilic material was exhibited by most of the yolk spheres contained in oocytes of A. elliotti, it appears unlikely that all are of a lipoid nature.

Many investigators have concluded that Golgi elements within insect oocytes are connected with or directly responsible for the production of lipoid yolk. In oocytes of Periplaneta Nath and Mohan (1929), Gresson (1931), and Nath et al (1958a) reported that lipoid yolk is formed by the Golgi apparatus. Essentially in agreement were the findings of Ries and van Weel (1934) concerning Pediculus oocytes and those of Nath and Mehta (1929) on Luciola gorhami. Recently, Jamieson and Palade (1966) have described yet another function of the Golgi apparatus. They found it to be active in the intracellular transport of secretory proteins.

The numerous osmiophilic bodies present in the cytoplasm of vitellogenic oocytes of A. elliotti resemble Golgi bodies as described by Nath and Mohan (1929) and the L_1 bodies of Nath et al (1958a). Although these sites appear to increase in size as the oocyte becomes older, it could not be ascertained whether they were responsible for forming the large globules of osmiophilic material in the proximal and penultimate oocytes. Perhaps additional study using the specialized techniques such as labeled isotopes

and the electron microscope would aid in uncovering a possible connection between the Golgi complex and lipid synthesis in insect oocytes.

The periodic acid-Schiff test is based upon two well documented reactions. Upon treatment of sugar molecules with periodic acid, there is a conversion of 1, 2 glycols into dialdehydes. A substituted dye complex is formed by reacting the resulting dialdehydes with Schiff's reagent.

Hotchkiss (1948) has pointed out that a reaction is not given by compounds in which the glycol groups are substituted. For this reason, the pentose portion of DNA or RNA will not react with Schiff's reagent, nor will the hydroxyamino acid residues of proteins.

Pearse (1953) listed five groups of compounds that can be expected to react with the PAS test in sections prepared by the paraffin method. These compounds are: 1) polysaccharides (glycogen), 2) acid and neutral mucopolysaccharides, 3) muco- and glycoproteins, 4) glycolipids, and 5) unsaturated lipids and phospholipids.

That hydroxyl groups of 1, 2 glycols in ovaries of A. elliotti were made visible by the PAS-method was proven by the reversible acetylation technique of McManus and Cason (1950). Upon treatment with acetic anydride + pyridine the hydroxyl groups of 1,2 glycols are prevented from giving a positive PAS reaction. When the acetyl groups were removed by treatment of ovarian sections with NH_4OH, the PAS-positive localizations were restored (Table I). This test provides corroborating evidence for the assumption that the PAS reaction in the ovarioles is due to complexed

carbohydrates and not as a result of the presence of amino groups.

It is concluded that glycogen is not present in ovarioles of adult A. elliotti, although two of the tests used for its demonstration displayed a coloration that is usually typical of glycogen. Because the coloration depicting presence of glycogen in sections stained with Best's carmine and the Bauer-Feulgen methods could not be removed with malt diastase or salivary ptyalin, it appears that something other than glycogen is being stained. Bonhag (1956) in a study of PAS-reactive substances in oocytes of A. maritima found that glycogen, depicted by the Best's carmine method, could not be verified by use of salivary amylase treated controls. The elimination of a PAS reaction upon pre-treatment with dimedone provides further evidence that glycogen is not present in ovarioles of A. elliotti (Table I). Brief dimedone treatment abolishes the PAS reaction of other normally reacting substances but does not affect the reaction of glycogen granules (Bulmer, 1959).

Radecka (1962) demonstrated the presence of glycogen in oocytes of Tachycines as did Bonhag (1956) in Anisolabis. However, glycogen was not found in oocytes of Periplaneta (Kugler et al, 1956; Bonhag, 1959) or in oocytes of Oncopeltus (Bonhag, 1955a). Eschenberg (1965) noted that diastase-sensitive yolk was not present in oocytes of Gerris remigis.

Results recorded in Table I show that the PAS-positive reaction displayed by compounds within the ovarioles is not from aldehyde groups of glycolipids. It appears very likely that other lipoid materials, which were not removed by the paraffin method, would be extracted by prolonged

treatment with the methanol-chloroform solution. Hence, no lipoid substances should be contributing to the positive PAS reaction appearing in ovarioles of A. elliotti.

A reduction in the staining intensity of the deutoplasm in oocytes pretreated with pepsin could mean that yolk containing uncomplexed protein is contributing to part of PAS-positive reaction (Table I). Although Hotchkiss (1948) maintains hydroxyamino acids will not yield a positive PAS reaction, Gregg, Clermont and LeBlond (1952) disagree. These authors stated, "the presence of the hydroxyamino acids, serine or threonine at the end of protein chains, or of hydroxylysine at any position in the chain would give rise to periodic acid-Schiff reactions since the α-amino alcohol groups are exposed". In spite of this statement, these authors felt that such amino acid arrangements must be uncommon because upon testing a number of proteins know to contain hydroxyamino acids, a positive PAS reaction could not be demonstrated.

Schrader and Leuchtenberger (1951) suggested that a negative PAS reaction after pepsin digestion, "may be due to the secondary depolymerizing action and loss of the polysaccharides from cellular material after union of the carbohydrate-protein is split by pepsin". Taking this into account, the reduction of the PAS reaction due to prior pepsin digestion appears to be strong evidence for presence of protein-carbohydrate complexed compounds in at least a portion of oocyte yolk in A. elliotti. This supposition is supported by results of the Hg-BPB test for proteins, of the PAS-naphthol yellow S test for carbohydrates and proteins, and of the various

aforementioned pretreatments.

The fact that pepsin-treated ovarian sections did not yield a totally PAS-negative reaction is good reason to think that some other substance or substances are contributing to the PAS-positive reaction. Upon consideration of the previous results received in this study, the only other compounds that are known to react to the PAS test are mucopolysaccharides.

A few investigators have found that acid mucopolysaccharides react positively to the PAS test. They are, however, in the minority. McManus and Hoch-Ligeti (1952) are of the opinion that experiments of investigators proclaiming a PAS-positive reaction with acid mucopolysaccharides are largely contradictory. The reason given by McManus and Hoch-Ligeti was that the samples of mucopolysaccharides used in these studies were variable and, in some cases, of questionable purity.

Because follicle cells of A. elliotti were found to be strongly orthochromatic when stained with toluidine blue and PAS-negative, it would appear that in this case acid mucopolysaccharides are not stained with the PAS technique. The alcohol-labile metachromasy exhibited by oocytes experiencing yolk deposition suggests that acid mucopolysaccharides are not as abundant as in the surrounding follicle cells. King and Koch (1963) found the exochorion of the egg cuticle of Drosophilia to be rich in acid mucopolysaccharides. Perhaps the major portion of the acid mucopolysaccharides in follicle cells of A. elliotti is deposited in the egg chorion rather than in the yolk.

By using the process of elimination, it appears that the PAS-positive

compounds not removed by pepsin or the other pretreatments must be a neutral mucopolysaccharide fraction. Consequently, it can be concluded that yolk of ovariole-contained oocytes is most likely made up of muco- or glycoproteins and mucopolysaccharides in addition to a lipid fraction.

The PAS-reactive yolk material within oocytes of Gerris remigis was tentatively classified as mucoprotein, glycoprotein, or neutral mucopolysaccharide in complex with protein (Eschenberg, 1965). PAS-positive yolk granules of Oncopeltus and of Anisolabis were concluded by Bonhag (1955a, 1956) to consist of protein-carbohydrate complexed substances. However, he found no evidence of acid mucopolysaccharides in ovarioles of Oncopeltus. The absence of acid mucopolysaccharides in Oncopeltus is interesting because it was suggested that the protein-carbohydrate material of which the yolk consists may be a mucoprotein. According to White, Handler and Smith (1964), acid mucopolysaccharides are usually present in tissue combined with proteins.

Although the origin of small PAS-positive yolk granules appearing first at the periphery of the oocytes is unknown, a relationship to the adjacent follicle cells is suggested. If follicle cells are responsible for producing these small yolk granules, then the PAS-negative reaction displayed by these cells is even more puzzling. Perhaps the concentration of yolk granules or yolk precursors in any one follicle cell is so low that the PAS technique is not sensitive enough to detect it.

I would again like to stress the significance of the follicular epithelium concerning its role in the process of vitellogenesis. It

would appear that follicle cells, especially in panoistic ovarioles, provide the egg with more than a chorion. The evidence shows that the metabolic activity within these cells is such that it is not consistent with the views of investigators who advocate a relatively passive role for the follicular epithelium during vitellogenesis. It is my opinion that the assembly of yolk granules from extracellular material takes place in the oocyte and that follicle cells participate in the acquisition and possibly conversion of these nutrients before they enter the oocyte.

Photoperiodism

Results obtained from the experiment to test the effect of varied photoperiods upon rate of oocyte maturation and oocyte resorption were inconclusive. From the analysis of the data it appears that the inherent variability of female A. elliotti was such that a response to a changing photoperiod under these experimental conditions could not be determined. This may be due in part to the criterion used for obtaining the data, although other workers have successfully used this technique.

Middlekauff (1964) examined the effect of short day and long day photoperiods upon oocyte maturation in Melanoplus devastator. It was discovered that a short day ($9\frac{1}{2}$ hours) photoperiod enhanced egg development, whereas it was retarded by a long day ($12\frac{1}{2}$-14 hours) photoperiod. Norris (1957) has found that sexual maturation in S. gregaria progresses more rapidly in females reared under short day photoperiods. Conversely, in N. septemfasciata, Norris (1962) noted that when females were reared under a 12 hour photoperiod and then shifted to a 13 hour photoperiod

after adult emergence, reproductive diapause could be averted.

Though the present study did not demonstrate increased oocyte resorption in females submitted to aberrant photoperiods, investigators have shown that certain unfavorable environmental conditions will induce resorption of eggs. In Locusta migratoria Singh (1958) demonstrated that oocyte resorptions would increase upon a decrease in the optimum rearing temperature or food withdrawal. Oocytes of dytiscid beetles continually form and resorb during the winter hibernating months (Joly, 1945). De Wilde (1954) also observed successive maturation and resorption of oocytes in hibernating Leptinotarsa. Highnam et al (1963) concluded that the neurosecretory system in S. gregaria controlled oocyte resorption. Any factor which will depress the neurosecretion controlling haemolymph protein concentration will likewise decrease the amount of protein available to the developing oocytes. It was suggested that resorption in oocytes occurs when adequate amounts of haemolymph protein are not available.

Although the oviposition rate of only a small number of individuals was examined, there appears to be a trend for these females to lay fewer eggs as daylengths decrease (fig. 54). On the other hand, a decrease in the number of eggs laid during a 28-day period was not exhibited by females receiving only one hour of light per day. If the curve represented in Figure 54 is not the result of sampling error due to the small number of individuals examined, then an interesting situation exists. It would appear that for optimum egg production in this insect a one hour

photoperiod is equally as good or better than 23 hours of light per 24 hour day.

If indeed oviposition rate is a function of daylength, then it appears that there are two critical periods within a 24 hour cycle where female A. elliotti are the most photosensitive. The first period of photosensitivity is between 24 hours and 23 hours of light per day and the second critical period falls somewhere between a three and a one hour photoperiod.

Sharply defined critical daylengths, by which the timing mechanism of an organism makes the appropriate metabolic changes, have been observed to occur in insects as well as many other animals. Norris (1965) has shown that the critical period of photosensitivity for Nomadacris septemfasciata, when imaginal diapause is induced, is located between a 12 and a 13 hour daylength. In Pectinophora it was found that diapause could be induced in individuals by maintaining them under a daylength of 13 hours or less and averted in individuals having 13.25 hours or more light per day (Adkisson, 1964).

Bunning (1960) has set forth an hypothesis in which he postulates an endogenous rhythm of cellular activity that consists of two half-cycles, one of them "light requiring" (or photophil) and the second "dark requiring" (or scotophil). Photoperiodic induction by long days is effected when daylight extends into the scotophil part of the endogenous cycle; it fails when it is restricted to the photophil. Short day photoperiodic induction fails when the photoperiod extends into the scotophil and succeeds when it

is restricted to the photophil.

Because no eggs were laid by females exposed to constant light, it would appear that the photoperiod must be interrupted by a period of darkness in order that oviposition might occur. Not only must the photoperiod be interrupted by darkness, the period of darkness must occur at the precise time of day when the insect can be stimulated by it. It is possible that the dark period did not come at the right time during the 24 hour cycle for those females reared under a 3 hour photoperiod. Hence, these individuals received no stimulation to induce them to oviposit their eggs. The decrease in oviposition rate with shorter daylengths could be due to a reduction in the sensitivity of the photoreceptor organ in response to a dark period occurring at the wrong time of day. Therefore, the stimuli affecting the neurosecretory mechanism controlling oviposition would also decrease resulting in the reduction of egg production by A. elliotti females. Additional study using a histochemical staining technique for neurosecretory material could possibly determine the validity of this hypothesis. With the use of such a technique the relative amounts of neurosecretory material in the brain and corpora cardica of ovipositing A. elliotti exposed to different daylengths could be compared. It may be that females laying fewer eggs would also display a corresponding decrease in neurosecretion.

Because females submitted to 24 hour and 3 hour photoperiods laid no eggs, it would be expected that there would be total resorption of proximal oocytes throughout the 28-day laying period. However, total

resorption was not observed in females maintained under these photoperiods in the prior study where the rate of vitellogenesis and oocyte resorption was examined. Oocyte resorption has been observed to occur at any time during yolk deposition up until the chorion has been formed. It is possible that proximal oocytes of the non-ovulating individuals were resorbed after the arbitrary seven-day criterion used in the previous study.

There is a possible selective advantage for ovipositing A. elliotti females to lay more eggs with the longer daylengths. It would seem beneficial to the species if most of the eggs were laid before the onset of shorter daylengths accompanied by colder temperatures not optimal for embryonic development. It is suggested that more study should be undertaken examining the relationship between photoperiod, temperature, and oviposition to determine the validity of this hypothesis.

APPENDIX.

TABLE IV

Raw data concerning the mean proximal oocyte length measured in millimeters, total number of resorbed oocytes per female, and total number of ovarioles for each female obtained from the photoperiod study.

Cage No.	♀ No.	Hours of daylight	Ovary A	Ovary B	Mean oocyte length- A & B	Total No. resorptions	Total No. ovarioles
1	001	23	female anomolous, no ovarioles				
1	002	23	2.9	3.1	3.0	1	10
1	003	23	2.7	2.9	2.8	5	10
1	004	23	1.2	1.2	1.2	0	10
1	005	23	3.4	3.4	3.4	3	9
1	006	23	4.0	3.9	3.9	3	10
2	007	23	4.1	4.2	4.2	3	10
2	008	23	3.5	2.0	3.0	7	10
2	009	23	4.9	4.9	4.9	5	10
2	010	23	4.0	4.0	4.0	7	10
2	011	23	4.5	4.5	4.5	4	10
3	012	21	3.8	3.9	3.8	3	10
3	013	21	3.9	4.0	4.0	6	11
3	014	21	4.1	4.2	4.2	4	10
3	015	21	4.4	3.0	3.5	2	11
3	016	21	2.9	0	2.9	8	10
4	017	21	4.9	5.0	5.0	1	10
4	018	21	4.0	3.9	3.9	5	10
4	-19	21	4.6	4.5	4.5	3	10
4	020	21	4.6	4.2	4.3	1	10
4	021	21	1.9	1.8	1.9	0	10
5	022	19	3.4	3.2	3.3	2	10
5	023	19	4.1	3.4	3.7	5	10
5	024	19	4.9	5.0	5.0	2	10
5	025	19	2.2	2.4	2.3	1	10
5	026	19	4.9	5.0	4.9	3	10
6	027	19	4.5	4.6	4.5	3	10
6	028	19	3.3	3.4	3.3	5	10
6	029	19	4.3	4.2	4.2	1	10
7	030	17	4.0	4.2	4.1	1	11
7	031	17	3.9	4.0	4.0	4	10
7	032	17	3.3	3.4	3.4	4	10
7	033	17	4.0	3.9	4.0	1	10
7	034	17	4.9	4.9	4.9	5	10
8	035	17	4.0	4.0	4.0	1	9
8	036	17	4.8	4.9	4.9	5	10
8	037	17	4.3	4.3	4.3	4	10
8	038	17	4.3	4.2	4.3	5	10

TABLE IV
(Continued)

Cage No.	♀ No.	Hours of daylight	Ovary A	Ovary B	Mean oocyte length- A & B	Total No. resorptions	Total No. ovarioles
8	039	17	4.9	4.9	4.9	3	10
9	040	15	3.6	3.2	3.4	2	8
9	041	15	3.5	3.5	3.5	2	10
9	042	15	4.5	4.5	4.5	3	10
9	043	15	4.1	4.5	4.3	4	10
9	044	15	4.2	4.3	4.2	3	10
10	045	15	4.1	0.0	4.1	6	10
10	046	15	0.0	0.0	0.0	10	10
10	047	15	3.8	3.9	3.9	2	10
10	048	15	3.1	3.1	3.1	2	10
10	049	15	3.1	3.0	3.1	2	10
11	050	13	4.6	4.1	4.3	8	10
11	051	13	3.0	3.2	3.1	4	10
11	052	13	4.0	4.1	4.0	2	10
11	053	13	4.5	4.3	4.4	7	10
11	054	13	4.1	4.8	4.4	5	10
12	055	13	2.3	2.4	2.3	1	10
12	056	13	4.4	4.5	4.4	5	10
12	057	13	4.2	4.1	4.1	5	10
12	058	13	3.5	4.4	4.0	4	11
12	059	13	4.4	4.4	4.4	4	10
13	060	11	4.8	5.1	5.0	2	10
13	061	11	4.8	4.9	4.8	3	10
13	062	11	4.0	4.1	4.0	2	10
13	063	11	5.1	5.0	5.1	6	10
13	064	11	4.1	4.1	4.1	2	10
14	065	11	4.1	4.0	4.1	2	10
14	066	11	4.4	4.4	4.4	2	8
14	067	11	4.7	4.6	4.6	5	10
14	068	11	3.0	3.0	3.0	7	10
14	069	11	4.8	5.0	4.9	6	11
15	070	9	4.1	4.2	4.2	1	8
15	071	9	2.7	3.1	2.9	0	10
15	072	9	4.0	4.0	4.0	3	10
15	073	9	4.0	4.1	4.1	4	10
15	074	9	4.9	5.0	4.9	2	10
16	075	9	2.9	2.8	2.8	1	10
16	076	9	no noticeable yolk deposition				10
16	077	9	2.4	2.4	2.4	0	9
16	078	9	3.1	3.0	3.0	1	11
16	079	9	4.0	4.0	4.0	3	10

TABLE IV
(Continued)

Cage No.	♀ No.	Hours of daylight	Ovary A	Ovary B	Mean oocyte length- A & B	Total No. resorptions	Total No. ovarioles
17	080	7	5.3	5.5	5.4	1	10
17	081	7	3.2	0.0	3.2	9	10
17	082	7	2.0	2.0	2.0	0	11
17	083	7	4.8	4.7	4.8	3	10
17	084	7	4.8	4.9	4.9	1	8
18	085	7	4.6	0.0	4.6	8	10
18	086	7	4.3	4.2	4.3	4	11
18	087	7	4.7	4.9	4.8	5	9
18	088	7	3.5	3.8	3.6	3	10
18	089	7	4.0	3.9	4.0	7	10
19	090	5	3.9	3.8	3.8	4	10
19	091	5	4.0	4.0	4.0	1	10
19	092	5	4.3	4.3	4.3	2	10
19	093	5	0.0	3.8	3.8	10	12
19	094	5	3.4	3.4	3.4	6	8
20	095	5	2.7	2.5	2.6	0	10
20	096	5	5.0	5.0	5.0	3	10
20	097	5	4.9	4.9	4.9	5	9
20	098	5	4.4	4.2	4.3	3	10
20	099	5	4.6	4.5	4.5	2	10
21	100	3	3.1	3.3	3.2	2	10
21	101	3	2.1	2.6	2.4	4	10
21	102	3	3.0	3.0	3.0	0	10
21	103	3	5.0	5.0	5.0	1	10
21	104	3	2.9	3.1	3.0	3	10
22	105	3	4.7	4.6	4.7	1	10
22	106	3	5.2	5.2	5.2	3	10
22	107	3	4.6	4.6	4.6	4	10
22	108	3	3.9	4.0	4.0	6	12
22	109	3	no noticeable yolk deposition				10
23	110	1	5.0	5.0	5.0	2	10
23	111	1	4.0	4.1	4.1	6	10
23	112	1	5.0	4.9	4.9	3	10
23	113	1	4.2	4.2	4.2	4	10
24	114	1	4.0	4.0	4.0	2	10
24	115	1	5.4	5.4	5.4	5	10
24	116	1	3.9	0	3.9	8	10
24	117	1	5.0	5.1	5.0	3	9
25	118	24	4.9	4.7	4.8	4	10
25	119	24	3.4	3.3	3.4	4	10

TABLE IV
(Continued)

Cage No.	♀ No.	Hours of daylight	Ovary A	Ovary B	Mean oocyte length- A & B	Total No. resorptions	Total No. ovarioles
25	120	24	2.8	2.9	2.9	6	11
25	121	24	no noticeable yolk deposition				10
25	122	24	4.0	4.3	4.1	5	10
26	123	24	4.0	4.2	4.1	5	11
26	124	24	0	3.1	3.1	7	10
26	125	24	4.2	4.1	4.1	3	10
26	126	24	3.9	3.9	3.9	3	10
26	127	24	4.7	4.9	4.8	4	10
					Mean = 3.98	Mean = 3.4	Mean = 9.9

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