INTERNAL ANATOMY AND SALIVARY GLAND ULTRASTRUCTURE OF THE SPINOSE EAR TICK (OTOBIUS MEGNINI DUGES) WITH NOTES ON WATER VAPOR UPTAKE AND SALIVARY GLAND DEGENERATION

BY

KERRY WADE STRICKER

Bachelor of Agriculture
Oklahoma State University
Stillwater, Oklahoma
1988

Master of Science
Oklahoma State University
Stillwater, Oklahoma
1990

Submitted to the Faculty of the
Graduate College of the
Oklahoma State University
in partial fulfillment of
the requirements for
the Degree of
DOCTOR OF PHILOSOPHY
December, 1993

INTERNAL ANATOMY AND SALIVARY GLAND
ULTRASTRUCTURE OF THE SPINOSE EAR
TICK (*OTOBIUS MEGNINI* DUGES)
WITH NOTES ON WATER VAPOR
UPTAKE AND SALIVARY
GLAND DEGENERATION

Thesis Approved:

Robert W. Barker
Thesis Advisor

Charlotte L. Ownby

Richard Berberet

John R. Sauer

Thomas C. Collins
Dean of Graduate College

ACKNOWLEDGEMENTS

I would like to thank Dr. Robert W. Barker for his patience, generous help, and good advice in the completion of my research and preparation of this manuscript. Also, much thanks are extended to the other members of my committee, Dr. John R. Sauer, Dr. Richard C. Berberet, and Dr. Charlotte L. Ownby for their helpful guidance.

I also wish to thank Marie Montelango and Bill Tyner for their help in raising ticks and procuring other equipment and supplies necessary for my work. Also, much gratitude is owed to Janice Pennington Green and Ginger Baker at the Oklahoma State University Electron Microscope Laboratory for their help in preparing material for microscopic study.

Most importantly, much love and gratitude are extended to my parents, Tom and Judi Stricker. Without their much needed moral and financial support, I could not have achieved what little success I have achieved. Thank you, once again, mom and dad.

TABLE OF CONTENTS

Chapter Page

I. INTRODUCTION. 1

II. LITERATURE REVIEW 6

Internal Anatomy 6
Salivary Gland Ultrastructure. 7
Water Vapor Uptake 9
Salivary Gland Degeneration. 11

III. MATERIALS AND METHODS 12

Colony 12
Internal Anatomy 12
Salivary Gland Ultrastructure. 13
Water Vapor Uptake 14
Salivary Gland Degeneration. 16

IV. RESULTS 17

Internal Anatomy 17
Initial Observations. 17
Digestive and Excretory Systems 20
Gastric System 20
Foregut and Midgut and Hindgut . . 22
Salivary Glands. 25
Coxal Glands 27
Reproductive System 29
Male Reproductive Organs 29
Female Reproductive Organs 34
Respiratory System. 37
Muscular System 42
Nervous System. 54
Circulatory System. 58
Water Vapor Uptake 58
Salivary Gland Ultrastructure. 66
Salivary ducts. 66
Type A Acini. 69
Type B Acini. 72
Salivary Gland Degeneration. 77

V. DISCUSSION. 86

Internal Anatomy 86

Chapter Page

Digestive and Excretory Systems 86
Gastric System 86
Foregut and Midgut and Hindgut . . 87
Salivary Glands. 89
Coxal Glands 91
Reproductive System 93
Male Reproductive Organs 93
Female Reproductive Organs 97
Respiratory System. 100
Muscular System 102
Nervous System. 107
Circulatory System. 109
Water Vapor Uptake 111
Salivary Gland Ultrastructure. 116
Salivary Ducts. 116
Type A Acini. 117
Type B Acini. 120
Salivary Gland Degeneration. 124

IV. SUMMARY AND CONCLUSIONS 130

Internal Anatomy 130
Water Vapor Uptake 132
Salivary Gland Ultrastructure. 133
Salivary Gland Degeneration. 134

REFERENCES 136

APPENDIXES 145

APPENDIX A - FIXATION PROCEDURE USED TO PREPARE SALIVARY GLAND TISSUE FOR ELECTRON AND LIGHT MICROSCOPIC EXAMINATION. . . 146

APPENDIX B - ABBREVIATIONS USED FOR INTERNAL ANATOMY STUDY. 147

APPENDIX C - ABBREVIATIONS USED FOR SALIVARY GLAND ULTRASTRUCTURE STUDY 152

APPENDIX D - ABBREVIATIONS USED FOR SALIVARY GLAND DEGENERATION STUDY 154

LIST OF TABLES

Table Page

I. Comparison Over Time of Average, Percent, Weight Change for Ten, 9 Month and Ten, 1 Month Old *O. megnini* Adult, Male ticks. 64

II. Comparison Over Time of Average, Percent, Weight Change for Ten, 9 Month and Ten, 1 Month Old *O. megnini* Adult, Female ticks. 65

LIST OF FIGURES

Figure | Page

1. Gross, internal anatomy of a female _O_. _megnini_, dorsal aspect 18

2. Dorsal view of extended gastric system of an adult _O_. _megnini_ 21

3. Dorsal view of adult, _O_. _megnini_ digestive system . . 24

4. Salivary glands from _O_. _megnini_ ticks. (A) Adult (B) Nymph 26

5. Coxal glands from (A) nymph and (B) adult _O_. _megnini_ 28

6. Dorsal view of male _O_. _megnini_ reproductive system. . 30

7. Male _O_. _megnini_ accessory gland. (A) Dorsal view and (B) ventral view. 32

8. Dorsal view of female _O_. _megnini_ reproductive system 35

9. Capitular areas of (A) adult female and (B) adult male _O_. _megnini_ 38

10. Respiratory system of _O_. _megnini_. 39

11. Respiratory system of _O_. _megnini_. (A) Brain tracheal system and (B) ventral anastomosis 41

12. Interior view of dorsal _O_. _megnini_ cuticle, showing postions of muscle attachments 43

13. Internal architecture of the ventral cuticle of a female, adult _O_. _megnini_ 44

14. Anterior muscular system of an adult _O_. _megnini_, female, dorsal view 46

15. Selected anterior muscles and posterior muscular system of and adult _O_. _megnini_, female, dorsal view 47

Figure | Page

16. Muscular system of _O. megnini_. (A) Endosternum and (B) inferior genital muscles of male (dorsal view) 48

17. Synganglion of _O. megnini_ (adult, dorsal view). . . 55

18. Circulatory system of adult _O. megnini_ (dorsal aspect) 59

19. Average, percent, weight changes over time for ten, 9 month old, normal, adult _O. megnini_ and ten, adult _O. megnini_ with mouthparts wax covered 60

20. Average, percent, weight changes over time for ten, 1 month old, normal, adult _O. megnini_ and ten, adult _O. megnini_ with mouthparts wax covered. . . 61

21. Salivary glands from adult _O. megnini_ showing type A acini. A) 500X B) 400X. 67

22. Ultrastructure of salivary gland ducts from adult _O. megnini_ salivary glands. A) Main salivary duct (13,000X) B) Efferent salivary duct (10,500X) 68

23. Peripheral lamellate cells from type A acini of adult _O. megnini_ salivary glands showing infoldings of basal plasma membrane. A) 38,500X B) 17,500X 70

24. Ultrastructure of adult _O. megnini_ salivary glands showing trachae and nerves located between type A acini. A) 16,500X B) 10,500X 71

25. Salivary glands from fed _O. megnini_ nymphs showing cell types within the type B acini. (A and B both 400X) 73

26. Ultrastructure of type Ba cells in type B acini of _O. megnini_ nymph salivary gland. A) Granule of type Ba cell (12,500X) B) Lumen of type Ba cell with microvilli (15,500X) 75

27. Ultrastructure of _O. megnini_ nymph salivary gland. A) Type Bb cell granules and interstitial cell (15,500X) B) Type Ba cell and interstitial cell (12,500X). 76

28. Ultrastructure of type B acini of _O. megnini_ nymph salivary gland at 2 days post-removal. A) 400X B) Type Ba cell granules (12,500X) 78

Figure | Page

29. Degeneration of type B acini of *O. megnini* nymph salivary gland. A) Type Bb cell granules at 2 days post-removal (5,000X) B) Type B acini at 5 days post-removal (400X) 79

30. Ultrastructure of type B acini of *O. megnini* nymph salivary gland at 5 days post-removal. A) Large vacuoles in type Ba cells (10,000X) B) Vacuoles and membrane whorls (9,500X). 81

31. Degeneration of type B acini of *O. megnini* nymph salivary gland. A) Type Bb and interstitial epithelial cells at 5 days post-removal (9,500X) B) Type B acini at 8 days post-removal (400X) 82

32. Ultrastructure of type B acini of *O. megnini* nymph salivary gland at 8 days post-removal. A) Extracellular space of degenerating B acinus (7,500X) B) Shriveled type Ba and Bb cells (14,500X). 84

33. Cytoplasm of degenerating cell of *O. megnini* nymph salivary gland, type B acinus at 8 days post-removal showing membrane whorls and other cellular debris (14,500X) 85

CHAPTER I

INTRODUCTION

Otobius megnini Duges is a member of the tick family, Argasidae. Ticks in this family are commonly called "soft ticks." The other major tick family, Ixodidae, contains the "hard ticks." The more widespread and familiar hard ticks are often pests of man and livestock and vector several important diseases to both man and animals. The family, Argasidae, is relatively small with only 140 species in five genera (Krantz 1978). The family, Ixodidae, is much larger, with approximately 650 species in 13 genera (Krantz 1978).

Ticks in the family, Argasidae, are generally secretive and live in or near the nests or burrows of their hosts, which are generally birds or small mammals (Krantz 1978). Virtually all species of Argasidae are multiple feeders. Thus, they come to the host intermittently, take a quick bloodmeal, then retreat to a hiding place. Because of these habits, members of the Argasidae are seldom seen and are not frequently studied, although some species can be economically important pests of livestock and can vector serious diseases to man (Hoogstraal 1966, 1973).

The new world genus, *Otobius*, contains two species, *Otobius megnini* and *Otobius lagophilus* (Cooley and Kohls).

The former was first described by Duges (1884) from Guanajuato, Mexico. Townsend (1893), in one of the earliest reports from the United States, referred to it as *Argas americanum* Packard. Marx (1895) published the name, *Ornithodoros megnini* (Duges). Finally, Banks (1912) assigned the tick to a new genus and presented the name, *Otobius megnini* (Duges), by which the spinose ear tick is currently known. These two species are very unusual for the Argasidae, because they are one-host ticks that remain attached as larvae and nymphs for lengthy periods of time (as much as 7 months or more in *O. megnini*). This feeding behavior is more typical of ticks in the Ixodidae. Most of the other Argasidae attach to the host only long enough to take a bloodmeal, anywhere from 2 minutes to 2 hours.

Otobius megnini and *O. lagophilus* are also unusual in that they do not feed as adults. The larvae and nymphs are found on the same host. The mature nymphs then leave the host and molt to adults on the ground. Most of the other Argasidae take multiple bloodmeals as adults. *Otobius megnini* is economically important because it frequently parasitizes domestic as well as wild ungulates, but it can attack a wide range of hosts (Wanchinga and Barker 1986).

A few pathogens have been isolated from *O. megnini*. Stiles (1944) discovered anthrax bacteria in ticks that had fed on infected cattle, and Jellison *et al*. (1948) discovered that this species was the only soft tick species able to harbor organisms of the rickettsia, *Coxiella*

burnetti, which causes Q fever. However, O. megnini has not been proven to be a vector of any pathogen to its assorted hosts, most likely because of its one-host feeding strategy. Howell et al. (1943) were unable to show transmission of anaplasmosis either transstadially or transovarially. Likewise, Ewing et al. (1990) could not demonstrate experimentally that O. megnini could transmit Ehrlichia canis to dogs.

The preferred feeding site of O. megnini is deep within the outer ear canals and pinnal folds of its host, where heavy infestations may cause severe irritation and permanent damage to the delicate tissues of the ear or possibly predispose the host to screwworm attack or infections that could cause the death of young or weakened hosts (Rich 1957, Hooker et al. 1912, Stiles 1944).

Otobius lagophilus, limits itself to wild lagomorphs in the western and northwestern United States (Cooley and Kohls 1944, Beck 1955, Hopla 1955, and Loomis 1961), and Mexico (Silva-Goytia and Elizondo 1952). The geographic range of O. megnini is somewhat greater due to the inadvertent assistance of man. The distribution of O. megnini now includes: parts of North America, including Oklahoma, as well as South, and Central America (Hooker et al. 1912 and Cooley and Kohls 1944) and has been introduced into Africa (Theiler and Salisbury 1958) and India (Chellappa 1973).

Information on the biology of O. megnini was reported by Hooker (1908), Hooker et al. (1912), Herms (1917), Davis

(1934), and Koshy _et_ _al_. (1979). Wanchinga and Barker (1986) detailed colonization and laboratory development of this tick and Wanchinga (1983) examined the external ultrastructure of _O_. _megnini_. Finally, Stricker (1990) described some aspects of this tick's reproductive behavior. The spinose ear tick has received only cursory mention in other publications. To date, no research has been undertaken to describe the internal anatomy or salivary gland ultrastructure of this unique tick species. Consequently, this study was undertaken to examine the internal anatomy of _O_. _megnini_ and describe the internal ultrastructure and some physiological aspects of the salivary glands. Specific objectives were to: 1) characterize the internal anatomy of adult and nymphal _O_. _megnini_ by describing the appearance and arrangement of organs and organ systems as completely as possible and to compare its internal anatomy with published information on other tick species in the family Argasidae; 2) describe the internal ultrastructure of the salivary glands using light and electron microscopy; 3) determine if the mouthparts are the location of water vapor uptake in adult _O_. _megnini_ and whether age has an effect on uptake of water vapor; and 4) observe and describe the process of salivary gland degeneration as _O_._megnini_ nymphs mature to adults. A more thorough understanding of this tick's internal anatomy will add to the limited body of knowledge compiled for this pest species, and may lead to the discovery of novel features,

especially in the salivary glands, that could inspire practical future research.

CHAPTER II

LITERATURE REVIEW

Internal Anatomy

With the notable exception of a few pest species, the internal anatomy of few tick species has been studied in detail. More species in the hard tick family, Ixodidae, have received attention because of their more widespread distribution and well-documented disease transmission capabilities. Detailed papers of internal anatomical studies have been published for several species, including: *Dermacentor andersoni* Stiles (Douglas 1943), *Boophilus annulatus* Say (Williams 1905 and Allen 1905), *Ixodes reduvius* Latreille (Nordenskiold 1908, 1909, and 1911), *Ixodes ricinus* L. (Samson 1909), *Rhipicephalus appendiculatus* Neumann (Till 1961), *Dermacentor variabilis* Say (Zebrowski 1926), and *Haemaphysalis flava* Neumann (Saito 1960). Limited anatomical studies of *Rhipicephalus annulatus* (Say) and *Hyalomma aegyptium* (L.) were presented in Christophers (1906).

Relative to hard ticks, the internal anatomy of species in the Argasidae has been less studied. Robinson and Davidson (1913, 1913a, and 1914) published an excellent description of the internal anatomy of *Argas persicus*

(Oken). A comprehensive anatomical study of Ornithodoros kelleyi Cooley and Kohls was also presented by Sonenshine and Gregson (1970) and Sonenshine (1970). Less complete works dealing with internal anatomical aspects of Ornithodoros savignyi (Audouin) and Ornithodoros coriaceus Koch were published in Christophers (1906) and True (1932), respectively. The morphology of respiratory, digestive, and reproductive organ systems of four species in the genus Argas were compared in Roshdy (1961, 1962, 1963, and 1966). Bonnet (1907) and Balashov (1968) dealt with the internal anatomy of ticks as a group, with that of Balashov containing anatomical descriptions of a few species in the Argasidae. Many additional papers dealing only with the histology and anatomy of separate organs or organ systems in ticks have also been published.

Salivary Gland Ultrastructure

Perhaps the best studied organs of ticks are the salivary glands. This is understandably due to their essential role in feeding and importance in disease transmission. As a result, the ultrastructure of the glands of a few species of ticks has been described in detail. Megaw and Beadle (1978) related the structure of the salivary glands of Boophilus microplus Canestrini. The fine structure of the salivary glands from Dermacentor variabilis was examined by Coons and Roshdy (1973), and the structure of the acini of Amblyomma americanum (L.) was described by

Krolak _et_ _al_. (1982). Chinery (1965) examined the salivary glands of _Haemaphysalis_ _spinigera_ Neumann, and Kirkland (1971) described how the ultrastructure of the glands of _Haemaphysalis_ _leporispalustris_ Packard changed during feeding and Barker _et_ _al_. (1984) noted ultrastructural changes in the type I salivary gland acini of _A_. _americanum_ during attachment, feeding, and mating. Binnington (1978) presented a similar study on the salivary glands of _B_. _microplus_. It should be noted that these ticks were all species of the Ixodidae.

In only three species of Argasidae, _Argas_ _arboreus_ Kaiser, Hoogstraal, and Kohls, _Argas_ _persicus_, and _Ornithodoros_ _moubata_ (Murray), has the salivary gland ultrastructure been examined in detail. The glands of _A_. _arboreus_ were studied in Guirgis (1971) and Roshdy and Coons (1975) and those of _A_. _persicus_ detailed in Roshdy (1972). Dzhafarov (1965) published an investigation of _O_. _moubata_ salivary secretory acini using the electron microscope. Drawings of the salivary gland acini of _O_. _savignyi_, _O_. _kelleyi_, _O_. _coriaceus_, and _Ornithodoros_ _papillipes_ (Birula) were presented in Christophers (1906), Sonenshine and Gregson (1970), True (1932) and Balashov (1968), respectively. However no ultrastructural information other than the appearance of basic cell types was presented. No information has been previously published on the salivary gland morphology or ultrastructure of _O_. _megnini_.

Water Vapor Uptake

The ability of arthropods to absorb water vapor from unsaturated atmospheres has been well documented in both insects and ticks. Machin (1979 and 1983) and Machin et al. (1982) as well as Noble-Nesbitt (1970), Rudolph (1982) and Rudolph and Knulle (1982) have demonstrated this ability in various species of insects. Lees (1946) was the first to show that a tick (I. ricinus) displayed this ability as well. However, he believed that the cuticle must have been the site of absorption (Lees 1948). Further evidence that ticks could sorb water vapor was identified in Hyalomma dromedarii Koch and O. savignyi (Hafez et al. 1972) and for D. variabilis (Knulle and Devine 1972).

Later experiments conducted by Rudolph and Knulle (1974) and McMullen et al. (1976) showed that the mouth, not the cuticle was the site of uptake in A. americanum and Amblyomma variegatum (Fabricius). McMullen et al. (1976) proposed that the salivary glands were responsible for initiating the process of water vapor uptake. Furthermore, Rudolph and Knulle (1974) observed that, in relatively low humidities, ticks secreted a clear salivary fluid that quickly dried to a white, crystalline solid. When the ticks were placed into atmospheres with the relative humidity higher than approximately 75%, the crystalline material absorbed water, redissolved, and was reconsumed by the tick. The crystalline solid was discovered to be composed of potassium, sodium, and chlorine ions. Rudolph and Knulle

(1974) and McMullen *et al*. (1976) suggested that the consumed water and ions were reabsorbed through the gut epithelium. Sigal *et al*. (1991) has completed additional work on this ion-containing salivary secretion. Most recently, attention had centered on the type I acini of the salivary glands as a key structure in the process of water vapor uptake. Balashov (1968) and Kirkland (1971) first suggested the possibility and their speculations were reinforced by later authors (Rudolph and Knulle 1974 and McMullen *et al*. 1976). Needham and Coons (1984) more recently showed that the ultrastructure of the type I acinus changed as dessicated ticks took in water from the atmosphere. These changes were in accordance with cells that were undergoing a high rate of secretion. Kahl *et al*. (1990) also showed that the morphology of *I*. *ricinus* salivary glands changed in relation to active uptake.

Species of Argasidae have also been shown to be capable of water vapor uptake. Browning (1954) proved that *O*. *moubata* individuals were able to actively sorb water vapor. Hefnawy *et al*. (1975) discovered that *A*. *arboreus* ticks also gained weight, presumably through water uptake at high humidities. Balashov and Filippova (1964) examined four species of *Ornithodoros*, three species of *Argas*, and *Alveonasus* *lahorensis* Neumann and discovered that all species except *A*. *lahorensis* could sorb water at relative humidities greater than 80%. No information on the water

uptake capabilities of *O. megnini* has been previously published.

Salivary Gland Degeneration

The process of salivary gland degeneration has only been studied in a few species of the Ixodidae. Till (1961) reported that histological disruption of the salivary glands of *R. appendiculatus* females occurred within a few days following detachment from the host. Harris and Kaufmann (1981) reported the appearance of numerous autophagic vacuoles in the secretory labyrinth of the type III acinus of *Amblyomma hebraeum* Koch within 1 day post-repletion. However, in small, partially-fed ticks, forcibly removed from the host, autophagic vacuoles did not appear for at least 4 to 5 days. For *A. hebraeum*, neural and hormonal factors, identified as ecdysteroids, have been proven to be factors in salivary gland degeneration (Harris and Kaufmann 1981, 1984, and 1985). Barker *et al*. (1984) examined ultrastructural changes within the type I acinus, including degeneration, during attachment, feeding, and mating in *A. americanum*. To date, no information has been published on salivary gland degeneration for any species of the Argasidae.

CHAPTER III

MATERIALS AND METHODS

Colony

Adult and nymphal O. megnini used in these studies were reared according to Wanchinga and Barker (1986) at the Oklahoma State University tick research facility. Sheep were used as hosts for larval and nymphal stages. Fully engorged nymphs were collected from the ears of sheep with forceps and placed individually in 2 dram glass vials. Both nymphs and adults were held at 21°C and 90% humidity, in constant darkness until used. Sex was determined after the nymphs had molted to adults.

Internal Anatomy

The general arrangement of organs as organ systems was drawn to scale using an ocular grid in a Bausch and Lomb stereozoom 7 dissecting microscope. For this purpose, dissections were performed by embedding live or freshly killed (by freezing for 24 hours) O. megnini adults or nymphs in a wax lined, 9 cm diameter, petri dish. All nymphs used were near maturity and had been feeding for more than 35 days. Ticks were placed dorsal surface up and the dorsal cuticle removed by cutting along the periphery of the

tick with a single-edged razorblade and dissecting scissors. The cuticle was then gently lifted off with forceps. Dissections were initially performed in a 0.09% NaCl solution to temporarily preserve the internal tissues in their natural state for preliminary observations. Afterwards, the saline solution was replaced with 90 to 95% ethyl alcohol for long term preservation of the dissections. Drawings of the organs and systems of each tick dissected were prepared and then final final composite drawings were completed from three to five dissections for each organ system studied. Drawings of the nervous and muscular systems required two or three additional dissections to obtain accurate composite drawings.

Salivary Gland Ultrastructure

The salivary glands from three adults (two female and one male) and three nymphs were excised and the structure and ultrastructure examined with light and electron microscopy. After removal of the dorsal cuticle and overlying gut tissue, the remaining tissues were rinsed with 0.09% saline and then immediately fixed *in situ* with 2% gluteraldehyde in 0.27M cacodylate buffer (pH 7.2). The glands were then removed and transferred to a 2 dram vial containing more gluteraldehyde and buffer and allowed to remain for 2 hours. The glands were then prepared using the procedure described in Appendix A. After processing, the glands were embedded in poly/bed 812 resin and sectioned

with a glass knife on a Porter-Blum MT-2 microtome. Thick sections approximately 0.5 to 1.0 μm in thickness, were stained with Mallory's Azure II Methylene Blue, and observed and photographed with a Minolta SRT 10T camera mounted on an Olympus BH-2 compound microscope. Thin sections, approximately 90 nm in thickness, were made and placed on 200 mesh copper grids. The sections on the grids were then stained (Appendix A) and observed on a JEOL JEM-100CX transmission electron microscope. The internal ultrastructure of the acini composing the salivary gland was then compared to published findings for other species in the family, Argasidae.

Water Vapor Uptake

Twenty adult male and female _O. megnini_ (10 of each sex) were weighed on a Mettler Type H-5 balance to the nearest 0.1 mg and placed into individual 2 dram vials. The vials were then placed into a 2 liter capacity glass bowl containing dry $CaCl_2$, and the bowl covered with a cut glass lid. The $CaCl_2$ maintained a relative humidity of near 0%. The relative humidities were measured with an Airguide humidity indicator. At 10 day intervals, the ticks were reweighed and weight changes were recorded. The ticks were maintained at near 0% R.H. for 30 days total. Five females and five males were then selected at random and their mouthparts covered with melted bee's wax. All 20 ticks were then placed into another glass bowl that contained a

saturated $CuSO_4$ solution (Winston and Bates 1960) which maintained a near constant relative humidity of 97%. Ticks were weighed every 24 hours for a period of 15 days or until no further increase in average weight was noticed. Ticks were then weighed approximately every 5 days for an additional 30 days. The total experiment length was 80 days. When ticks were not being weighed, the glass bowls were kept in a environmental chamber at a constant 25 C and continuous darkness.

The experiment was performed twice, once with 9 month old ticks and once with 1 month old ticks, to compare water uptake abilities. All weight changes from measurement to measurement were converted to percentages of initial weight and graphs of percent weight change over time for both age groups were created. Additionally, the percent weight changes of the control and test ticks of both age groups were compared at 30 days dehydration and 5, 10, and 15 days post rehydration at 97% R.H. The two age groups were also compared at 15 and 25 days after the wax was removed from the test ticks (still at 97% R.H.). Variances were checked for significant differences using and F-test, and the mean percent weight changes for the two age groups at each day examined were compared using t-tests (Steele and Torrie 1960).

Salivary Gland Degeneration

The salivary glands were dissected from mature nymphs at 2 days, 5 days, and 8 days post removal from the host and processed as described in Appendix A. Thick and thin sections were prepared and observed on a JEOL JEM-100CX transmission electron microscope and micrographs made. Photomicrographs were also taken using a compound light microscope as described before. Any degenerative changes in the acini of the glands from age group to age group were noted and compared to information available in the literature.

CHAPTER IV

RESULTS

Internal Anatomy

Initial Observations

The gross, internal anatomy of O. megnini is illustrated in Figure 1. The abbreviations used for this drawing and others are listed in Appendix B. The hemolymph of the ticks was clear with a pale, yellow-brown tinge. In saline, the entire inner surface of the cuticle was lined with a translucent white epithelium in which many droplets, possibly lipids, that were not miscible in the saline could be seen. When alcohol was applied, many of these droplets were released and floated to the surface. An extensive gastric system with several caeca extende throughout the body cavity. Most of the caeca are completely or partially filled with a dark, red-brown, granular material in which could also be seen a few lipid-like droplets. Beneath the gastric system, the reproductive organs occupy a prominent postion in the center of the body (Figure 1). In saline, these organs are an opaque white, sometimes with a pale yellow tinge in the area of the testis or ovary. An extensive tracheal network extends throughout the body to

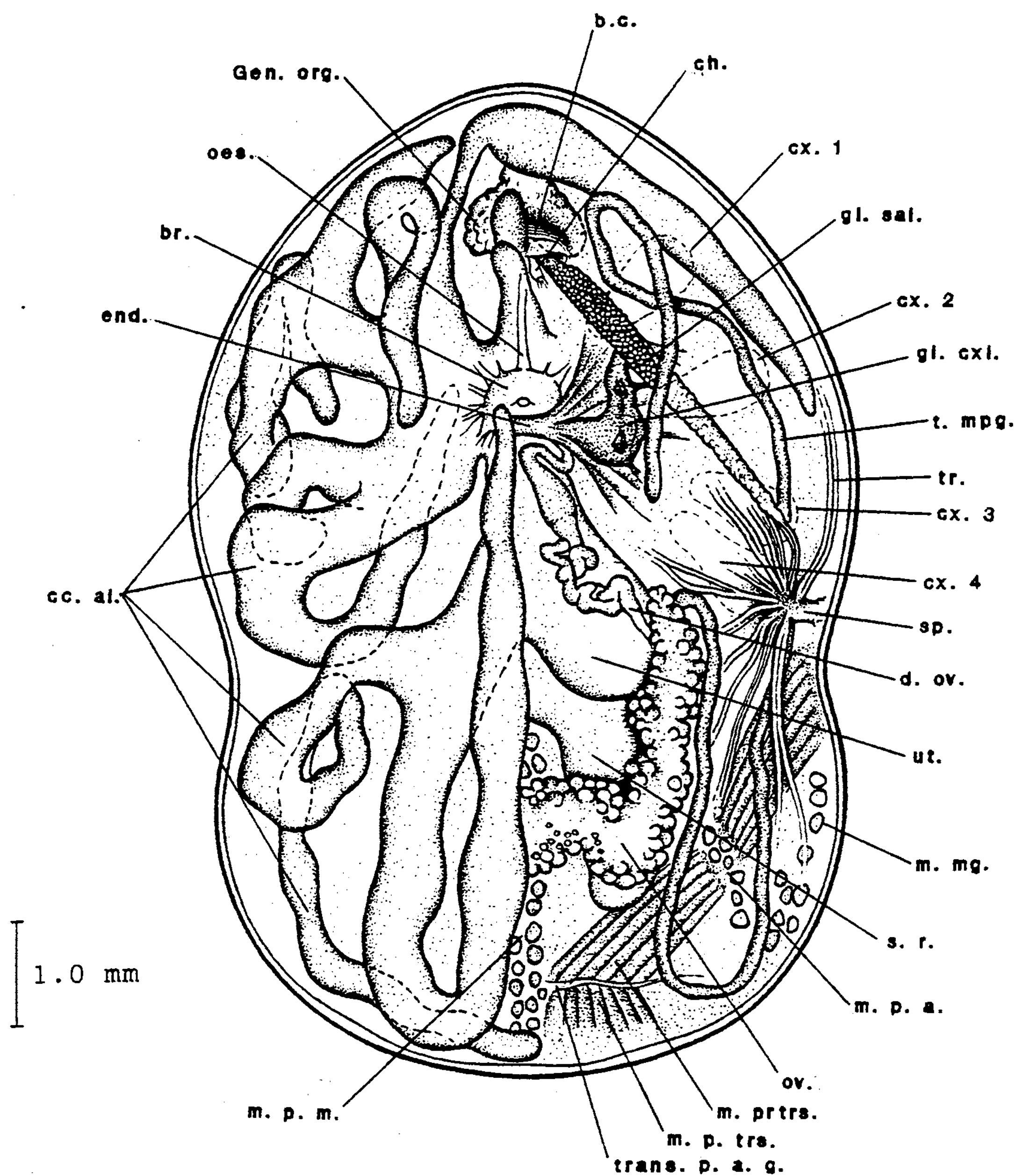


Figure 1. Gross, internal anatomy of a female <u>O</u>. <u>megnini</u>, dorsal aspect.

every organ and tissue. The spiracles are located laterally on the body wall near mid-body. Trachae are apparent initially because air trapped within gave them a silvery appearance. Eventually, this air was pushed out by the alcohol and the trachae became transparent.

Muscles and nerves were virtually invisible until application of alcohol, after which they turned an opaque white. Prominent lateral muscles connected mesally to the endosternum occupiy a large portion of the anterior half of the body. The coxal glands are positioned directly dorsal to these muscles (Figure 1). Dorso-ventral muscles predominate in the posterior region. Many of the dorso-ventral muscles pass upward through loops created by folds of the gastric caeca. Both the dorso-ventral muscles and trachae apparently function to hold the organs in place. The salivary glands are anchored posteriorly by trachae near the spiracle and pass obliquely and anteriorly into the oral opening. The bilobed rectal sac with its pair of Malpighian tubules was prominent in the posterior portion of the body. Both the rectal sac and Malpighian tubules are usually filled with white, crystalline compound. The synganglion occupies a cental location directly beneath the endosternum. In the adult females, the U-shaped, bi-lobed Gene's organ is found in the anterior apex of the body cavity. The gross anatomy of the mature nymphs with the exception of the salivary glands, coxal glands, and reproductive organs is virtually identical to that of the adults.

Digestive and Excretory Systems

Gastric system. The gastric caeca of *O. megnini* are comprised of four paired and one unpaired set of diverticula connected to an expanded central region (Figure 2). Each diverticulum has two secondary divisions or branches which were all tubular in cross section, but the sizes of these branches varied tremendously from individual to individual, depending on how much material remained within each branch. The central region, called the stomach proper, is roughly rectangular and approximately 3 to 4 mm in length and 1.5 to 2 mm in width. The unpaired antero-median diverticulum consists of two, relatively short branches, and attaches to the anterior end of the stomach near the midline. The dorsal branch of the antero-median division is the shorter, often less than 2 mm in length, and generally extends anteriorly. The longer ventral branch extends ventrally and is often deflected to the right or left along the antero-ventral body wall. In some of the nymphs, this branch is considerably more robust and extends further posteriorly beneath the endosternum and brain.

The paired antero-lateral diverticula are located directly on either side of the antero-median diverticulum. Both branches are comparable in length, usually between 3 and 5 mm. Occasionally, the anterior branch is somewhat longer and is deflected posteriorly along the anterior bodywall. The posterior branch is usually bent posteriorly along the dorsal body wall. The paired lateral diverticula

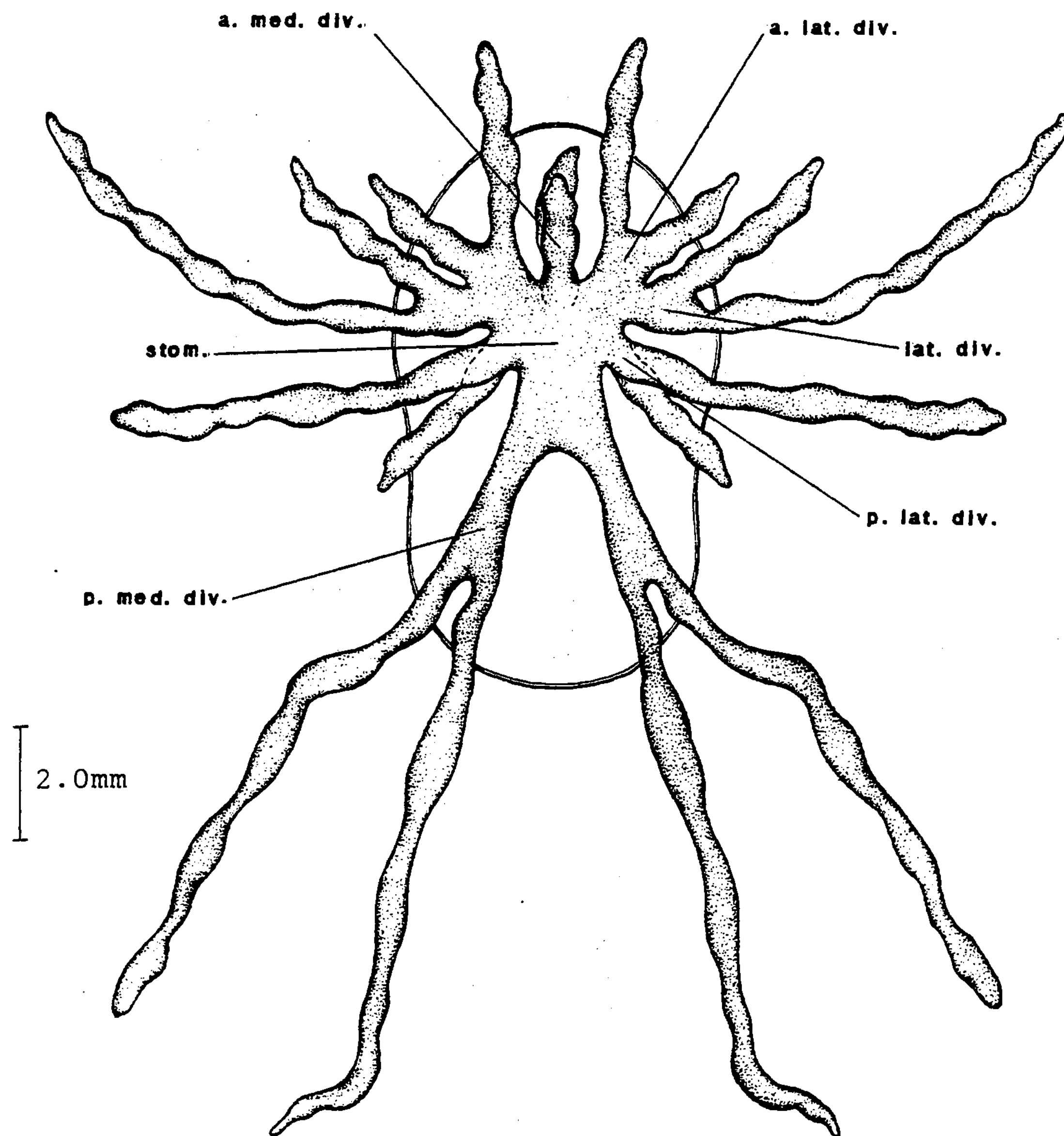


Figure 2. Dorsal view of extended gastric system of an adult _O_. _megnini_

are postioned immediately adjacent to the antero-lateral diverticula. The posterior branch is generally the longer and was 8 mm or more in length when fully extended. This branch, usually less than 4 mm in length, is frequently deflected postero-ventrally, then turned anteriorly. The anterior branch extends anteriorly along the lateral body wall.

The postero-lateral diverticula emerge from near the mid-point of the central stomach. As on the antero-median diverticulum, the branches are oriented in the vertical, not the horizontal plane. The dorsal branch was frequently the longer of the two and often extended to 7 mm or more in larger ticks. The ventral branch is only half as long on average. In the natural state, both branches are often found turned ventrally along the lateral and ventral body walls. The largest pair of diverticula are the postero-medians. These diverticula emerge from the postero-lateral edges of the stomach, and the outstretched branches often extend posteriorly for more than 10 mm in some individuals. The two branches are often of similar length, but in several individuals examined, the right branch is slightly longer. Undisturbed, the left branch is found coiled within the postero-lateral regions of the body cavity, and the right branch is frequently bent over itself and extends anteriorly over the reproductive organs.

<u>Foregut and Midgut and Hindgut.</u> The foregut consists of the pharynx, esophagus, and proventricular valve. The

esophagus is a narrow tube less than 0.1 mm in diameter in the largest individuals. It passes posteriorly from the capitular foramen through the anterior portion of the synganglion and enters the stomach slightly posterior to the antero-median diverticulum (Figure 3). The proventricular valve is visible as a slight indentation around where the esophagus enters the stomach. The pharynx was not studied. The midgut consists of the stomach and associated diverticula (described earlier), rectal tube, and rectal sac. The rectal tube extends from the stomach mid-line near the attachment point of the postero-lateral diverticula ventro-posteriorly to the anterior end of the rectal sac. The rectal tube is generally less than 2 mm in length and its diameter widenes as it approaches both the rectal sac and the stomach attachment points. In one nymph examined, the rectal tube was filled with the same dark, red-brown material as the diverticula. The rectal sac is a U-shaped, bilobed organ which is almost always filled with a white, crystalline compound in both nymphs and adults. The size of the lobes varies in relation to how much of the compound is contained within. However, one lobe is almost always larger than the other. A single nymph with the rectal sac almost completely empty was examined. In this case, the two lobes were the same length, approximately 1.5 mm, and were wrinkled, somewhat resembling a deflated balloon.

A single pair of Malpighian tubules originate ventro-laterally from the rectal tube a short distance anteriorly

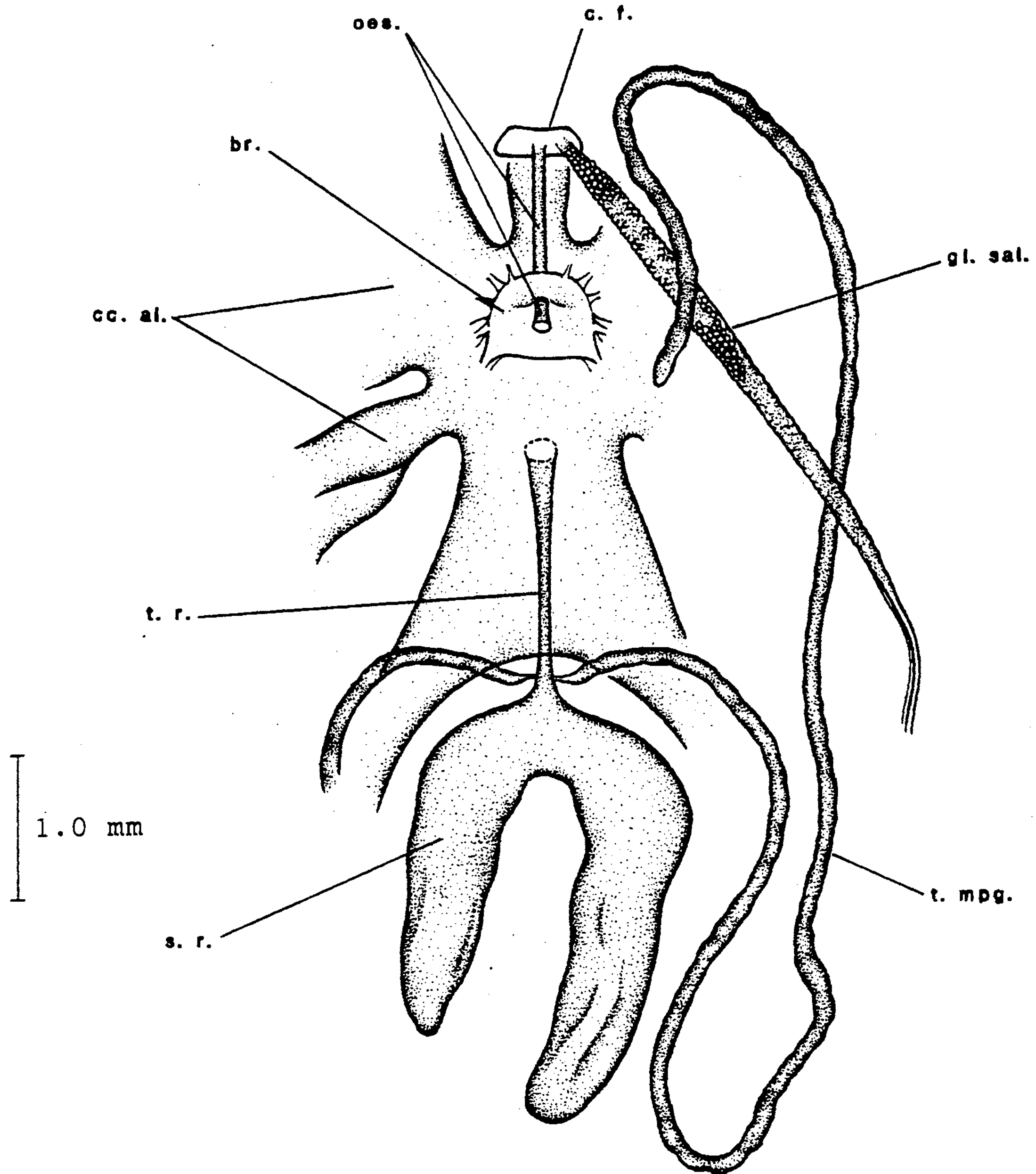


Figure 3. Dorsal view of adult, _O. megnini_ digestive system.

from the tube's attachment point to the rectal sac. Each tube extends posteriorly almost to the posterior limit of the body cavity, then turns anteriorly ventral to the salivary gland, to the anterior end of the body, and then extends posteriorly again dorsal to the salivary gland. The tubules eventually terminate at a position over the stomach (Figure 3). In one female, the entire length of one tubule exceeded 15 mm. In the nymphs, the tubules are proportionately the same length but are generally one-third larger in diameter.

The hindgut consists of the rectal canal and anus (not illustrated). The rectal canal is a short tube of approximately the same diameter as the narrowest portion of the rectal tube, but is barely one third of the length. The rectal canal attaches ventrally to the rectal sac near the point of attachment for the rectal tube and extends almost vertically downward to the anus. The anus is a simple circular bivalved structure.

Salivary Glands. The salivary glands are of the acinar type, and ranged from 2.5 to 3.0 mm in length in most individuals. In the nymphs, two types of acini were observed (Figure 4). The larger type averages 0.06 to 0.10 mm in diameter and are tightly clustered along most of the gland length. The second, much smaller type is found only on the anterior one-half of each gland and are grouped on the mesial surface. Tracheoles were observed among the acini throughout the gland. In the adults, only one type of

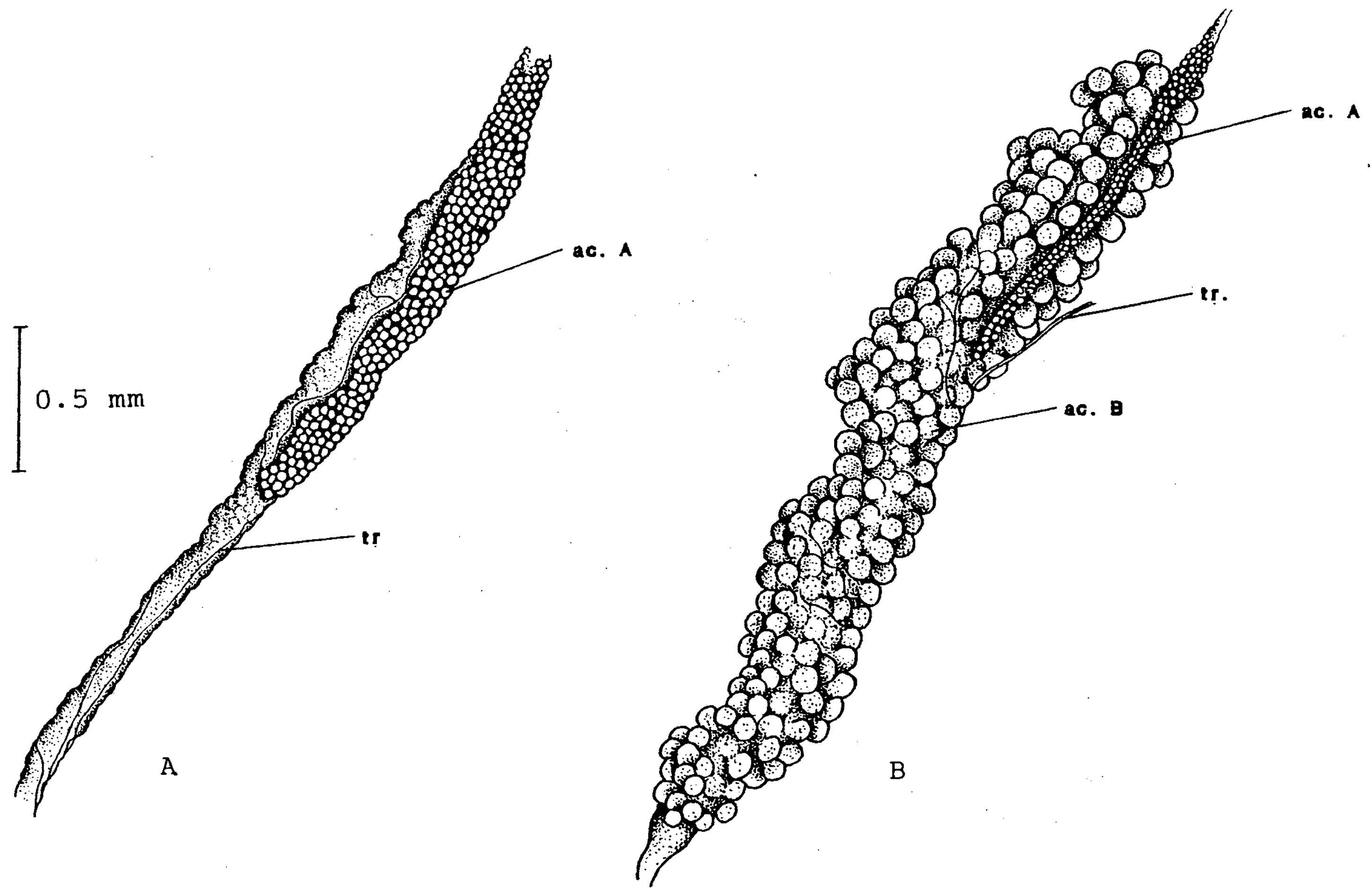


Figure 4. Salivary glands from O. megnini ticks. (A) Adult (B) Nymph.

acinus is clearly visible. These acini are roughly 0.02 to 0.05 mm in diameter and, as on the nymphs, are clustered mesially on the anterior portion. Near the location of the capitular foramen, the acini are found around the entire circumference of the glands. The remainder of each gland appeared to be primarily amorphous tissue, however on closer examination, the faint outlines of a few, slightly larger acini were seen (Figure 4). In both the nymphs and adults, the acini are present to the capitular foramen and proceed for a short distance inside.

Coxal Glands. The structure of each coxal gland of the nymphs consists of a U-shaped, coxal tubule which is covered with many irregularly shaped globules of varying size (Figure 5). The coxal tubule rests on and is anchored mesially to the large muscles attached to the endosternum. Laterally, the coxal tubule attenuates into a short coxal duct that passes ventrally between the endosternal muscles and opens to the coxal pore (not illustrated), which is positioned near the base of the first coxal projection (Figure 5). The body of the coxal tubule is suspended laterally by the three lateral coxal gland muscles and dorso-ventrally by the four dorso-ventral coxal gland muscles (only two illustrated). One pair originates from the dorsal body wall and inserts on the coxal tubule. The other originates on the ventral body wall and inserts on the vental wall of the coxal tubule.

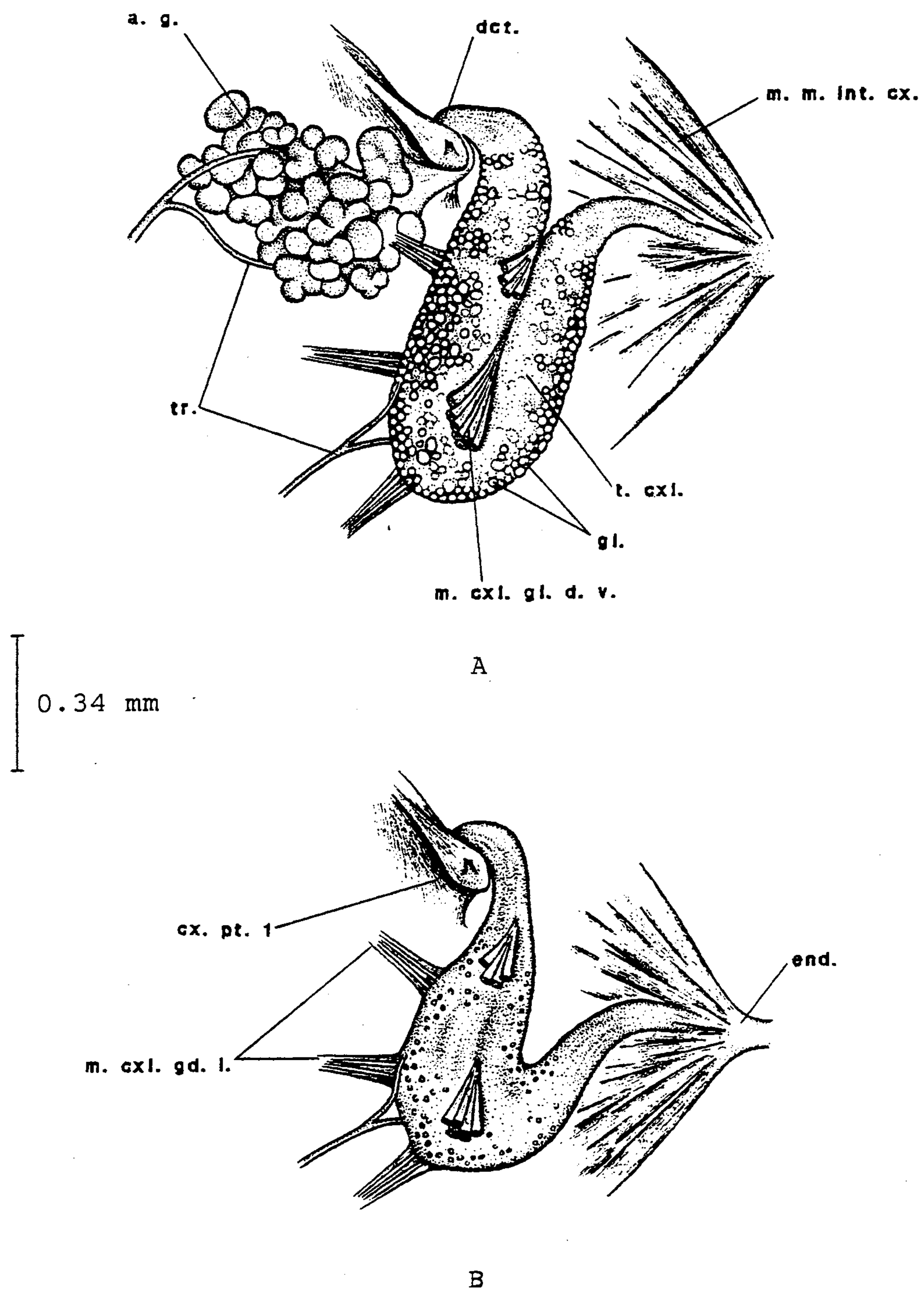


Figure 5. Coxal glands from (A) nymph and (B) adult <u>O</u>. <u>megnini</u>.

An accessory gland consisting of what appeared to be large, irregular acini is also associated with each coxal tubule. The accessory gland is generally positioned between the first and second coxae with a duct leading to the coxal tubule. The accessory gland duct merges with the tubule duct slightly above the coxal pore.

The coxal tubule of the adult retained the U-shape but was much more flaccid. In addition, only a few, much smaller, globules could be seen (Figure 5). Furthermore, no accessory glands are found in the adults. All suspensory muscles observed in the nymphs are retained in the adult. A single tracheole was observed penetrating the coxal tubule of both nymphs and adults, and another tracheole supplies the nymphal accessory gland.

Reproductive System

Male Reproductive Organs. The male reproductive system consists of the testis, vasa deferentia, ejaculatory duct, genital aperture, and lobes of the accessory gland (Figure 6). The testis is tubular organ that is narrow distally and expanded proximally. The thin, distal section of the testis extends transversely across the body above the rectal sac. The expanded proximal portion of the testis maintains a constant diameter for some distance before gradually attenuating into the narrower distal portion of the vasa deferentia. The vasa deferentia are tightly coiled for most of their length. The proximal portion of the vasa

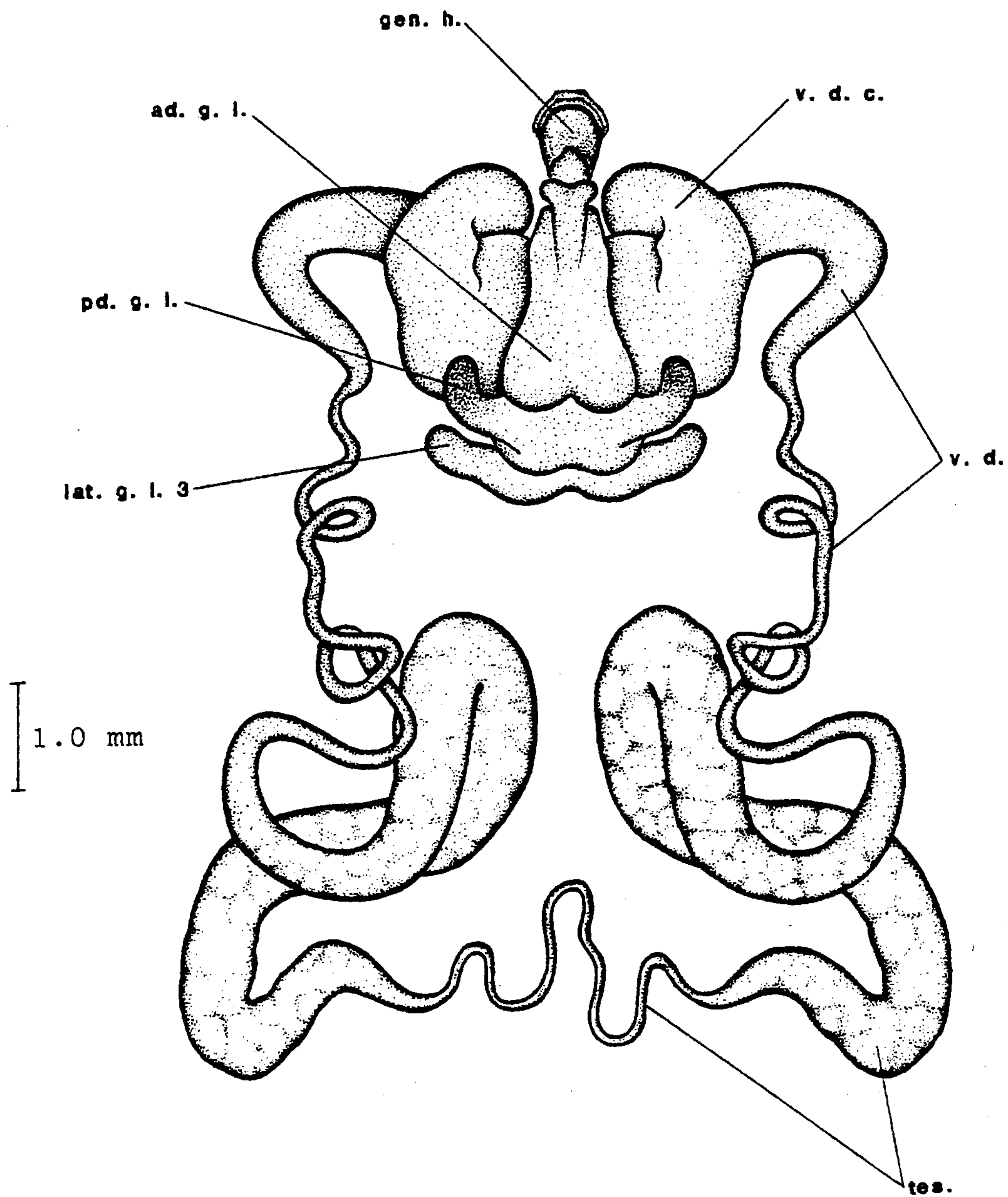
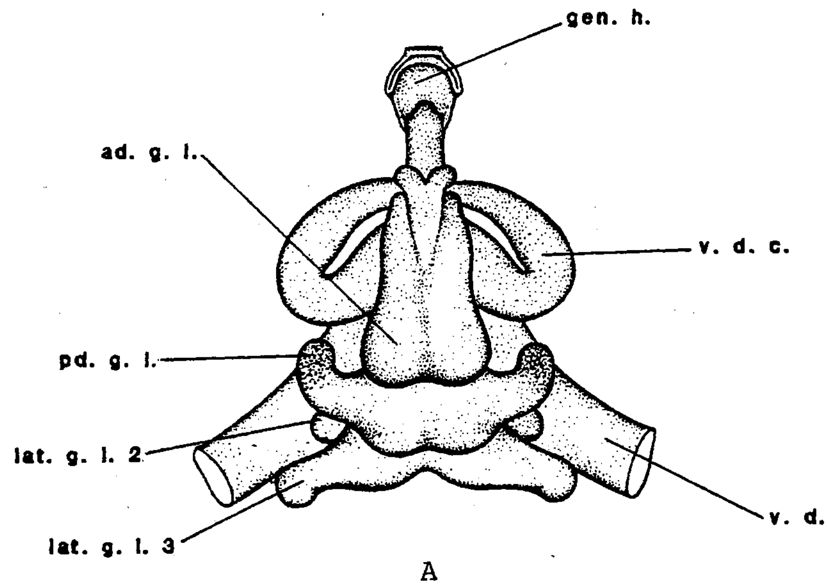


Figure 6. Dorsal view of male _O_. _megnini_ reproductive system.

deferentia are again greatly expanded to a diameter almost equal to the proximal regions of the testis. The expanded portions again narrow significantly as they merge with the terminal coils and ejaculatory duct (Figure 7A,B). The terminal coils merge antero-ventrally with the remainder of the vasa deferentia and ejaculatory duct, extend dorso-posteriorly for a short distance, then loop ventro-anteriorly again to join the ejaculatory duct dorsally. In adult males ,less than 1 month of age, the terminal coils are occasionally smaller and narrower in diameter. In older males, the terminal coils are frequently enlarged and obtrusive.

The ejaculatory duct is a simple tube that extended anteriorly a short distance before merging with the genital hood. This structure is a dome of cuticle which covers the genital aperture on the interior side of the ventral body wall. Normally, the hood is covered by a thin sheet of epithelial tissue and not visible.

The accessory gland of the male was complex and consists of ten granular lobes (four paired and two unpaired) and four spongy lobes (two paired) (Figure 7A,B). The lobes were labelled identically to similarly positioned lobes in other argasid species. The top most lobe is the antero-dorsal granular lobe which displays a shallow groove along the dorsal midline. At the apex, there are two bulbous projections often oriented to the left and right. The anterior third of the lobe which displays the



1.0 mm

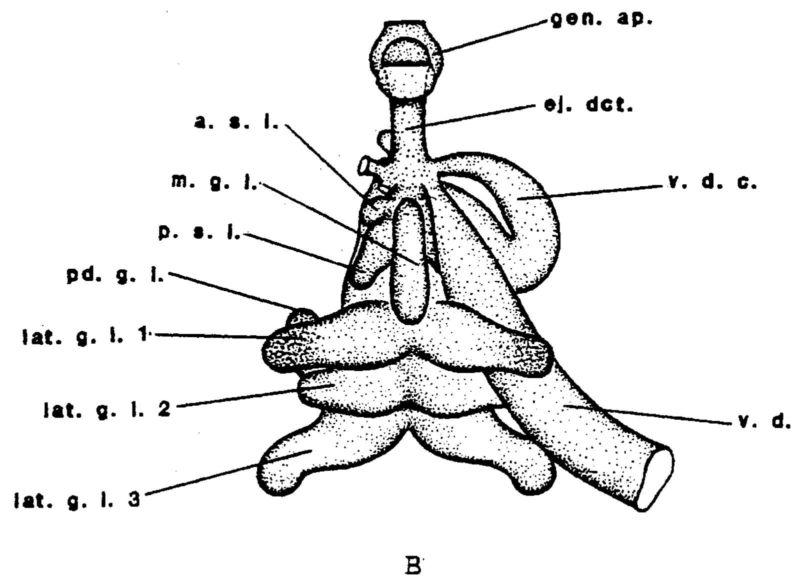


Figure 7. Male O. megnini accessory gland. (A) Dorsal view and (B) ventral view.

projections is also frequently clearly defined from the remainder of the antero-dorsal lobe (Figure 7A). Basally, the postero-dorsal granular lobes are fused with the posterior portion of the antero-dorsal lobe. However, the tubular distal portions project antero-laterally from each side of the postero-dorsal lobes.

The three pairs of lateral granular lobes are located beneath the postero-dorsal lobe. Like the postero-dorsal lobe, these lobes are joined with the others basally (Figure 7B). The first lateral granular lobes are located ventrally on the accessory gland and each lobe projects laterally or postero-laterally from the accessory gland. As in the postero-dorsal, granulation was often clearly visible at the apices of these lobes. The second pair of lateral granular lobes are the smallest and are located directly dorso-posteriorly to the first pair. These lobes are frequently tightly appressed to the remainder of the accessory gland. The third pair are the largest of the three and projected laterally or postero-laterally parallel to the ventral body surface.

The unpaired median granular lobe is a compact, oval lobe located mesially on the ventral side of the accessory gland (Figure 7B). The anterior and posterior spongy lobes are positioned antero-dorsally to the median granular lobe. These are the smallest lobes and are usually completely hidden by the vasa deferentia and their terminal coils. The anterior spongy lobe is roughly spherical and is located

directly anterior to the larger, pear-shaped posterior spongy lobe. On the ventral surface of the accessory gland, the ejaculatory duct is clearly visible as it emerges from the anterior region of the accessory gland and fuses with the genital hood. The genital aperture, which is visible as a semi-circular flap of cuticle, could only be seen on the external body surface, but was included in Figure 7B for clarity. In the mature nymphs, which were males, all organs of the reproductive system including the multi-lobed accessory gland are present and anatomically identical to that for the adults. However, the nymphal organs are only about one-half the size of those in the adults.

Female Reproductive Organs. The female reproductive system include the ovary, oviducts, uterus, vestibular and cervical vagina, accessory glands, genital aperture (not illustrated), and Gene's organ (Figure 8). Gene's organ is illustrated in Figure 1. The ovary resembles a flattened tube which extended transversely across the body in approximately the same location that the testis occupies in the male. Spherical ova of varying sizes were observed embedded in the epithelium of the ovary. The ova did not appear to encircle the ovary completely. The anterior surface of the ovary did not show any visible ova. Additionally, the ova appeared to decrease in size as they approached this region. The loosely coiled oviducts arose from each terminus of the ovary. The diameter of the oviducts remain fairly constant along the entire length with

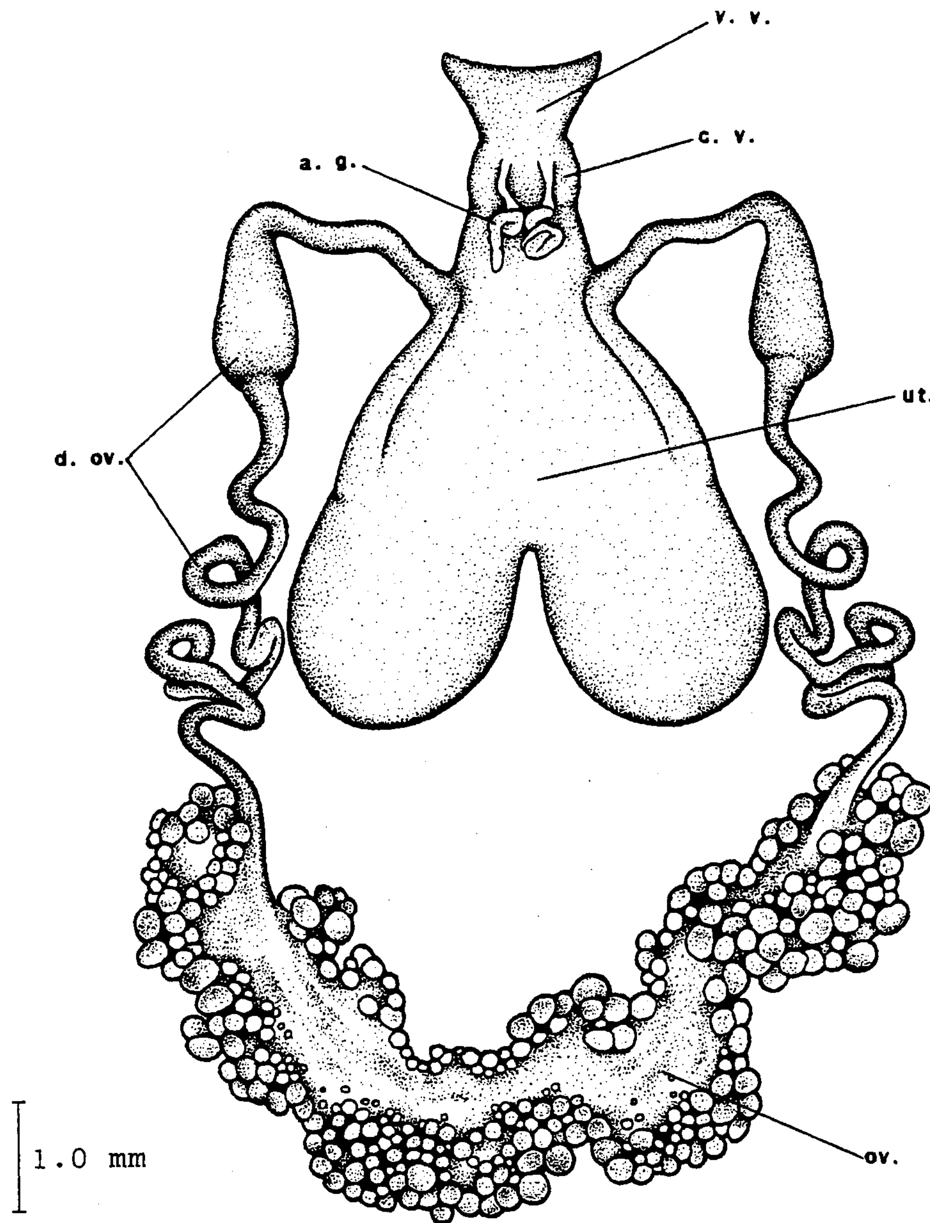


Figure 8. Dorsal view of female _O_. _megnini_ reproductive system.

the exception of a region near the juncture with the uterus where each expands abruptly to four or five times the previous diameter. The expanded portion of the oviducts gradually reduces in diameter as they approach their union with the uterus. The oviducts eventually empty into the uterus near the midline of that organ. However, the oviducts are fused with the uterus for some distance before emptying into the uterus. This results in the formation of an L-shaped bend in each oviduct (Figure 8).

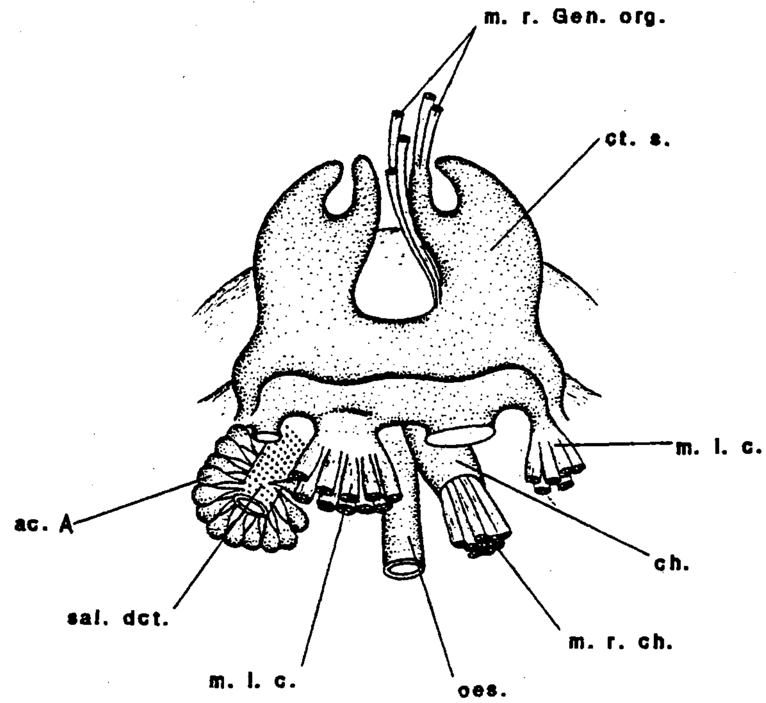
The large uterus displays two wide, rounded lobes posteriorly and narrows anteriorly as it unites with the cervical portion of the vagina. The tubular, cervical portion is visible as a slight enlargement near the anterior portion of the uterus. The vestibular portion of the vagina merges with the cervical portion posteriorly and widens greatly at its anterior end to cover the slit-like genital aperture (not illustrated). No cuticular genital structure, such as the hood in the males, was observed. However, a thin sheet of cuticle continuous with the cuticle around the genital aperature extends into the vestibule for a short distance. Two coiled, tubular accessory glands extend from the dorsal surface of the cervical portion (Figure 8).

Gene's organ is located at the anterior apex of the body cavity directly above the basis capituli and extends ventrally to a slit immediately above the basis capituli (Figure 1). The visible glandular portion is a heavily-wrinkled, bilobed structure. When the glandular tissue is

removed, the chitinous interior sac is exposed. The sac is also bilobed, but with each lobe bifurcated anteriorly. Retractor muscles of Gene's organ are found inserted on the inner arm of the bifurcations and near the bases of the lobes (Figure 9A). The chitinous sac is continous with the cuticle that surrounds the slit-like opening through which the organ extruds when in use. As in the male nymphs, the reproductive organs of the female nymphs are present but reduced in size. The glandular portion of Gene's organ is only present as an amorphous mass of tissue continuous with the epithelium of the anterior region of the body. However, the chitinous sac and retractor muscles are observed in reduced form.

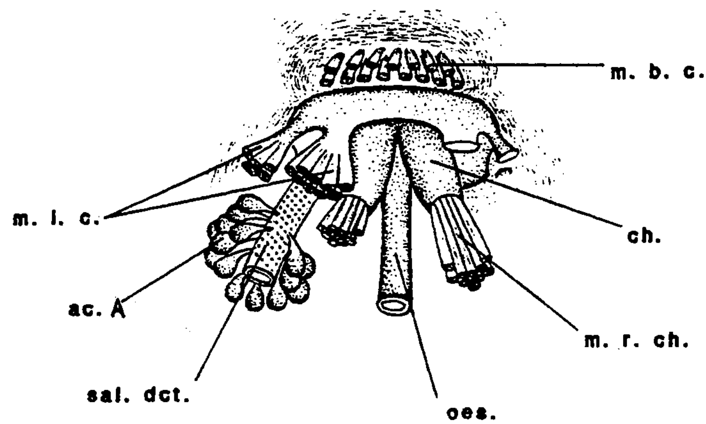
Respiratory System

The respiratory system of *O. megnini* consists of the paired spiracles, atria, and numerous trachae. The spiracles are located at mid-body approximately midway between dorsal and ventral surfaces. The atria, from which all trachae extend, is encased in a tube of cuticle. All of the trachae emerge from beneath this tube (Figure 10). The trachae are bundled into six definable groups. The antero-lateral groups consists of numerous trachae that supply various tissues, muscles, and organs on the antero-lateral regions of the body. Trachae from this group supply the muscles of the first and second pair of legs (pedal trachae 1 and 2) as well as the coxal gland and brain. Several small tracheoles



A

0.30 mm



B

Figure 9. Capitular areas of (A) adult female and (B) adult male _O_. _megnini_.

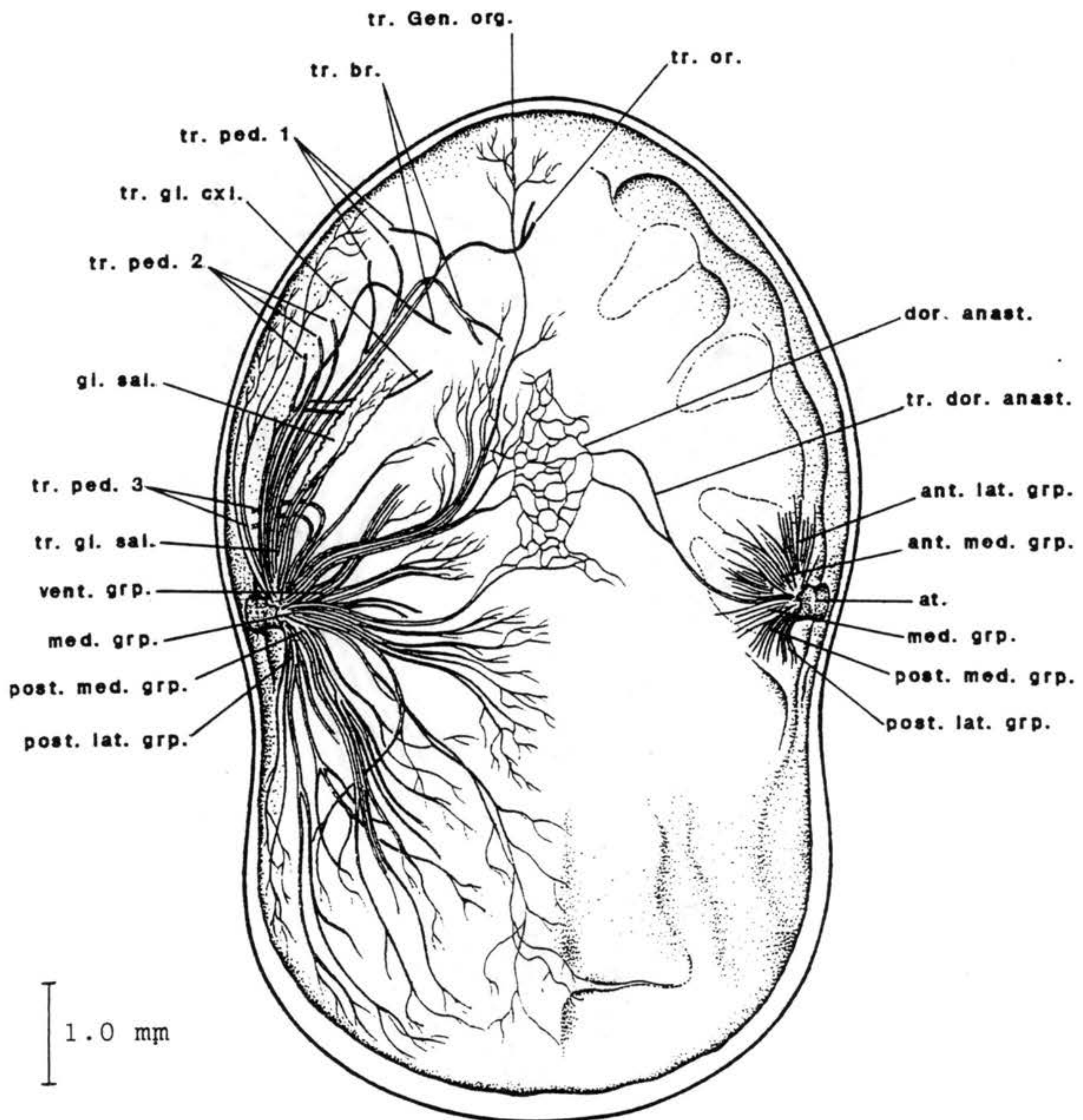


Figure 10. Respiratory system of O. megnini.

originate from these trachae and penetrate the tissue of the brain along the large pedal nerves. Once inside the brain, these tracheoles ramify into an intricate network of ever smaller tracheoles (Figure 11A). Three trachae that supply the salivary glands and one that travels to the oral tissues also originate in this group.

Moving clockwise, the next group is the antero-medial group. Trachae from this group supply the third and fourth pair of legs (pedal trachea 3, pedal trachea 4 not shown) in addition to Gene's organ, the anterior muscles, the dorsal anastomosis (in part), reproductive organs (in part), and muscles in the anterior middle region of the body. The dorsal anastomosis is an extensive network of tracheoles embedded in the dorsal epithelium directly above the stomach. The ventral group consists of only a few trachae which supply ventral tissues near the middle of the body. A few trachae also extend to antero-ventral regions of the reproductive organs. Continuing clockwise, the medial group was next. This group oxygenates the reproductive organs primarily (testis in males, ovary and uterus in females), but trachae from this group also extend to the dorsal anastomosis and postero-medial muscles.

The last two groups are the postero-medial and postero-lateral groups. These two groups supply the muscles and tissues throughout the postero-lateral quarters of the body. The postero-lateral group, which consists of only five or six trachae is the smallest of the six groups. The gastric

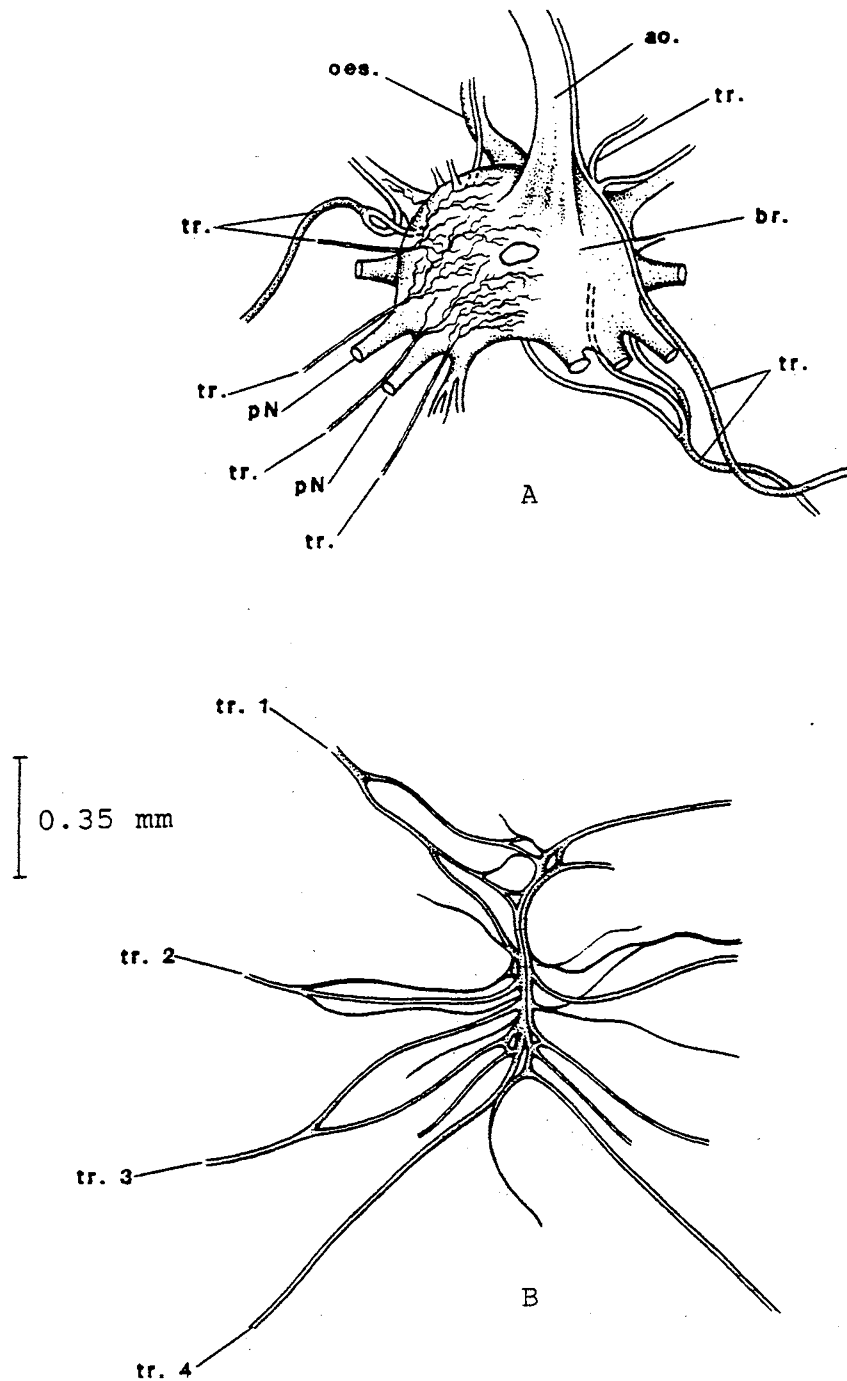


Figure 11. Respiratory system of <u>O</u>. <u>megnini</u>. (A) Brain tracheal system and (B) ventral anastomosis.

caecae are supplied with trachae from both the anterior and posterior groups. Likewise, the trachae supplying the Malpighian tubules could not be traced to any single group.

A ventral anastomosis is also located directly beneath the synganglion posterior to the genital opening (Figure 11B). The tracheal group supplying this tree-like anastomosis could not be located but tracheoles from this structure supply the muscles of all four pairs of legs and the epithelial tissues immediately adjacent to it. The tracheal system of the mature nymphs is virtually identical to that of the adults.

Muscular System

The muscular system of O. megnini consistes of both dorso-ventral and ventral muscles as well as appendicular muscles. The dorso-ventral and ventral muscles are attached to small circular areas called discs, which are located on the interior surfaces of both the dorsal and ventral integument. The discs found on the dorsal cuticle are illustrated in Figure 12 and the internal architecture of the ventral integument, which is identical in the males and females except for the genital opening, is shown in Figure 13 (discs not illustrated). The disc groups and folds on the ventral cuticle are named after the muscles associated with them. The ventral muscle origins are restricted to discs on the ventral cuticle. The extrinsic appendicular muscles originate from discs on the dorsal cuticle and

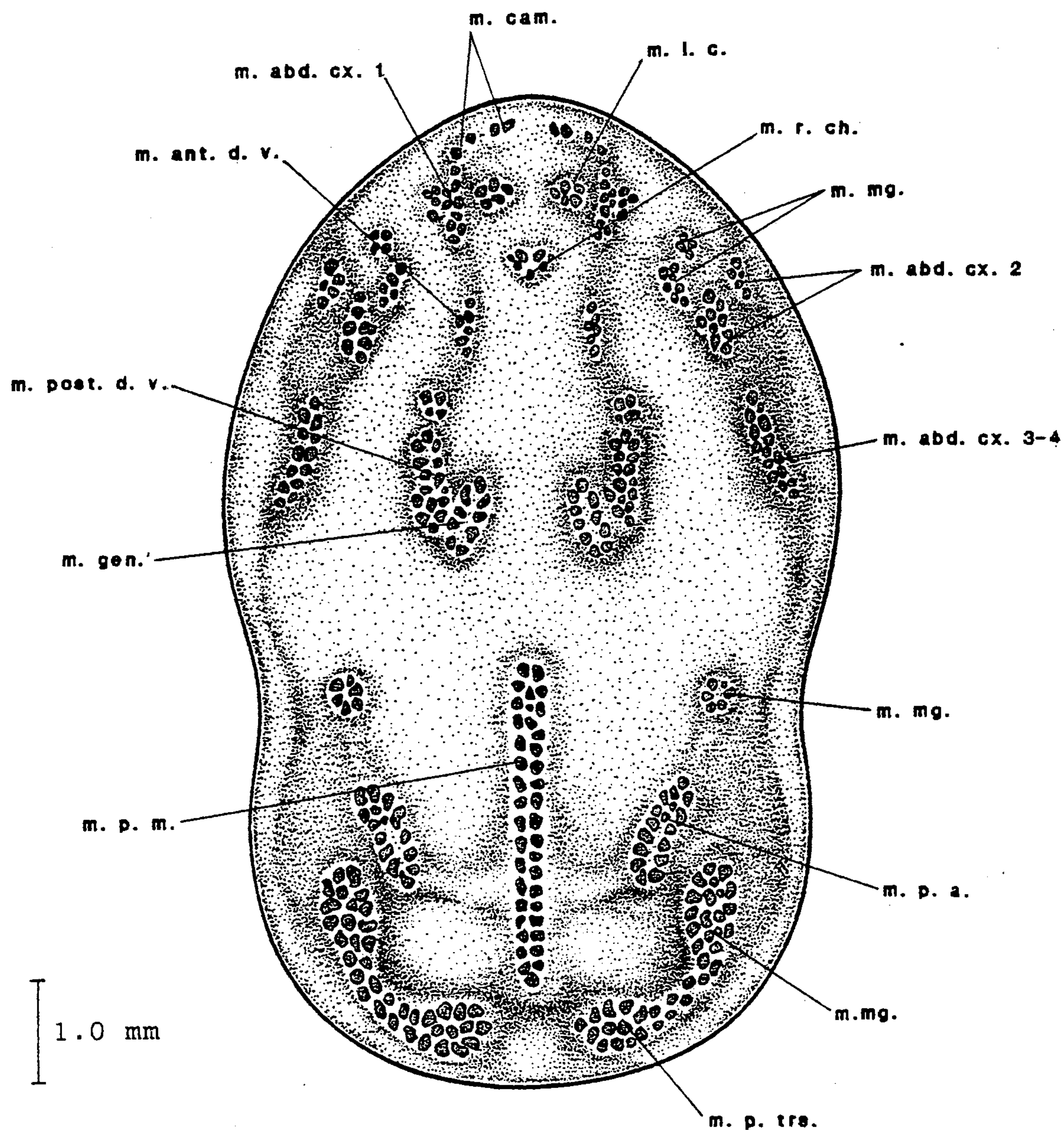


Figure 12. Interior view of dorsal _O. megnini_ cuticle, showing positions of muscle attachments.

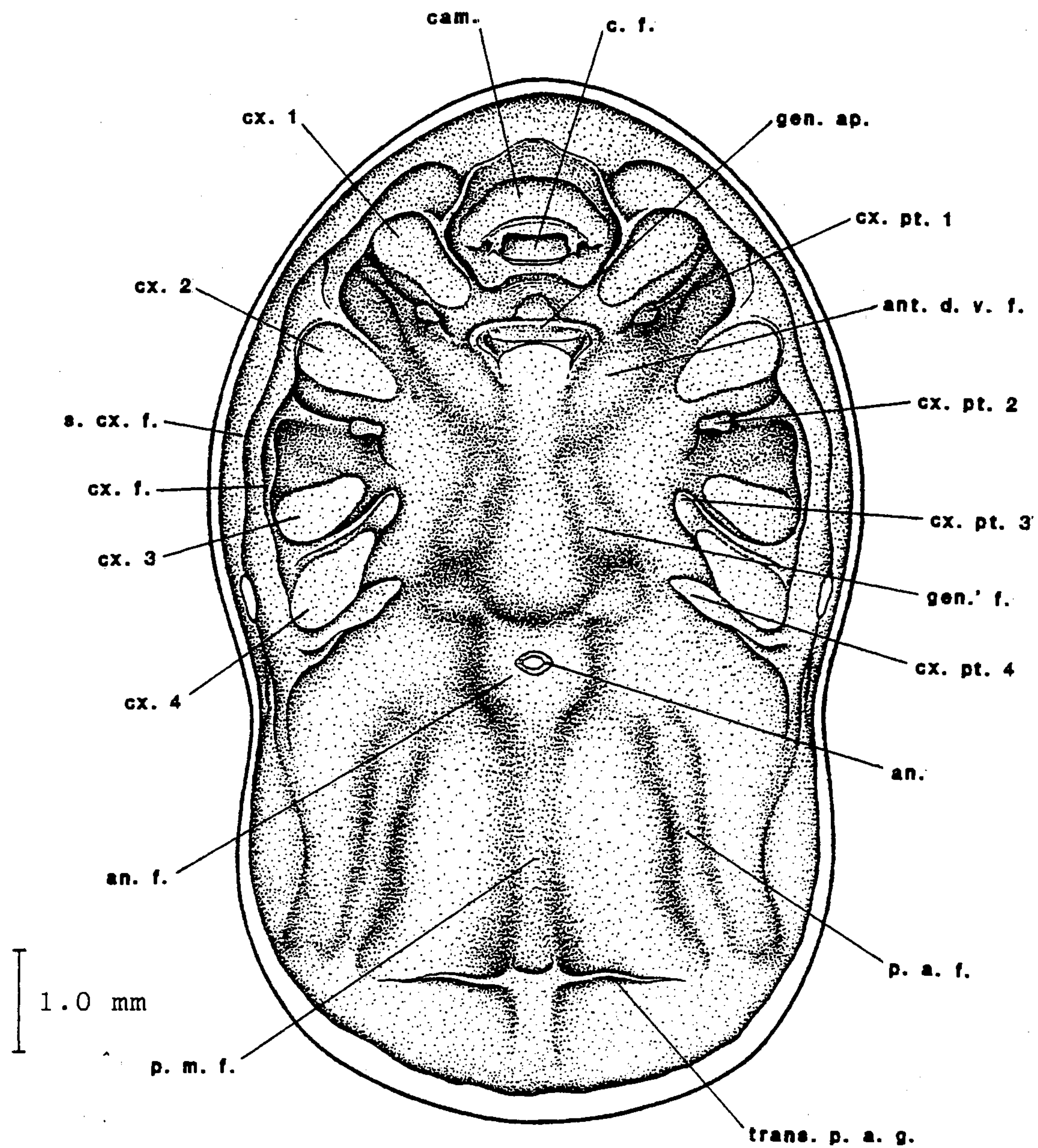


Figure 13. Internal architecture of the ventral cuticle of a female, adult _O. megnini_.

insert by means of tendons at the bases of the appendages. The intrinsic appendicular muscles are retained entirely within the appendages.

In the anterior one-half of the body, the dorso-ventral muscles are comprised of the dorso-ventral genital muscles, anterior and posterior dorso-ventral muscles, levator muscles of the capitulum, camerostomal muscles, muscles of the basis capituli, and marginal muscles (Figures 14 and 15). The dorso-ventral genital muscles are a pair of large muscles comprised of more than 20 fibers. This muscle pair is arranged in a two tier design with the dorsal fibers extending from a group of discs located centrally on the dorsal cuticle and ventral fibers extending from discs on the dorso-ventral genital fold of the ventral cuticle (Figure 12 and 13). Both groups of fibers insert on what appeared to be a thin sliver of cuticle located between the two groups of fibers (Figure 14).

The posterior and anterior dorso-ventral muscles also consist of a dorsal and ventral groups of fibers. Both muscles consist of 8 to 10 fibers in total which extend from discs on the dorsal and ventral cuticle and insert on the endosternum. The anterior dorso-ventral muscles originate from a small groups of discs located antero-centrally on the dorsal integument and insert on the arm of the endosternum. The posterior dorso-ventral muscles insert on the endosternal base (Figure 16A) and originate from a group of

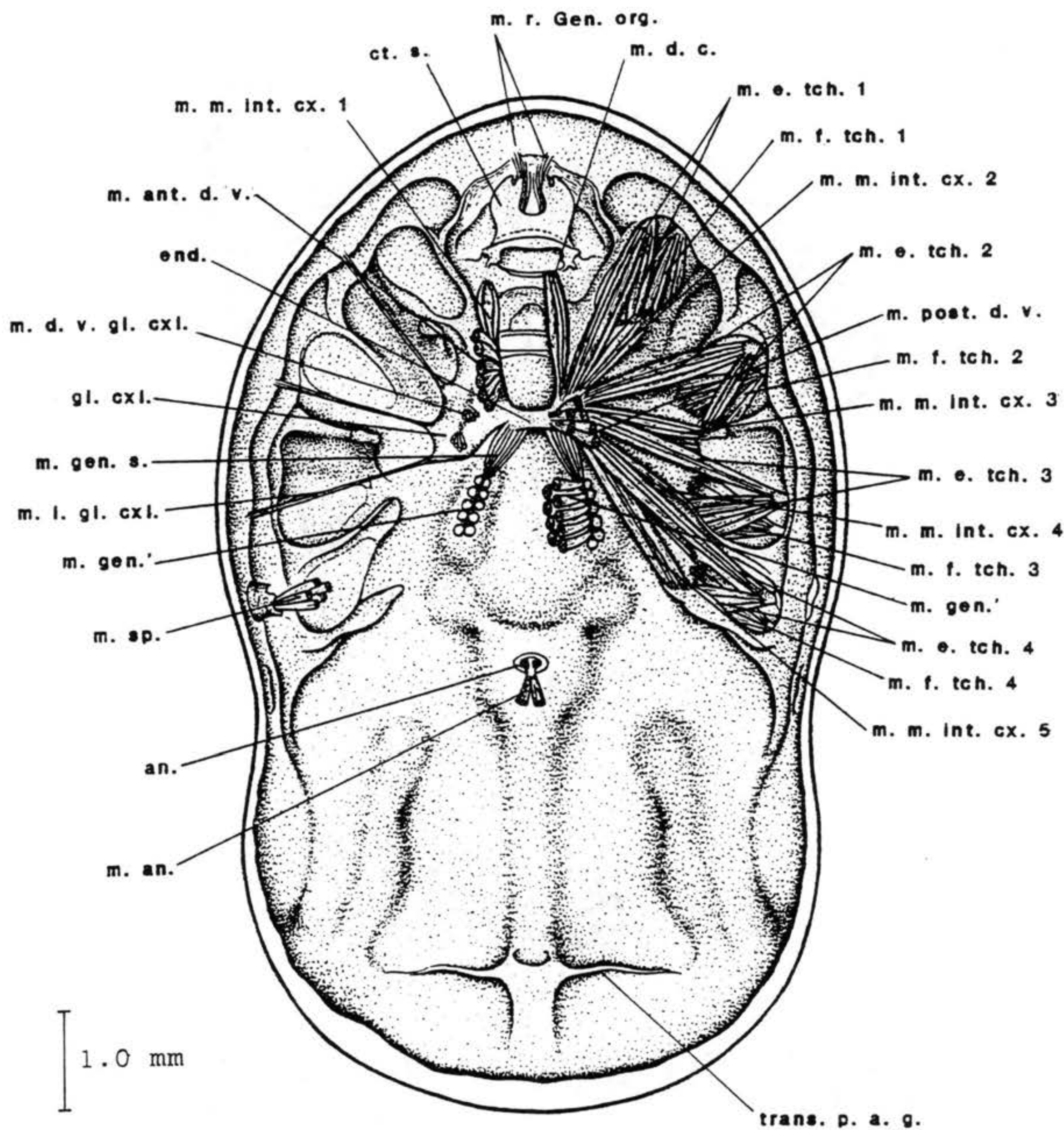


Figure 14. Anterior muscular system of an adult O. megnini, female, dorsal view.

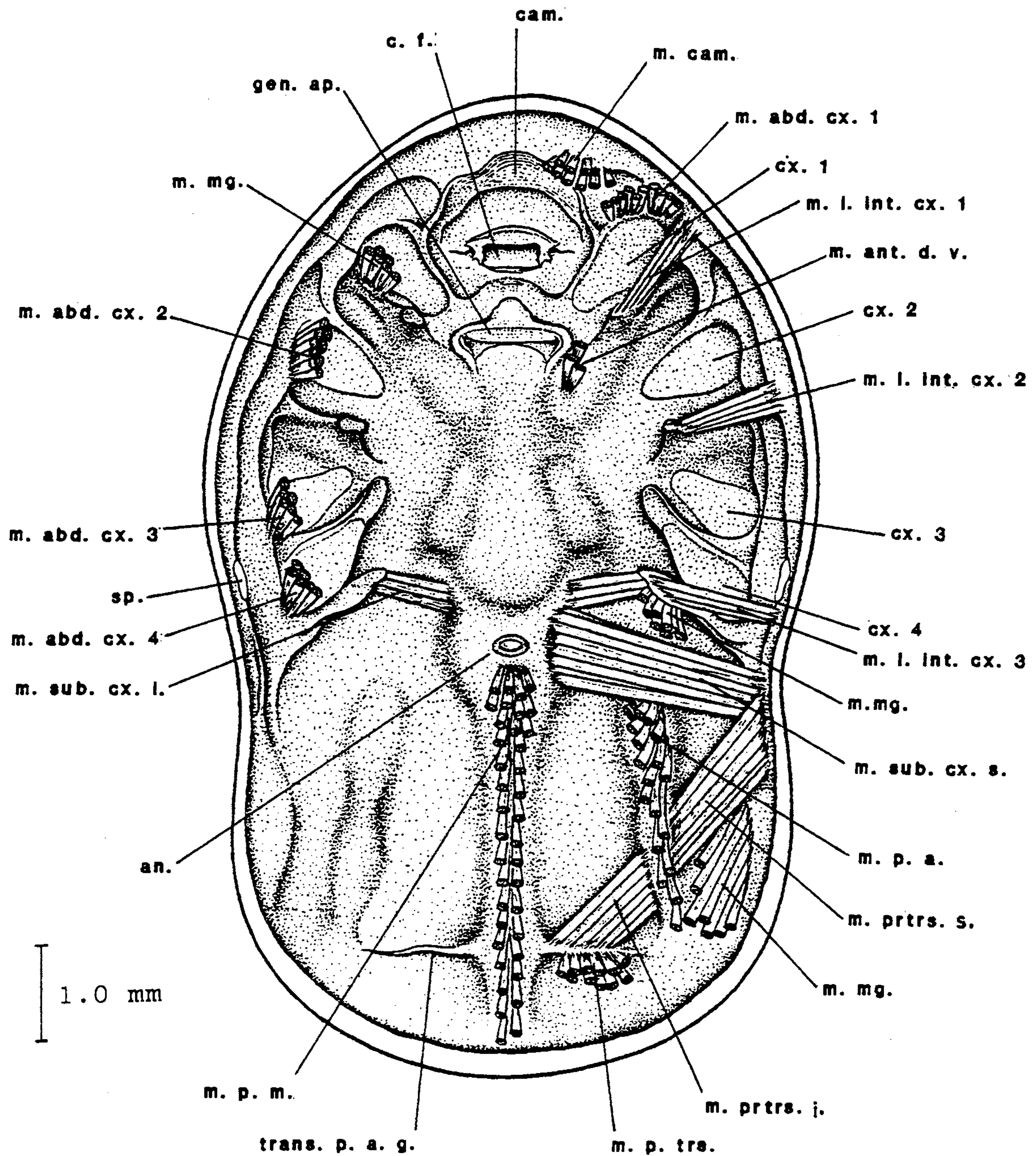
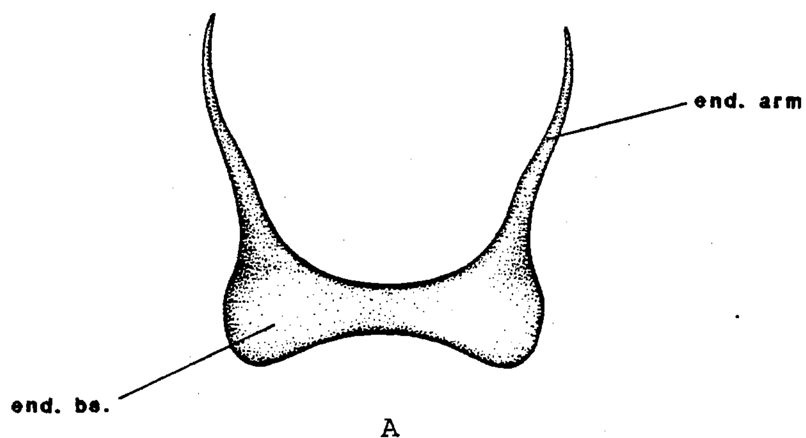


Figure 15. Selected anterior muscles and posterior muscular system of an adult O. megnini, female, dorsal view.



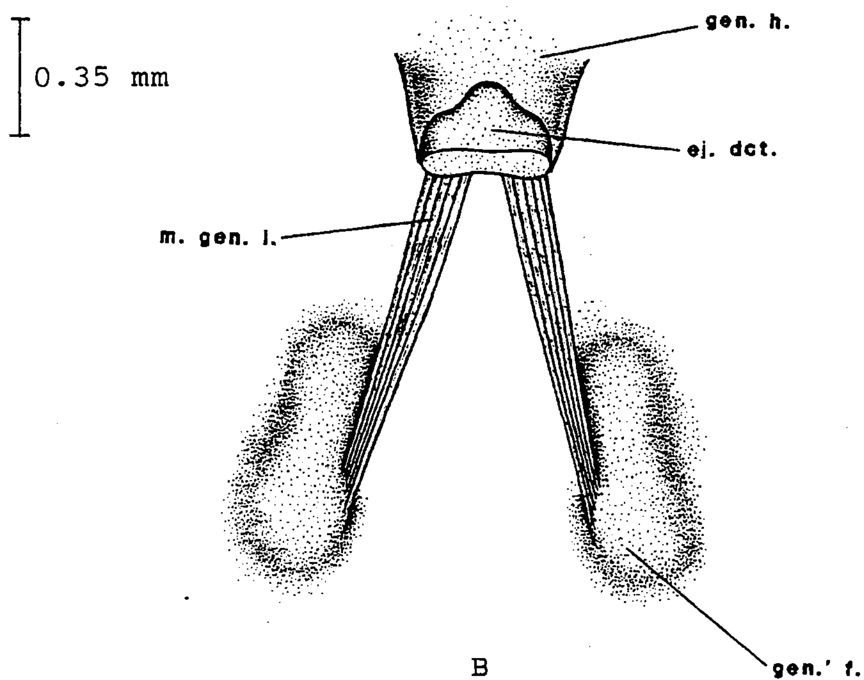


Figure 16. Muscular system of O. megnini. (A) Endosternum and (B) inferior genital muscles of male (dorsal view)

discs postioned directly anterior to the dorso-ventral genital discs.

The levator muscles of the capitulum divide into two small groups of fibers that insert on a flap of cuticle around the capitulum (Figure 9A,B) and extends dorsally to two small groups of discs located antero-mesially on the dorsal cuticle. The camerostomal muscles are a group of 9 to 10 fibers that originate from discs scattered along the antero-dorsal integument and attach to discs on the dorso-lateral margins of the camerostome. The muscles of the basis capituli are found only in the males. This muscle is comprised of 10 to 15 fibers arranged in two rows and attaches to the antero-dorsal cuticle dorsally and the basis capituli ventrally. On the basis capituli, these muscles are positioned immediately above the capitular foramen (Figure 9B). The single pair of marginal muscles attach to the ventral cuticle between the first and second coxae and extend to discs on the lateral dorsal cuticle (Figure 14).

The extrinsic appendicular muscles described include the paired abductors of the coxae and the cheliceral retractors. The abductors and adductors of the palps were not examined. The abductors of the coxae are composed of 6 to 10 fibers which attache to discs positioned laterally on the coxal fold (Figure 15) above each coxa and extend dorsally to discs on the lateral areas of the dorsal cuticle (Figures 12 and 15). No coxal adductors were found. The retractors of the chelicerae extend from discs on the

antero-medial region of the dorsal cuticle to the bulbous bases of the both chelicerae (Figure 9A,B). The fibers of the retractors are somewhat more robust in nymphs than in adults. The intrinsic appendicular muscles include the trochantor flexors and extensors and the flexors and extensors of the cheliceral digit and leg and palpal segments. The intrinsic muscles of the chelicral digit, leg and palpal segments were not studied. The trochantor extensor muscles are comprised of four pairs of muscles with approximately 20 fibers each. The fibers are separated into two distinct groups. The larger group originates on the endosternal base and extends laterally to insert on a small sclerite which was in turn connects to the upper edge of the trochantor. The smaller group originates on the four coxal projections and passes antero-laterally to insert on the same sclerite. The four pairs of trochantor flexors also consist of 15 to 20 slightly larger fibers which originate on a ridge of cuticle on the mesal margins of the coxae (Figure 14). The flexors extend laterally to insert on small sclerites connected to the ventral edge of the trochantor. The spiracular muscles (Figure 14) consist of five fibers each that originate from the posterior dorso-ventral group of discs and insert in the wall of the atrium.

The ventral muscles of the anterior body consist of the mesial intercoxal muscles 1-5, lateral coxal muscles 1-3, depressor muscles of the capitulum, superior genital muscles, and inferior genital muscles (Figure 14 and 15).

The first mesial intercoxal extend from the endosternal arm and pass anteriorly to attach to the ventral cuticle near the first pair of coxae. Mesial intercoxal muscles 2-5 all originate from the endosternal base and extend laterally to attach to the four coxal projections (Figure 12). The coxal projections are clearly illustrated in Figure 13. These structures, which projected dorso-laterally into the body cavity, are rigid outgrowths of the coxal fold. Projections 1 and 2 are near vertical columns of cuticle, and projections 3 and 4 are wider and slightly concave at their apices. The three pairs of lateral intercoxal muscles extend laterally from coxal projections 1,2, and 4 to attach to the supracoxal fold (Figures 13 and 14). The depressor muscles of the capitulum originate from the endosternum near the base of the cuticular arm and insert at the lower edge of the capitular foramen. The superior genital muscles are inserted posteriorly on the same strip of cuticle that joins the dorso-ventral genital muscle and attaches anteriorly to the endosternal base as well as the lateral walls of the vagina or ejaculatory duct (Figure 14). The inferior genital muscles (Figure 16B) are found only in the males. This pair of ventral muscles is attached posteriorly to the dorso-ventral genital fold of the ventral cuticle and extends anteriorly along the ventral surface to insert in the ventro-lateral wall of the ejaculatory duct near the genital aperture.

The large, paired muscles of the posterior region of the body are primarily dorso-ventral in nature. (Figure 15). The most prominent muscle is the unpaired post-medial muscle. This muscle is constructed of more than 35 fibers which extend from discs on the post-medial fold posterior to the anus, pass dorsally through the lobes of the rectal sac, and attach to discs on the dorsal cuticle midline. The fibers in the post-medial muscle are arranged in two rows which extend linearly from near the anus to near the posterior margin of the body.

The sub-coxal muscles are comprised of two groups, the inferior and superior sub-coxals. The larger superior sub-coxals extend from discs on the Y-shaped anal fold (Figure 13) near the anus and extend postero-laterally to the supracoxal fold. The inferior sub-coxals, which are one of the two ventral muscle pairs in the posterior region of the body, are less than one forth of the size of the superiors. These muscles project antero-laterally from the anal fold a short distance from the superior sub-coxals and attach to the ventro-lateral side of the fourth coxal projection (Figure 15).

The post-anal muscles are another large pair consisting of more than 20 fibers. These muscles originate on the post-anal fold midway between the post-medial fold and the lateral margins of the body. The fibers are arranged in a staggered row posteriorly and clumped anteriorly and extend dorsally to a group of discs located anterior to the

posterior marginal group of discs (Figure 12). The post-anal muscles extend anteriorly on the post-anal fold to a line near the anterior extent of the post-medial muscles and posteriorly to near the transverse post-anal groove. The pretransversal muscles are also divided into a superior and inferior group. The superior pretransversals extend antero-laterally from discs on the lateral edges of the post-anal folds to the supracoxal fold, slightly overlapping the superior sub-coxals. The inferior pretransversals are the second pair of ventral muscles. The fibers of these muscles project antero-laterally in line with the superior pretransversals. They originate posteriorly on discs on the transverse post-anal groove and insert anteriorly to discs on the mesial fold opposite of the posterior attachment site of the superior pretransversals. The post-transversal pair of muscles are comprised of 10 to 15 fibers which extend from discs on the transverse post-anal groove to the posterior region of the dorsal cuticle.

Two pairs of marginal muscles are present in the posterior region. The anterior pair originate on the postero-ventral side of the fourth coxal projection and project vertically to a circular group of discs on the dorsal cuticle. The posterior pair extend postero-dorsally from discs on the postero-lateral body wall to attach to the postero-lateral margin of the dorsal cuticle (Figure 15). The anal muscle consist of only two fibers. Both fibers pass ventrally from the post-medial group of discs on the

dorsal cuticle to insert on a strip of cuticle that was continous with the cuticle dividing the anus (Figure 14).

Nervous System

The nervous system of O. megnini adults and nymphs is comprised of a central nerve mass, or synganglion, and peripheral nerves which originate from it. The synganglion, which is a hemispherical shaped organ approximately 0.5 to 0.75 mm wide, is located directly beneath the endosternum at mid-body. The esophagus enters the brain antero-ventrally and emerges dorsally near the center of the organ (Figure 17). The synganglion is enclosed by the neurolemma, which is a thin sheath of tissue continuous with the tissue surrounding the nerves and esophagus. Except for size differences related to body size, the synganglia of adult females and males as well as mature nymphs are identical in external appearance.

The four pairs of pedal nerves are the largest. Each pedal nerve emerges radially from the lateral margins of the synganglion and extends to and embeds in the appendicular muscles of the bases of the four pairs of legs. Four pairs of hemal nerves emerge from the synganglion near the bases of the pedal nerves. Hemal nerve pairs 1, 3, and 4 are nearly the same diameter (approximately 0.02 mm), but the second hemal is roughly one-half the diameter. The hemal nerves extend to the lateral margins of the body where they innervate muscles and tissues located along the body walls.

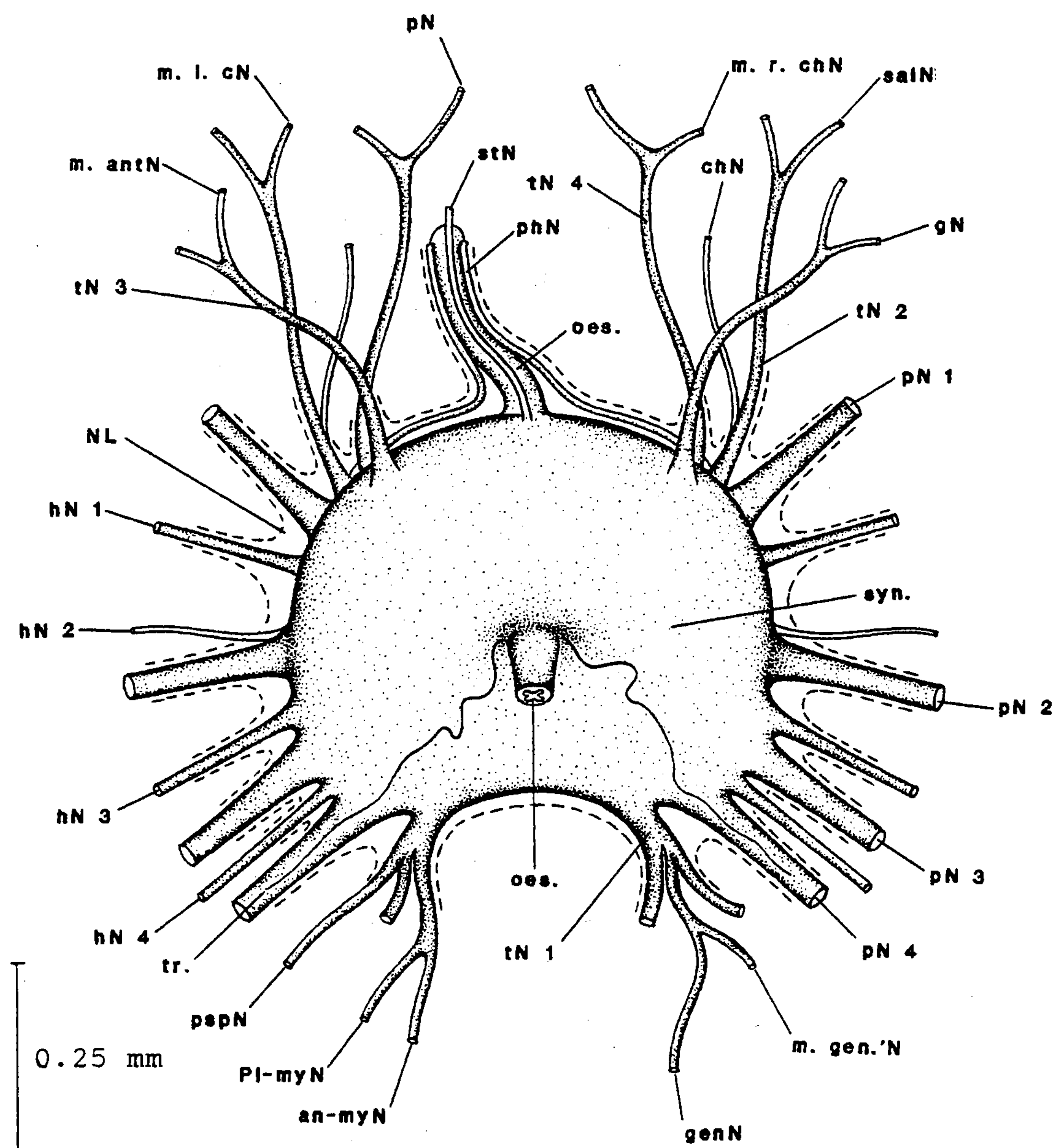


Figure 17. Synganglion of <u>O</u>. <u>megnini</u> (adult, dorsal view).

Two large nerve trunks (tN 1) are located on the postero-lateral margins of the synganglion. Several additional nerves branch from these trunks. The most lateral branches are the paraspiracular nerves (pspN, Figure 17). These nerves innervate the spiracular muscles and other tissues around the spiracles. The innermost branches split to become the post-lateral myosomal nerve (Pl-myN) and the anal-myosomal nerve (an-myN, Figure 17). The post-lateral myosomals innervate the muscles in the postero-lateral quarters of the body. The anal-myosomal nerves also innervate muscles and tissues in the postero-lateral areas as well as muscles in the postero-medial regions including the anal muscle. The genital nerve (genN) emerges ventro-laterally from the base of the paraspiracular nerve and extends a short distance before dividing into two additional nerves (Figure 17). The innermost enters the walls of the vagina or ejaculatory duct. The outer branch innervates the superior genital muscles and the dorso-ventral genital muscles.

Several nerve trunks emerge from the anterior margin of the synganglion. The most lateral pair (tN 2) extend from the synganglion near the bases of the first pedal nerves. This nerve produces two branches that project anteriorly and innervate the capitular levator muscles and the salivary glands. The salivary nerve (salN) extends posteriorly along the gland for most of its length. The small cheliceral nerve (chN) emerges from the base of the above nerve and

extends to the bases of the chelicerae (Figure 17). The pharyngeal nerves (phN) also emerge from the synganglion near the cheliceral nerves and extend to the capitular foramen along either side of the esophagus. These nerves remain within the tissue sheath surrounding the esophagus. Another nerve pair (tN 3) extends antero-dorsally from the antero-dorsal corners of the synganglion. This nerve also divides into two branches. The lateral nerve, which extends dorso-posteriorly to the stomach, is the gastric nerve (gN). The mesal branch (m. antN) extends to muscles in the anterior regions of the body.

A final pair of nerve trunks (tN 4) emerges from the synganglion anteriorly, directly below nerve trunk three. As in the other anterior nerves, this nerve pair also splits into two branches. The outer branch (m. r. chN) extends to the cheliceral retractor muscles and the inner nerve (pN) extends to the capitular foramen to innervate the palps. The unpaired stomodeal nerve emerges antero-medially from the synganglion and innervated tissues along the esophagus (Figure 17). Many fine nerves also intertwine with the intercoxal muscles and connect with the hemal nerves in several places. These nerves innervate the coxal glands. The remainder of the network could not be viewed in its entirety due to their position in the muscles and were not illustrated.

Circulatory System

The heart and dorsal aorta were the only organs of the circulatory system observed in dissections of *O. megnini* adults and nymphs. The flattened, triangular heart is an extremely obscure organ located directly beneath the dorsal anastomosis (Figure 18). The outlines of the heart are difficult to discern because of surrounding tissue, but many wavy tracheoles are observed on walls of the heart. The aorta extends antero-ventrally from the heart and merges with the neurolemma of the synganglion dorsally near the emergence point of the esophagus. The neurolemma forms a sinus encompassing the synganglion. The neurolemma also appeares to be continuous with the tissue around the esophagus.

Water Vapor Uptake

After 30 days of dehydration at near zero relative humidity (R.H.), the 9 month old test males lost 6.3 $\pm$ 1.43% of their initial, average body weight of 33.6 mg. The control males lost 10.85 $\pm$ 2.19% of their initial average weight of 29.6 mg. The 9 month control and test females lost 2.61 $\pm$ 0.68% and 2.43 $\pm$ 1.65% of their initial average weights of 42.4 and 50.3 mg, respectively (Figure 19). The 1 month old, test males and females lost 10.43 $\pm$ 1.22% and 3.0 $\pm$ 1.44% of their initial, average body weights of 36.1 and 59.6 mg, respectively (Figure 20). The 1 month old control males lost 7.52 $\pm$ 1.80% of their initial, average

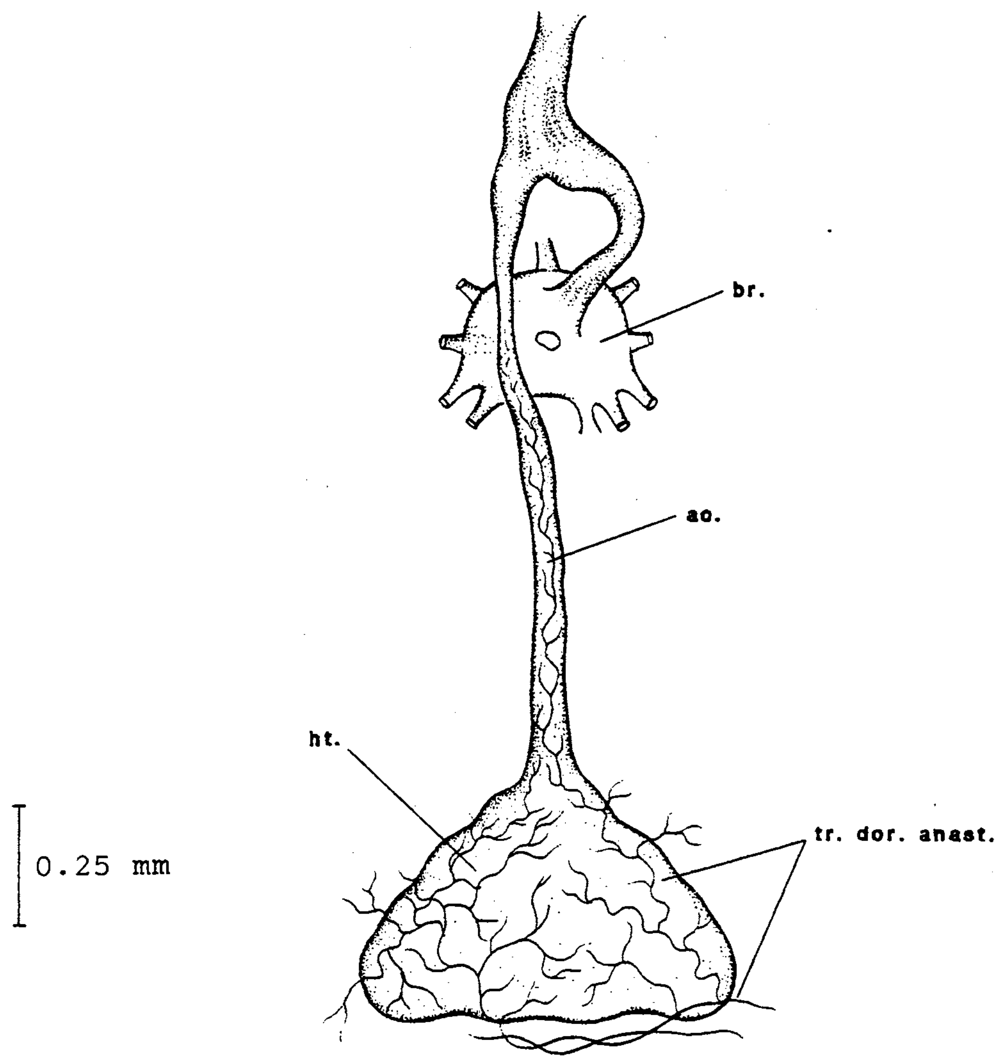
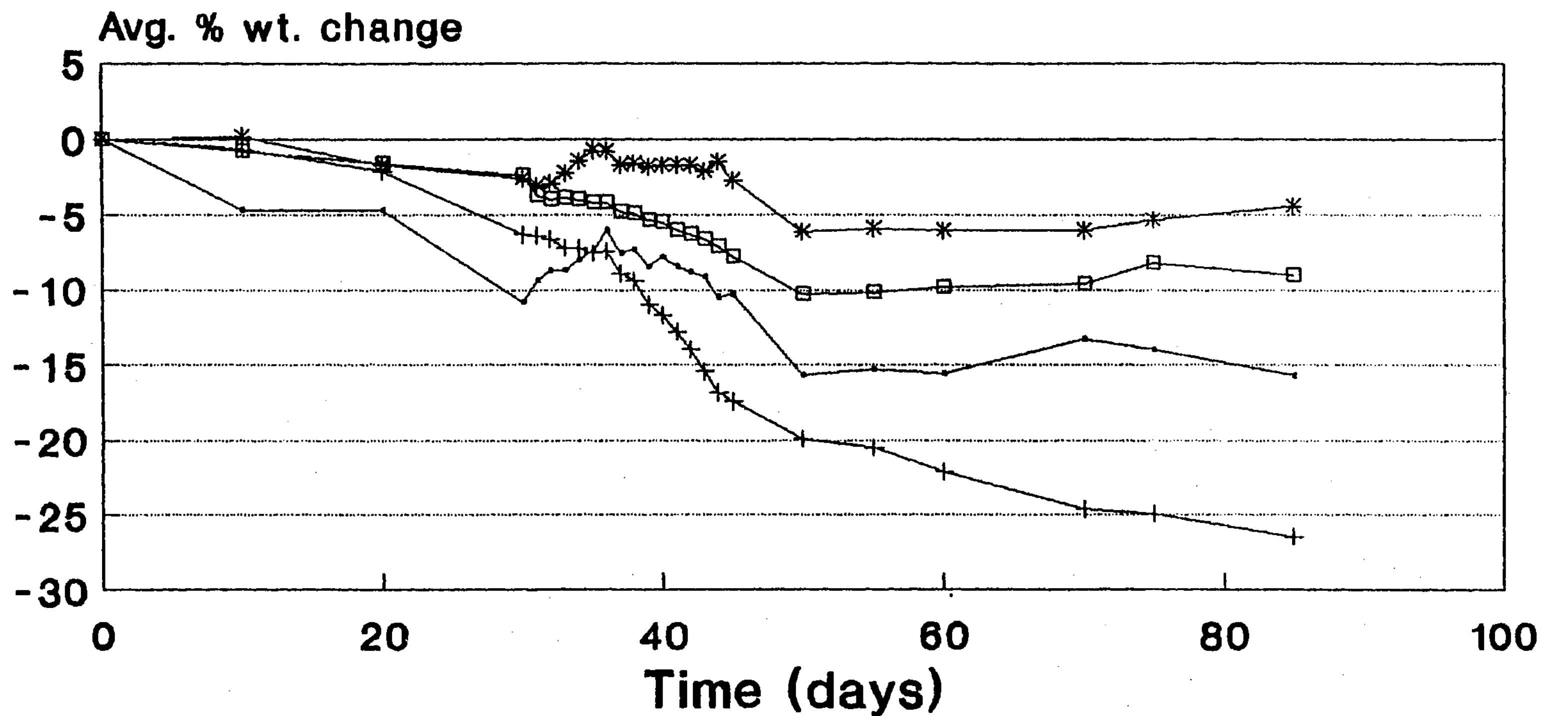


Figure 18. Circulatory system of adult O. megnini (dorsal aspect).



Test tick mouthparts wax covered
Dehydration through day 30
Wax removed on day 45

5 ticks/group

Cont. females
Test females
Cont. males
Test males

Figure 19. Average, percent, weight changes over time for ten, 9 month old, normal, adult O. megnini and ten, adult O. megnini with mouthparts wax covered.

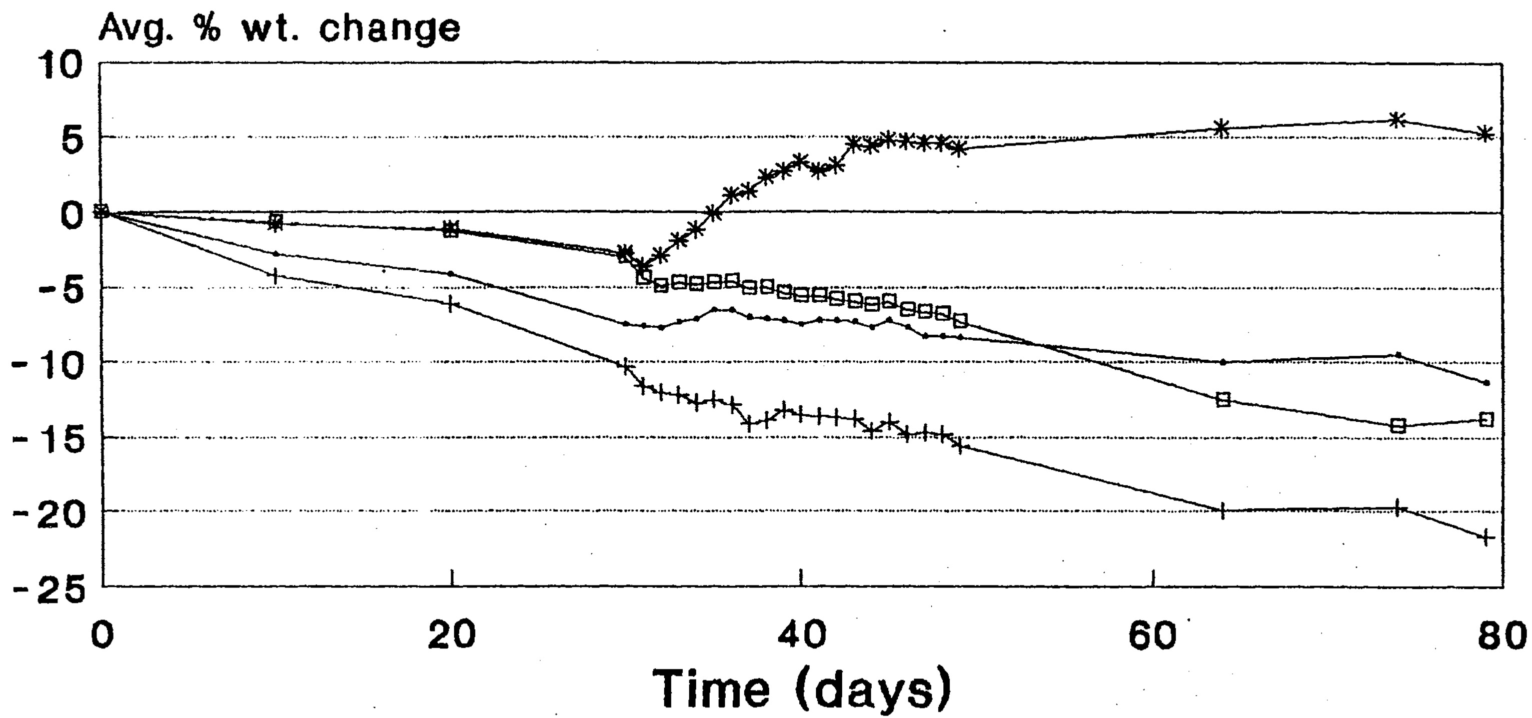


Figure 20. Average, percent, weight changes over time for ten, 1 month old, normal, adult O. megnini and ten, adult O. megnini with mouthparts wax covered.

body weight of 38.7 mg, and the five, 1 month control females lost 2.72 ± 1.8% of their initial average weight of 63.6 mg.

Both the 9 month control females and males reached maximal recovery after 6 days of rehydration at 97% R.H.. The males improved to within 5.98 ± 4.7% of their initial, average weight, and the females to 0.8 ± 3.36% of their initial, average weight. Thereafter, the average percent weight loss increased continually for the males, eventually reaching 15.7 ± 2.99% at the last weighing. The control females also continued to lose weight, reaching a loss of 6.08 ± 4.77% at 20 days post rehydration, then increased again to 5.3 ± 5.65% of average, initial weight at 45 days post rehydration.

The 9 month old test ticks, which had mouthparts wax covered, never increased in weight after rehydration. They continued to decrease at a fairly constant rate even after the wax was removed (Figure 19). At the end of the experiment, only three were alive and they had lost 26.5 ± 10.5% of their initial, average weight. The test females also lost weight continuously, but at a significantly reduced rate. However, after the wax was removed at day 15 of rehydration, the 9 month test females reduced their average weight loss from 10.36 ± 14.6% to 6.92 ± 12.5% of their initial, average weight at 30 days post wax removal.

After rehydration, the 1 month, control males reduced their average percent weight loss to a minimum of 6.5

± 2.4% at day 6 of rehydration. Afterwards, the average weight loss continued to increase irregularly reaching 11.32 ± 4.11% at the last weighing. The 1 month, test males showed no increase in body weight (Figure 20). The average, percent weight loss continued to increase even after wax removal. The percent weight loss eventually reached 23.32 ± 3.24% of initial, average weight.

The 1 month, control females increased in weight after initiation of rehydration, eventually reaching an average weight 3.28 ± 3.47% greater than the initial average weight at day 15 of rehydration. The average weight of these females continued to increase to a maximum of 6.18 ± 7.25% above initial, average weight at 44 days post rehydration. The 1 month, test females showed no decrease in average, percent weight loss. After the wax was removed, the test females continued to lose weight, reaching a maximum loss of 14.16 ± 4.9% at 25 days post wax removal. By the end of the experiment, at 30 days post wax removal, the average weight of the test females had recovered to 13.8 ± 7.6% of their initial, average weight.

Comparisons between the average, percent, weight changes of 9 and 1 month post-molt adult _O. megnini_ indicated few significant differences (Tabel I and Table II). Variances for each comparison were not significantly different at the 95% probability level (F-values not shown). The average, percent weight changes were significantly different for the 1 and 9 month control females at 15 days

TABLE I

COMPARISON OVER TIME OF AVERAGE, PERCENT WEIGHT CHANGE FOR TEN, 9 MONTH AND TEN, 1 MONTH OLD O. MEGNINI ADULT, MALE TICKS

	Test Ticks[1]			Control Ticks		
	Avg. % Wt. Change[2]			Avg. % Wt. Change		
Day	9 month[3]	1 month	$\|t\|$	9 month	1 month	$\|t\|$
30[4]	-6.30	-10.34	4.87[5]	-10.85	-7.52	2.66[5]
5[6]	-7.46	-10.96	1.62	-7.42	-6.54	0.41
10[6]	-11.66	-13.50	1.09	-7.84	-7.46	0.19
15[6]	-17.38	-13.96	0.85	-10.26	-7.12	1.36
15[7]	-22.10[8]	-19.92	0.95	-15.64	-10.04	1.44
25[7]	-24.58[8]	-19.72[8]	1.39	-13.33	-9.48	1.76

[1] Test tick mouthparts wax covered.

[2] Percentage of initial milligram weight.

[3] Age at beginning of experiment, 5 test and 5 control ticks in each age group.

[4] Dehydration at near 0% R.H.

[5] Significant at the 95% probability level.

[6] Rehydration at 97% R.H.

[7] Days after removal of wax from test ticks (97% R.H.).

[8] Average of only four ticks.

TABLE II

COMPARISON OVER TIME OF AVERAGE, PERCENT WEIGHT CHANGE FOR TEN, 9 MONTH AND TEN, 1 MONTH OLD O. MEGNINI ADULT, FEMALE TICKS

	Test Ticks[1]			Control Ticks		
	Avg. % Wt. Change[2]			Avg. % Wt. Change		
Day	9 month[3]	1 month	|t|	9 month	1 month	|t|
30[4]	-2.43	-3.00	0.72	-2.61	-2.72	0.63
5[5]	-4.24	-4.72	0.15	-0.73	-0.10	0.35
10[5]	-5.52	-5.56	0.01	-1.70	+3.28	2.26
15[5]	-7.80	-6.00	1.30	-2.73	+4.83	2.78[6]
15[7]	-9.84	-12.52	0.40	-6.04	+5.64	3.02[6]
25[7]	-9.62	-14.16	0.64	-6.04	+6.18	3.02[6]

1 Test tick mouthparts wax covered.

2 Percentage of initial milligram weight.

3 Age at beginning of experiment, 5 test and 5 control ticks in each age group.

4 Dehydration at near 0% R.H.

5 Rehydration at 97% R.H.

6 Significant at the 95% probability level.

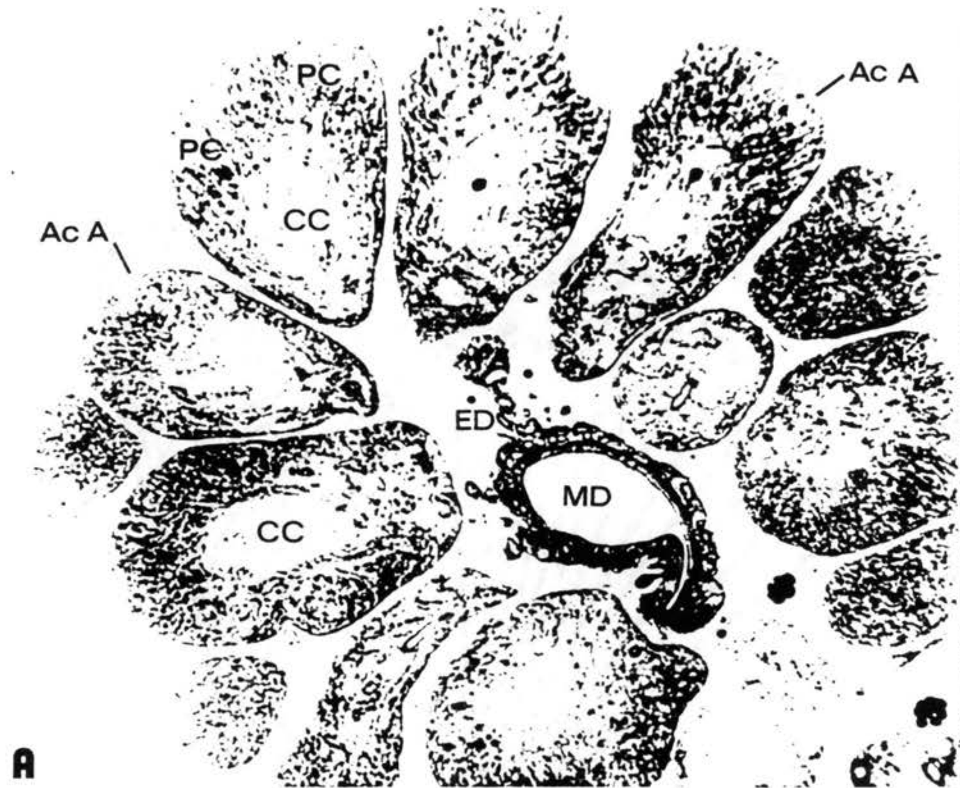
7 Days after removal of wax from test ticks (97% R.H.).

of rehydration and at 15 and 25 days after removal of wax from test ticks (55 to 59 days total at 97% R.H.). No other comparison was significant at the 95% probability level.

Salivary Gland Ultrastructure

Salivary Ducts

Abbreviations used to describe the ultrastructure of the acini are located in Appendix C. The main salivary ducts of adult and nymphal O. megnini were oval or comma shaped structures approximately 15 to 20 μm in diameter (Figure 21A,B). Each duct consisted of an inner layer of cuticle surrounded by a single layer of epithelial cells. The cuticular layer, generally highly folded, was comprised of a thin electron dense cuticular epicuticle surrounded by a larger less dense layer of fibrillar procuticle. Sections of spiral thread, similar in appearance to the reinforcing taenidia of insect trachae, were observed within the procuticle as darker, oblong shapes arrayed along the main duct (Figure 22A). The epithelial cells surrounding the main duct were characterized by granular cytoplasm with an ovoid or irregularly shaped nuclei, and the basement membrane around the epithelial cells was unusually tortuous. The secondary and efferent ducts had a structure identical to that of the primary duct, but were only about 5 μm in diameter (Figure 22B).



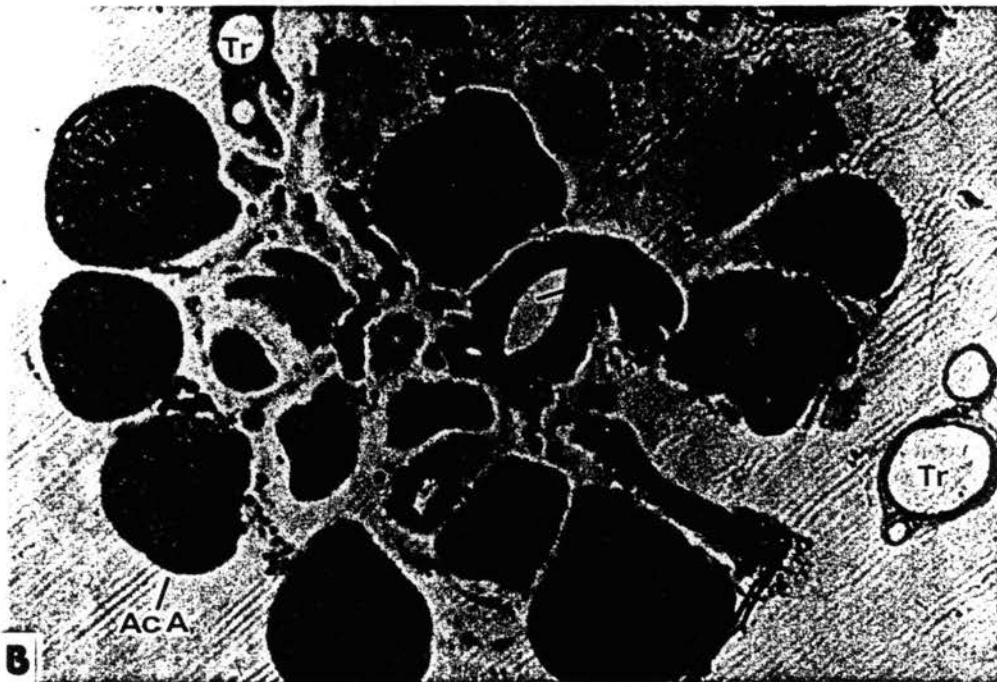
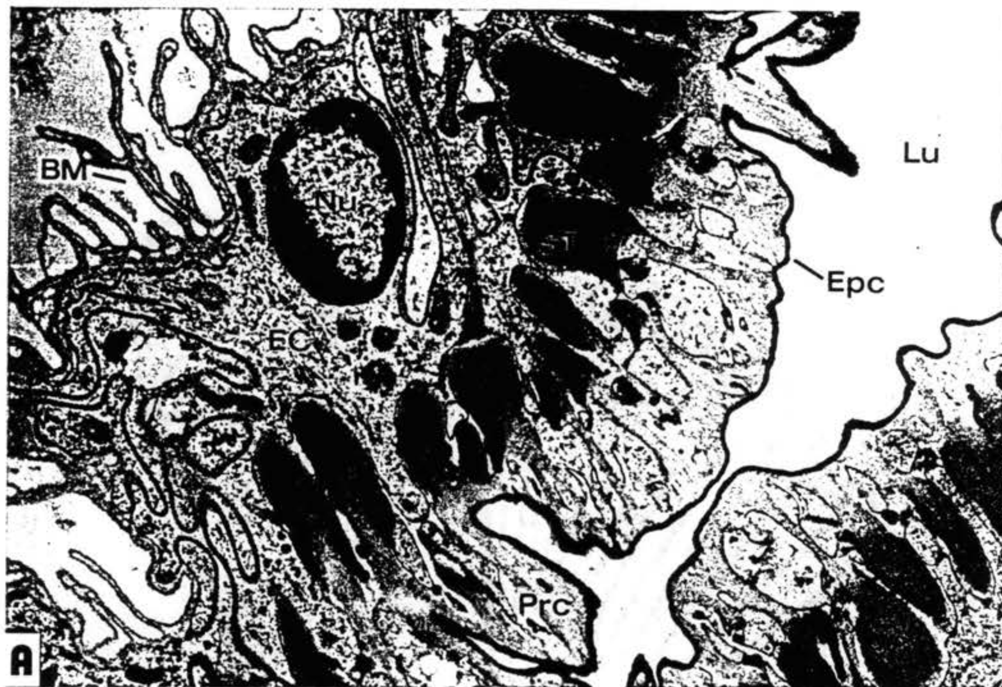


Figure 21. Salivary glands from adult O. megnini showing type A acini. A) 500X B) 400X



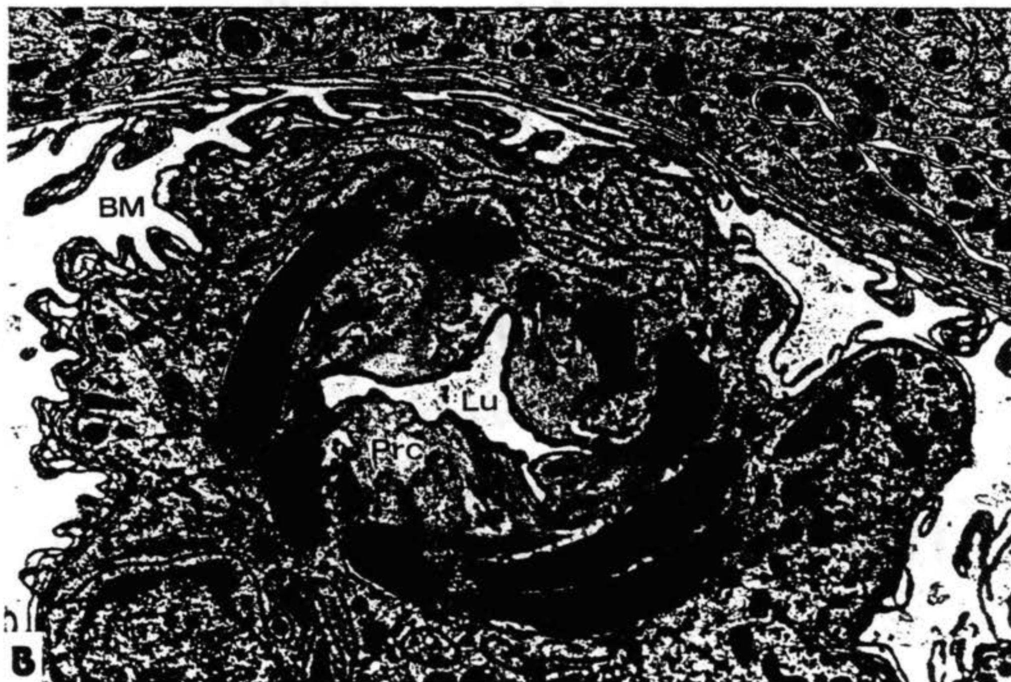
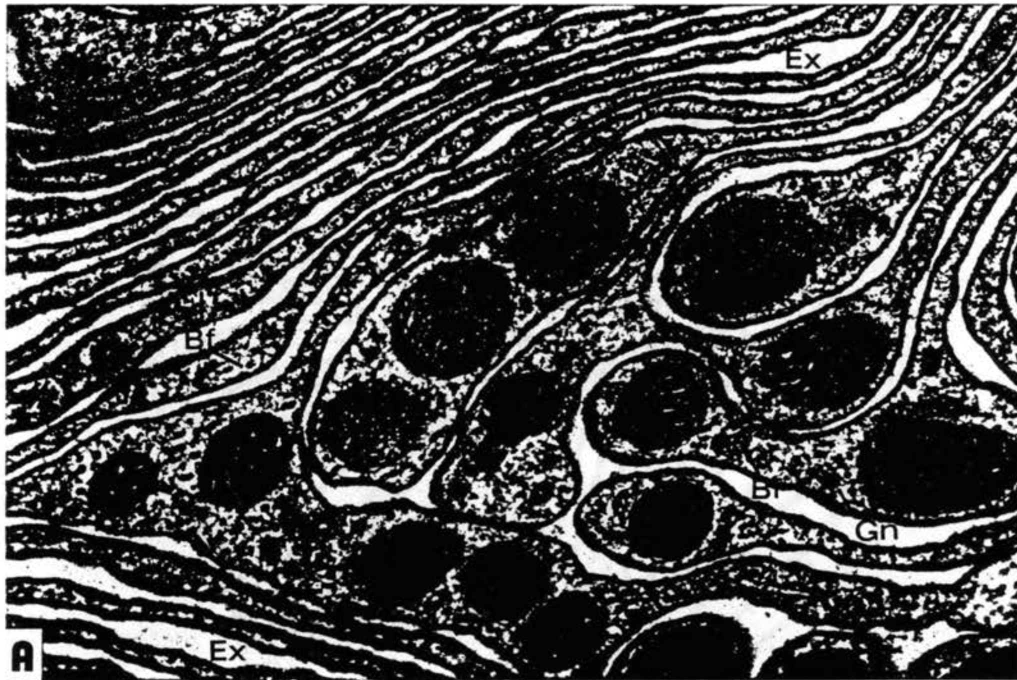


Figure 22. Ultrastructure of salivary ducts from adult O. megnini salivary glands. A) Main salivary duct (13,000X) B) Efferent salivary duct (10,500X)

Type A Acini

The type A acini of adult *O. megnini* were located only on the antero-mesial one-half of the gland and were connected directly to the primary salivary duct by a short efferent duct. Each acinus consisted of a large, clear central cell surrounded by an undetermined number of peripheral lamellate cells (Figure 21A). With application of Mallory's Azure II Methylene Blue, the type A acini stained an overall pale blue-violet (Figure 21B). The peripheral lamellate cells were characterized by intricate infoldings of the basal, plasma membrane that extended from the basal lamina to the central cells. The infoldings appeared to be anchored to the basal membrane by hemidesmosomes. The narrow infoldings surrounded equally narrow extracellular spaces. Many circular or oval mitochondria were also observed in enlarged areas of the infoldings (Figure 23A,B). Dark granules, similar in appearance to glycogen granules, were observed scattered throughout the peripheral lamellate cells. Many trachae and a few nerves were observed extending between the acini (Figure 24A,B).

The type A acini in the nymphs were less than a third of the size of those in the adults and were not examined at the ultrastructural level.



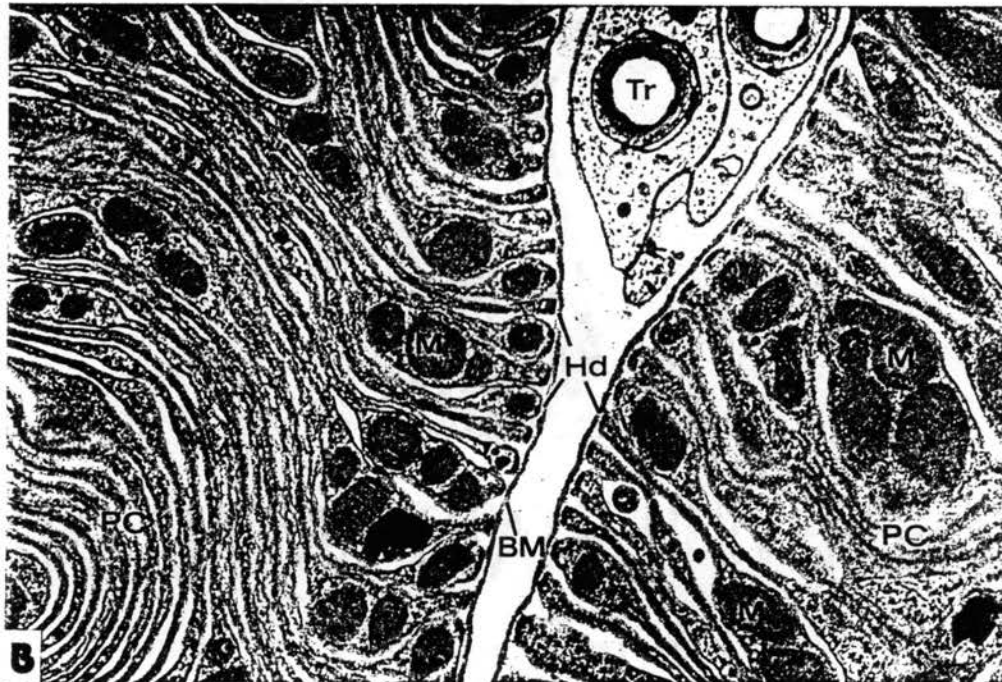
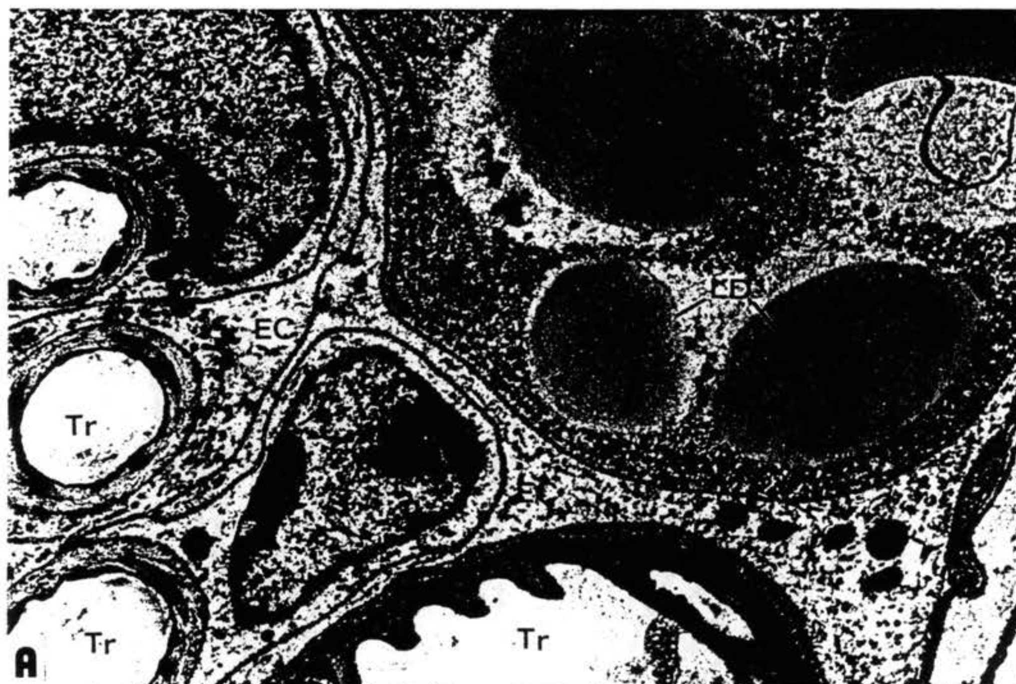


Figure 23 Peripheral lamellate cells from type A acini of adult O. megnini salivary glands showing infoldings of basal plasma membrane. A) 38,500X B) 17,500X



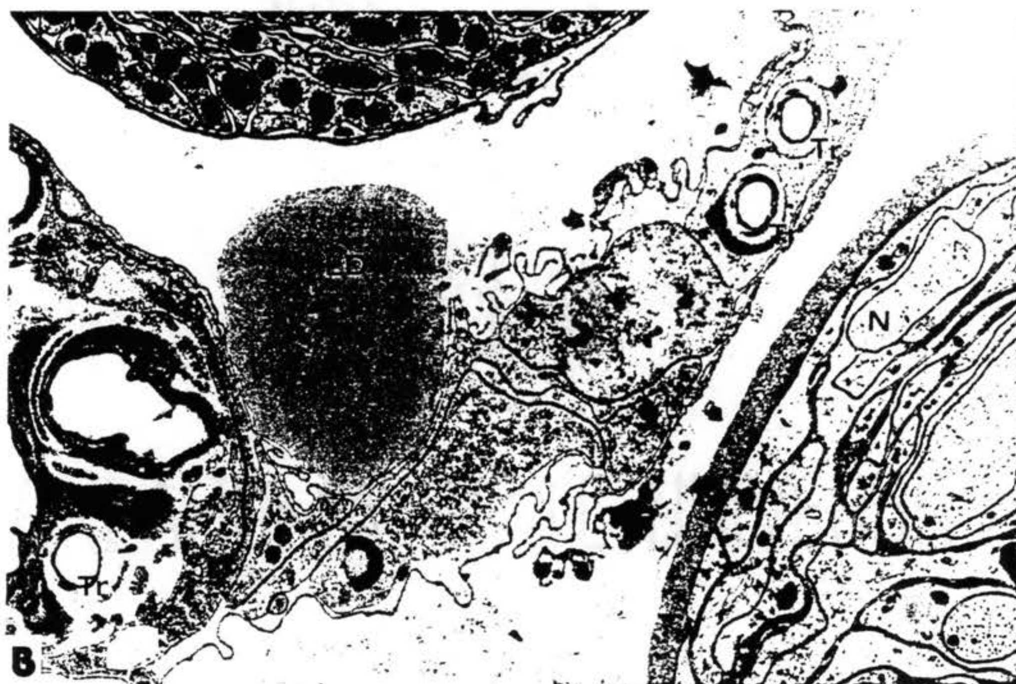


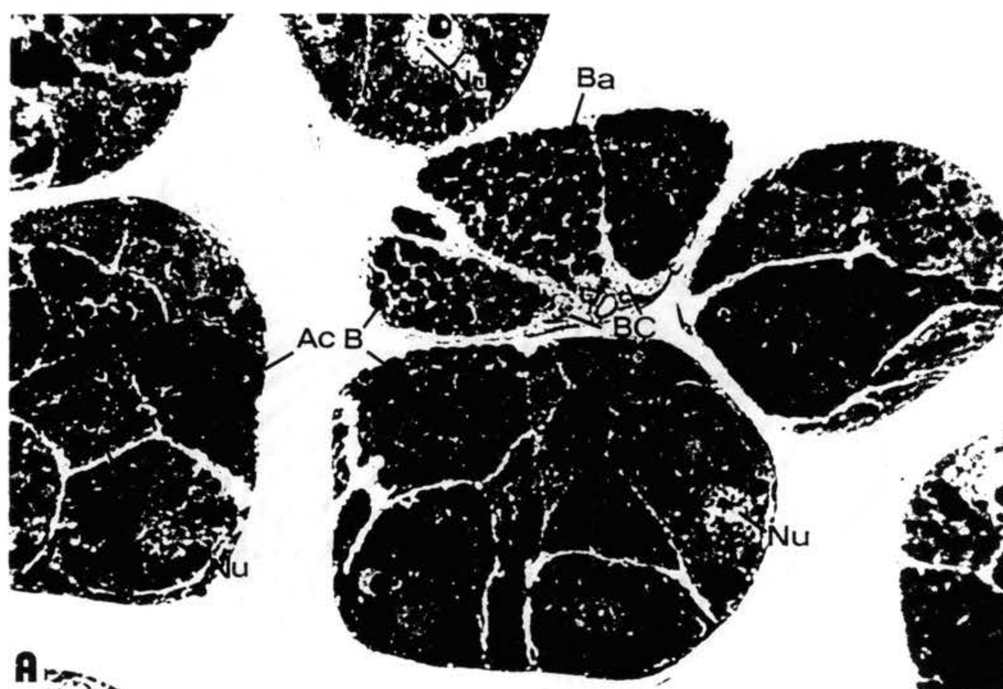
Figure 24. Ultrastructure of adult O. megnini salivary glands showing trachae and nerves located between acini. A) 16,500X B) 10,500X

Type B Acini

The type B acini comprised the bulk of the salivary glands in *O. megnini* nymphs. The acini were connected directly to the primary or secondary duct by short efferent ducts. The ultrastructure of the efferent ducts was identical to those for the type A acini of the adults.

Only one type of granular, type B acinus was found in the nymphs. These acini contained at least ten pyramidal cells that rested on a basement membrane (Figure 25A,B). After staining with Mallory's Azure II Methylene Blue, there appeared to be two types of granular cells within the acini based on granule size and staining characteristics. The bulk of each acinus was composed of cells, labelled Ba cells, with large, round granules up to 3 μm in diameter. These granules displayed a central core which stained a reddish blue-violet while the periphery stained a simple blue-violet. The remainder of the Ba cell bodies stained a pale reddish blue-violet. A few large, dark purple granules were also observed in some Ba cells. The nuclei were fairly large, measuring up to 8 μm in diameter. Some nuclei displayed a prominent dark blue nucleolus.

With the electron microscope, the two layers of these granules were very evident. The core of the granules was of a homogeneous electron dense material and the outer layer was of a less electron dense material. In some of the granules, smaller granules of the darker material could be observed in the lighter layer around the dark core (Figure



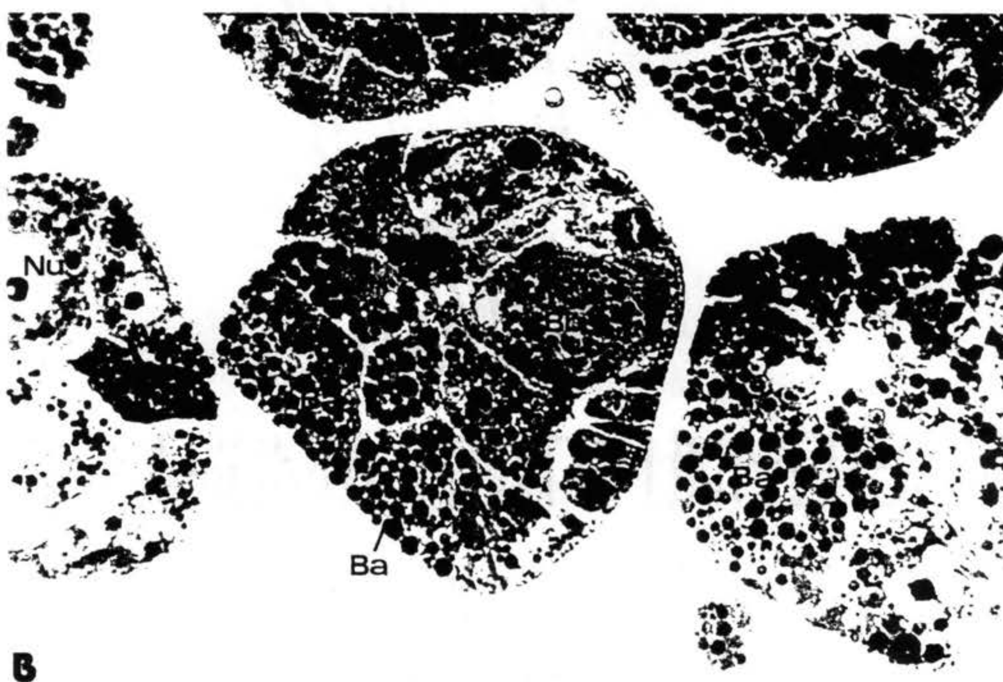


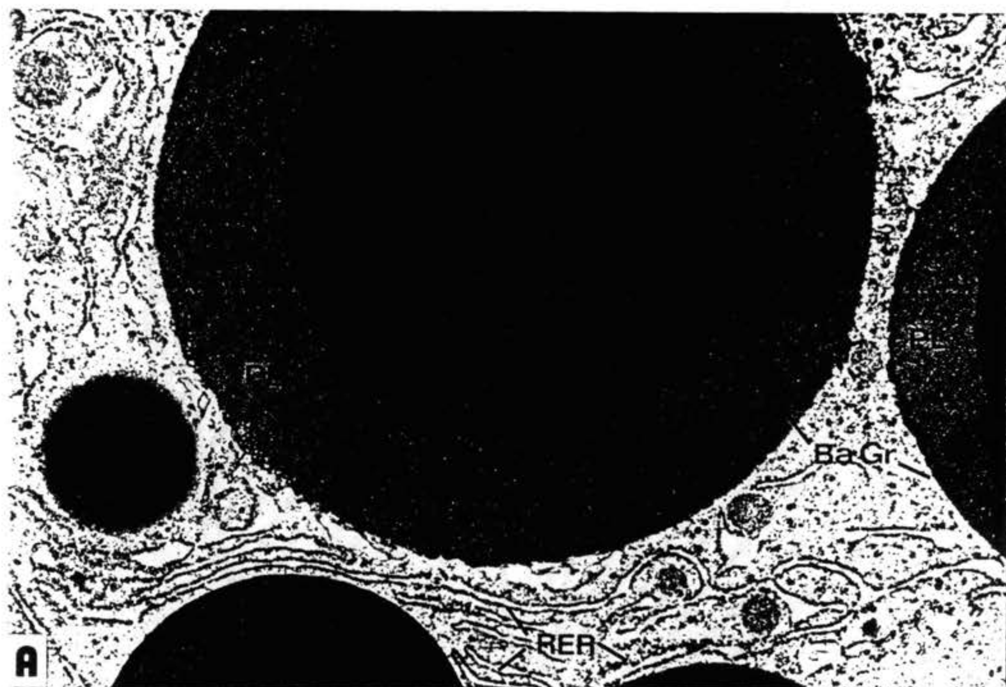
Figure 25. Salivary glands from fed O. megnini nymphs showing cell types within the type B acini. (A and B both 400X)

26A). Also, extensive rough endoplasmic reticulum was observed around the granules. The apices of the granular cells exhibited numerous microvilli that extended into a small luminal space (Figure 26B).

A second cell type, labelled Bb, contained granules which stained a very dark purple and reached a maximum size of 2 μm. These cells were generally found clustered together and sometimes extended to the center of the acini. On closer examination, some of these granules also exhibited a slight reddish tint at the cores.

In electron micrographs, the granules of the type Bb cells appeared as solid, electron dense bodies. Rough endoplasmic reticulum was also observed surrounding these granules, but the reticulum did not appear as organized as in the the type Ba cells (Figure 27A).

In additon to the granular cells, a few interstitial cells were found between granular cells and between granular cells and the basement membrane (Figure 27A,B). These cells were arranged in complex canaliculi which enclosed mitochondria and glycogen granules. There were also at least three cells with comparatively large nuclei located near the bases of the acini (Figure 25B). These cells appeared to surround the efferent duct and were notably free of granules.



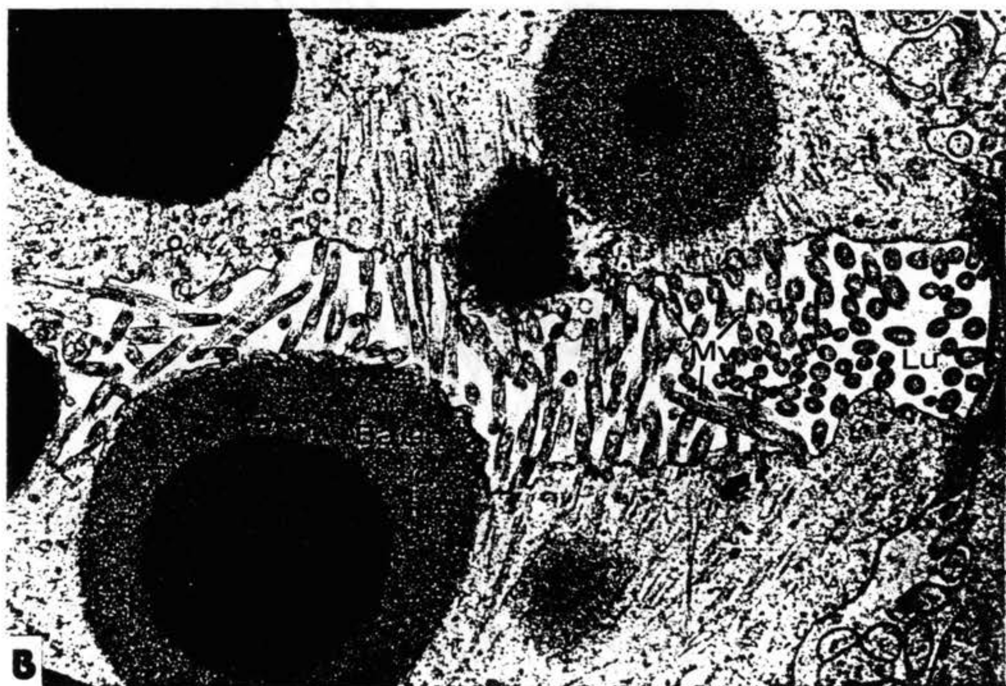
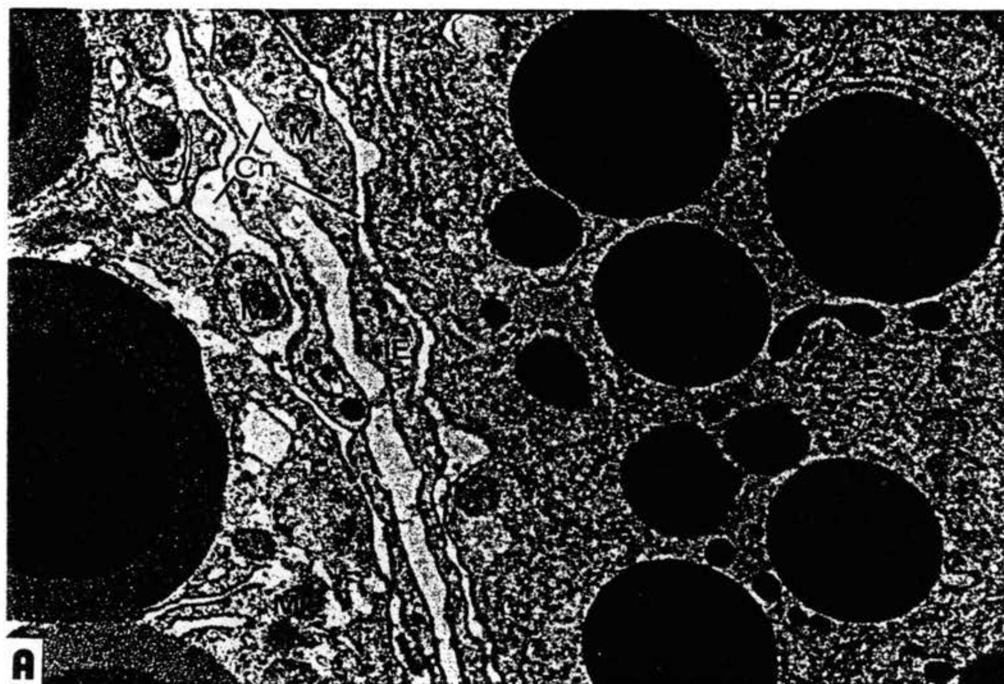


Figure 26. Ultrastructure of type Ba cells in type B acini of O. megnini nymph salivary gland.
A) Granule of type Ba cell (12,500X)
B) Lumen of type Ba cell with microvilli (15,500X)



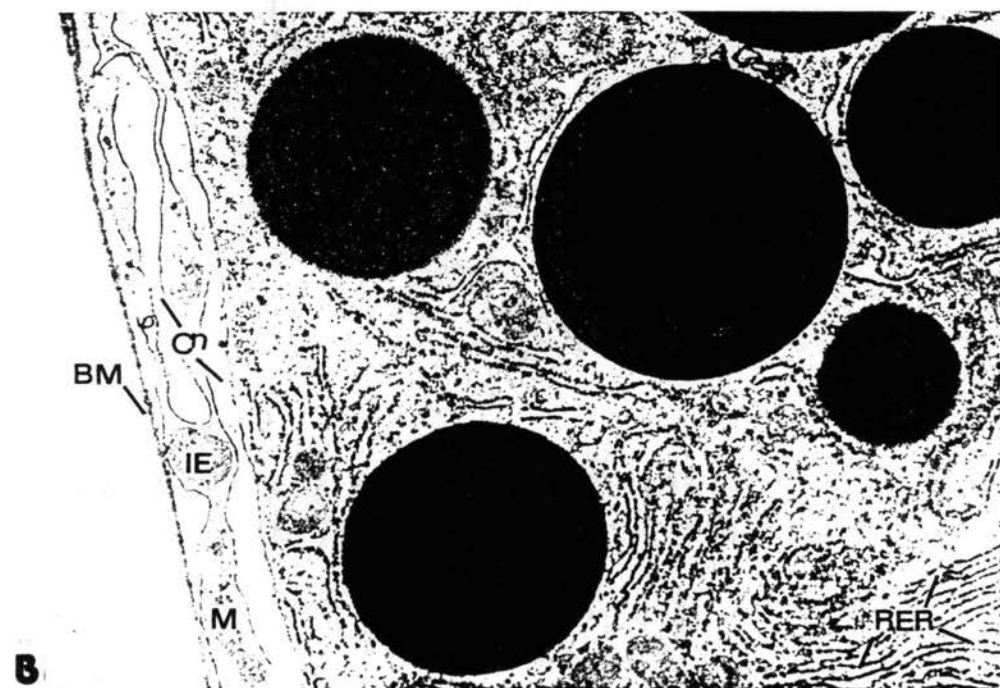


Figure 27. Ultrastructure of O. megnini nymph salivary gland. A) Type Bb cell granules and interstitial cell (15,500X) B) Type Ba cell and interstitial cell (12,500X)

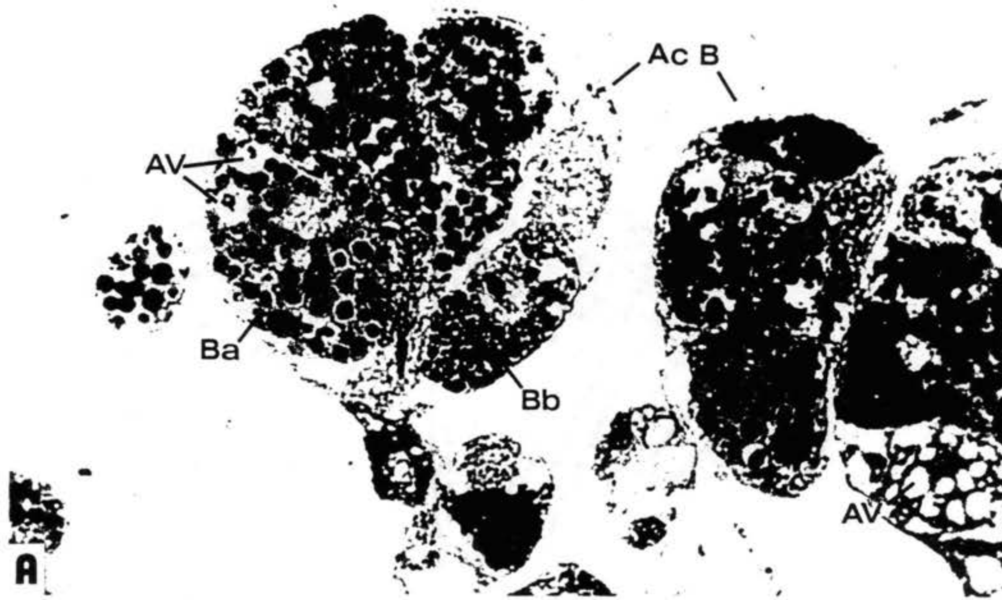
Salivary Gland Degeneration

At two days post-removal from the host, the individual cells within the type B acini of mature nymphs were still discernable, and the granular structure was still intact (Figure 28A). However, the cytoplasm of some cells contained large, clear areas when compared to light micrographs of the type B acini of actively feeding nymphs (Figure 25A,B). In some acini, the granules were visibly smaller, and the cell borders were ill-defined.

In electron micrographs, some of the type Ba cell granules could be observed disintegrating. The substance of some granules appeared to be less dense than usual, and the darker core could be seen dispersed around the granule as smaller, more electron dense granules. In addition, primary lysosomes and many autophagic vacuoles were observed containing electron dense inclusion bodies (Figure 28B). Intact rough endoplasmic reticulum was still visible in some cells.

The granules of the type Bb cells maintained their electron dense nature, but many were irregular in shape. Some of the granules appeared to be partially digested by nearby autophagic vacuoles (Figure 29A). The cytoplasm of the type Bb cells appeared to be full of cellular debris with little intact rough endoplasmic reticulum or mitochondria.

The interstitial epithelial cells of the type B acini also showed signs of degeneration. The canaliculi remained



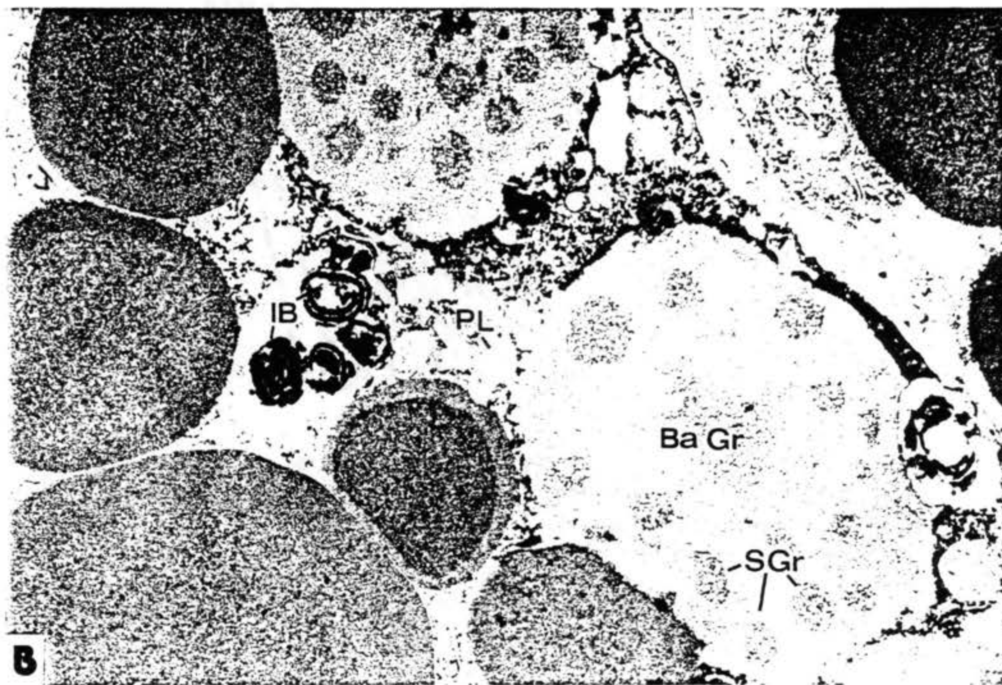
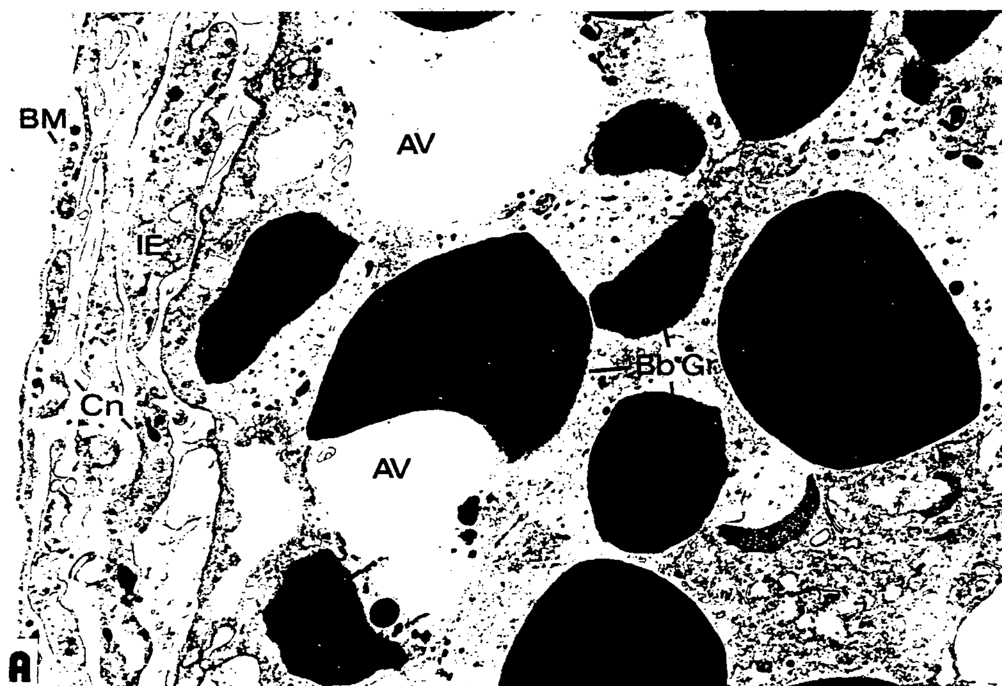


Figure 28. Ultrastructure of type B acini of O. megnini nymph salivary gland at 2 days post-removal. A) 400X B) Type Ba cell granules (12,500X)



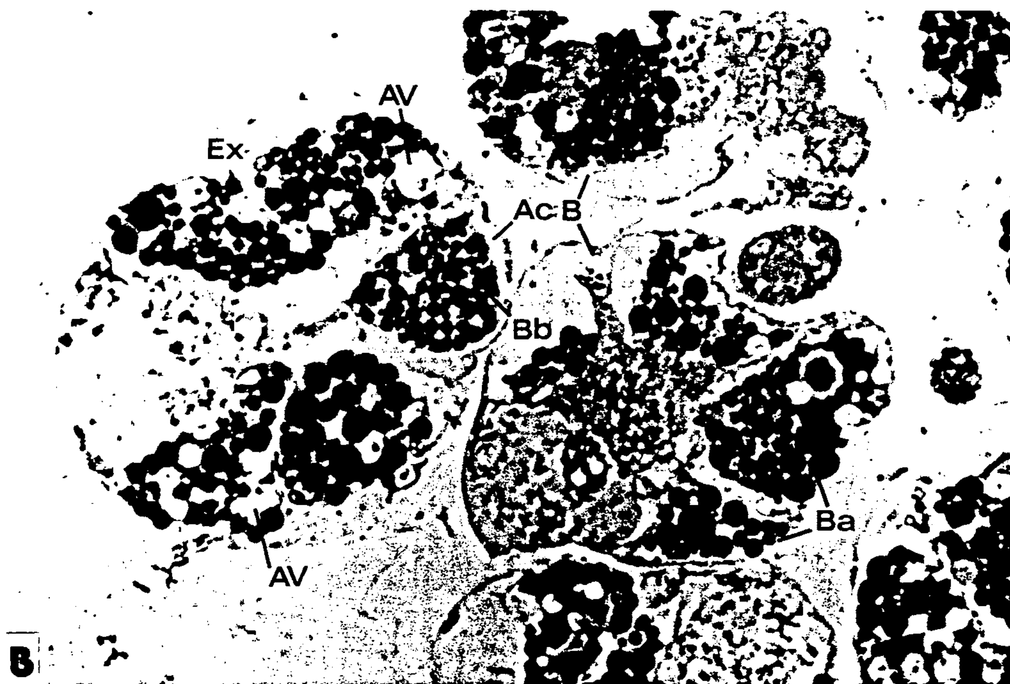


Figure 29. Degeneration of type B acini of <u>O</u>. <u>megnini</u> nymph salivary gland. A) Type Bb cell granules at 2 days post-removal (5,000X) B) Type B acini at 5 days post-removal (400X)

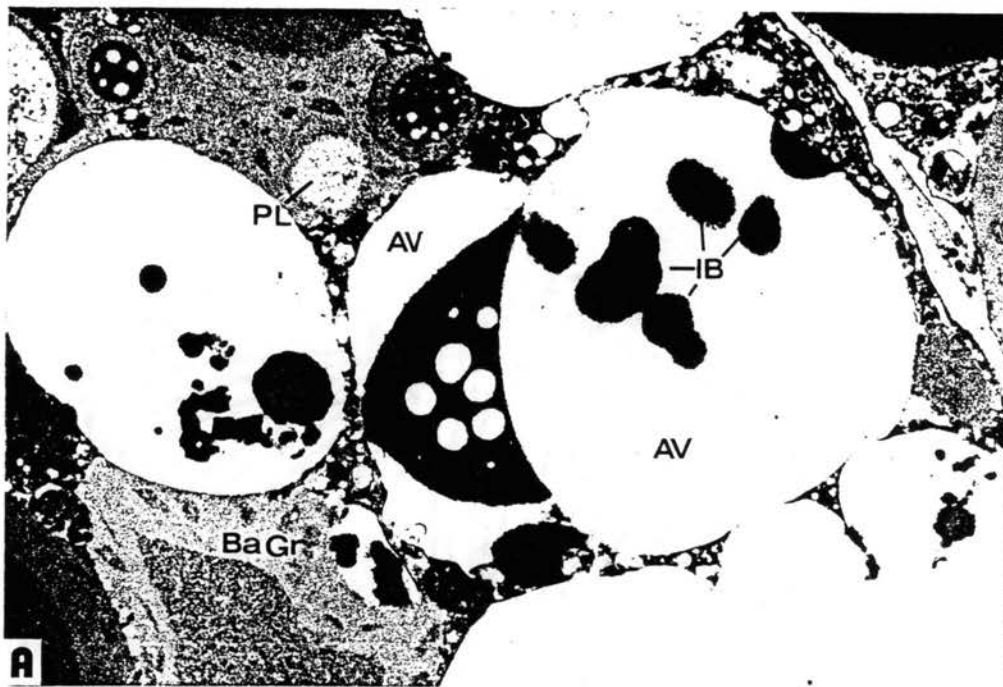
relatively intact, but the cytoplasm contained much cellular debris, large vacuoles, and few intact mitochondria.

At five days post-removal, the B acini of the nymphs superficially resembled that of the two day post-removal glands. The granular arrangement of the Ba and Bb cells was reasonably intact, but some shrinkage of the cells was evident as gaps between the cell and basement membrane of the acini (Figure 29B). As in the two day samples, degeneration in some B cell acini was more advanced than in others. In some acini, one or more of the cells were completely filled with merging vacuoles.

In electron micrographs, large, coalescing, autophagic vacuoles, often with inclusion bodies and granule remnants, were observed in the type Ba cells (Figure 30A). Also, unusual structures consisting of concentric rings of fine, whorled membrane were observed (Figure 30B). Type Bb cells were observed at five days post-removal.

Some of the interstitial epithelial cells of the B acini were almost completely degenerate. The canaliculi were virtually undetectable and the spaces where the epithelial cells had been were generally filled with residual bodies (Figure 31A).

At eight days post-removal, the type B acini of the nymphs exhibited extreme degeneration. The acini were visibly shrunken and they were almost devoid of intact cells. The few granular cells that remained were irregular in shape with ill-defined borders. Some intact cell



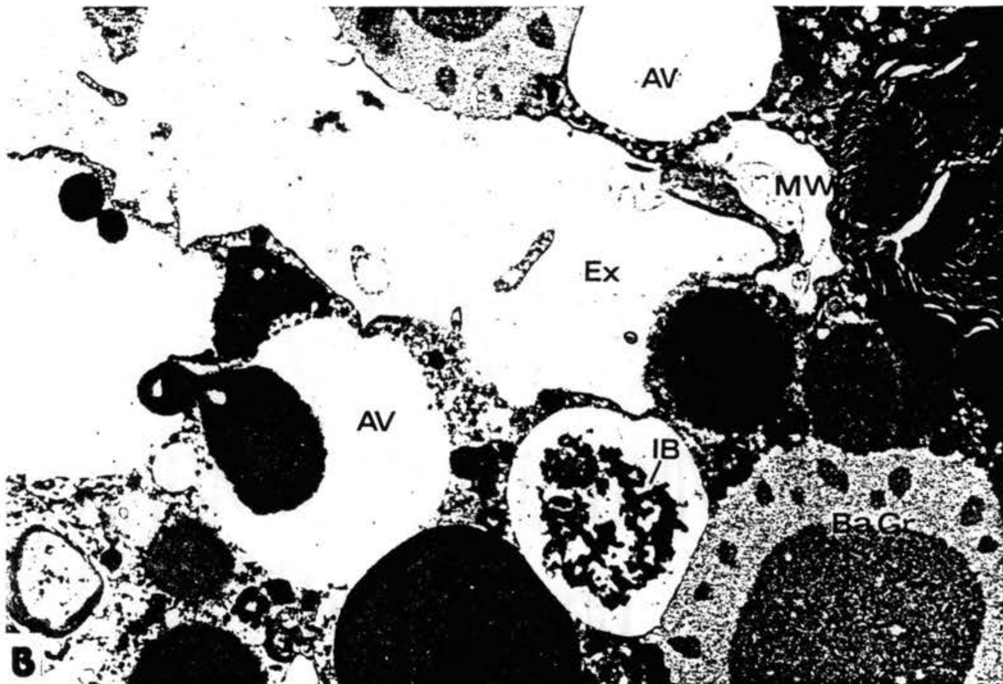
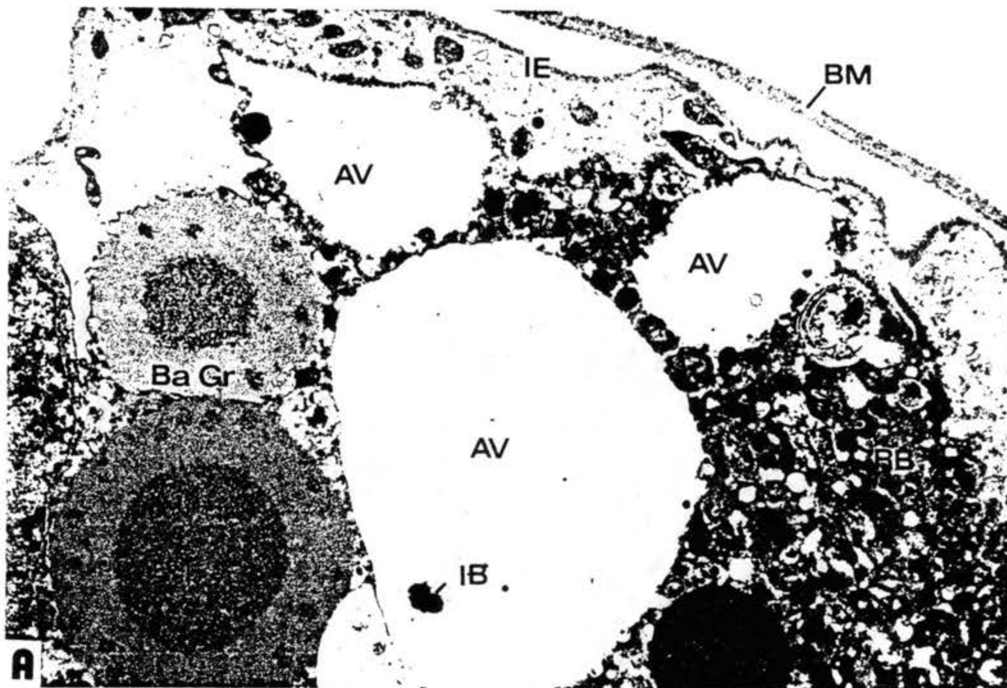


Figure 30. Ultrastructure of type B acini of O. megnini nymph salivary gland at 5 days post-removal. A) Large vacuoles in type Ba cells (10,000X) B) Vacuoles and membrane whorls (9,500X)



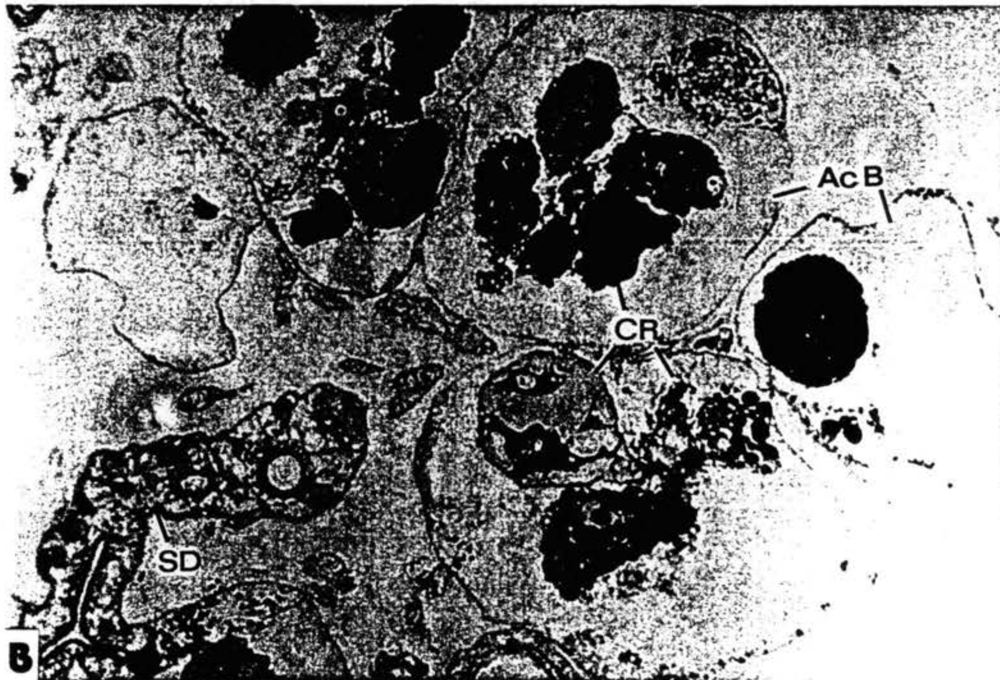
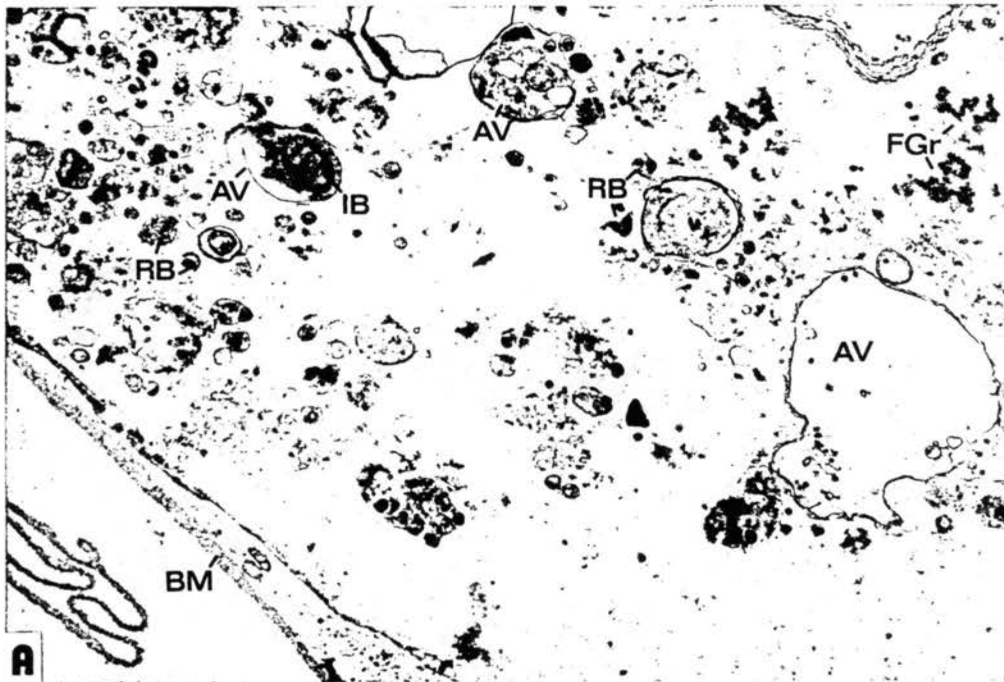


Figure 31. Degeneration of type B acini of O. megnini nymph salivary gland. A) Type Bb and interstitial epithelial cells at 5 days post-removal (9,500X) B) Type B acini at 8 days post-removal (400X)

granules were present, but most of the granular material had coalesced into large, amorphous masses that stained with uniformity (Figure 31B). As a result, the Ba and Bb cells could not be reliably identified as distinct cell types. At the light microscopic level, the interstitial epithelial cells appeared to be entirely absent. Much empty extracellular space was observed to exist between the cell remnants and the basement membranes of the acini. Also, at this time, the cytoplasm of the epithelial cells surrounding the secondary ducts contained vacuoles and appeared to be in the process of degeneration. However, the dark staining cuticular lining appeared to still be intact (Figure 31B).

Electron microscopy demonstrated that the empty areas of the acini observed by light microscopy consisted of abundant residual bodies, vacuoles surrounded by membrane whorls, and free granular material (Figure 32A). Granules were still present in some of the remnants, but many contained electroluscent autophagic vacuoles within the granules (Figure 32B). Within the cell remnants themselves, many membrane whorls and other unidentified debris (residual bodies) were observed (Figure 33). Only one Bb cell was found at eight days post-removal. It was severely distorted in shape and contained an equally distorted nucleus, but no granules were visible. The remains of a few interstitial epithelial cells were also found between some of the granular cell remnants (Figure 32B).



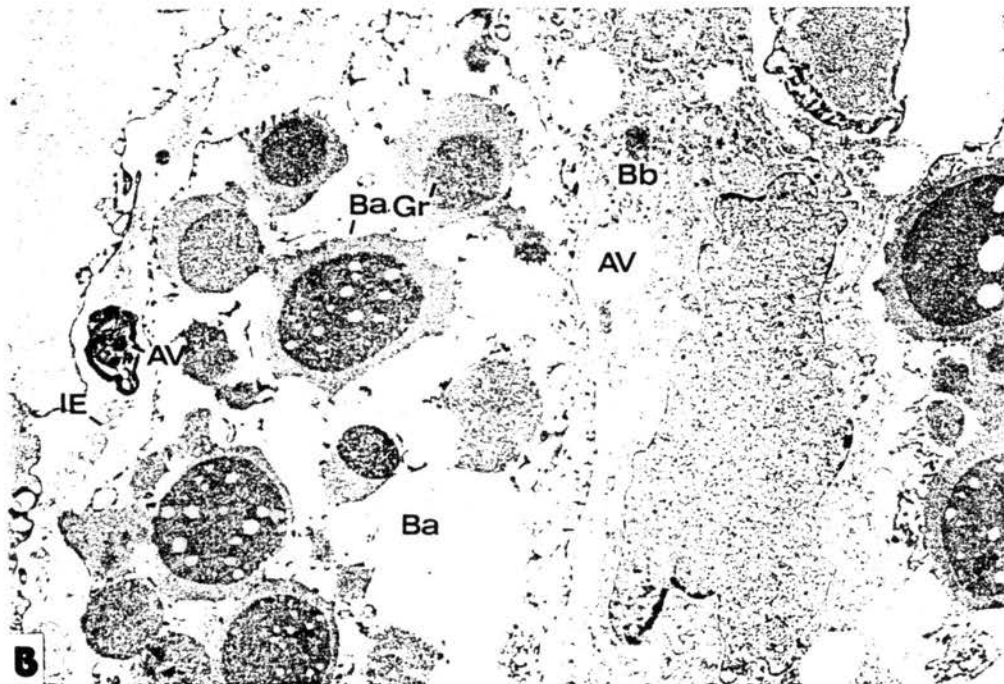


Figure 32. Ultrastructure of type B acini of O. megnini nymph salivary gland at 8 days post-removal. A) Extracellular space of degenerating B acinus (7,500X) B) shriveled type Ba and Bb cells (14,500X).

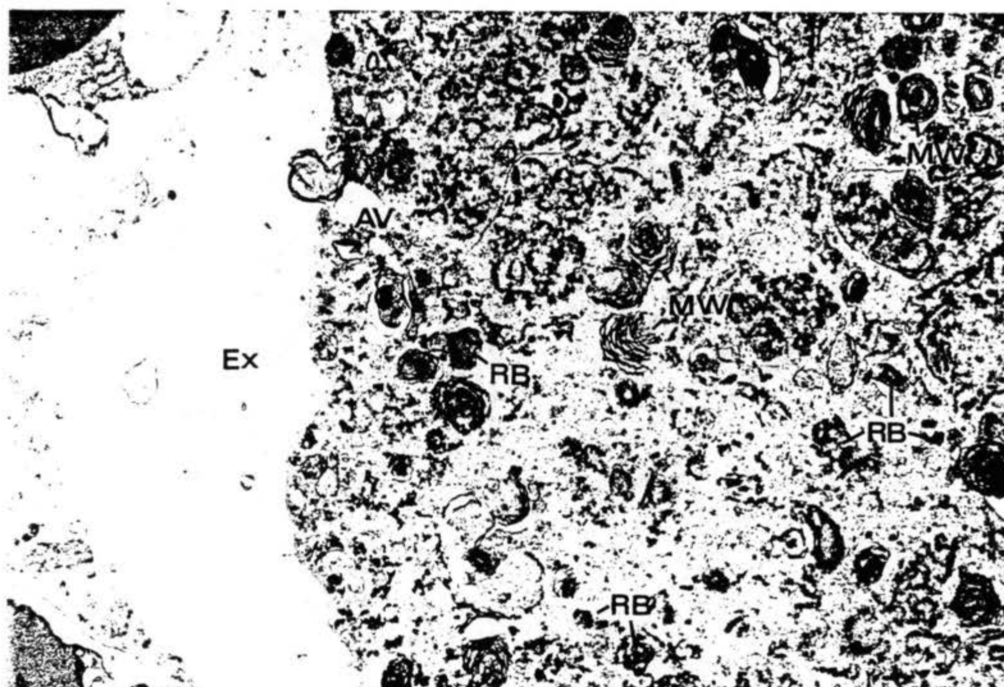


Figure 33. Cytoplasm of degenerating cell of <u>O. megnini</u> nymph salivary gland, type B acinus at 8 days post-removal showing membrane whorls and other cellular debris (14,500X)

CHAPTER V

DISCUSSION

Internal Anatomy

Digestive and Excretory Systems

Gastric System. The pattern of the diverticula in O. megnini closely resembles that of O. kelleyi (Sonenshine and Gregson 1970) and Ornithodoros alactogalis Isaakian and Ornithodoros coniceps canestrini (Balashov 1968). The diverticular branches are also uniformly tubular as in the above species. The branching pattern is also similar to O. kelleyi and O. coriaceus (True 1932) as well as O. alactogalis and O. coniceps. However, O. kelleyi and O. coniceps have only one branch on the postero-lateral diverticulum not two as in O. megnini and the other two Ornithodoros species. The branching patterns of species in the genus Argas are generally more complex with the branches often subdividing into many smaller, rounded lobes (Roshdy 1961, 1962, 1963, and 1966 and Robinson and Davidson 1913).

One feature of O. megnini not illustrated in any other Ornithodoros species is the single, double-branched antero-median diverticulum. Sonenshine and Gregson (1970) show a short, unnamed lobe at the antero-central position in

O. kelleyi. However, they labelled two unbranched lobes on either side of this central lobe as the antero-median divertucula. Two species of Argas, Argas vespertilionis Latreille and Argas boueti Roubaud and Colas-Belcour examined in Roshdy (1961) and Roshdy(1962), respectively, exhibit a similar anter-medially located diverticulum. In A. vespertilionis, this diverticulum is called the median dorsal lobe and is a single, unbranched, tubular diverticulum extending antero-dorsally. In A. boueti, this diverticulum has two branches, as in O. megnini, and was named the antero-median lobe. The branches of this diverticulum are similar to those of O. megnini in that the dorsal branch is shorter than the ventral branch.

Sonenshine and Gregson (1970) state that the arrangement of diverticula is most likely not an indicator of metameric relationships in ticks because of the great morphological variation in the diverticula from species to species and variation within species due to differences in the amount of material stored in the diverticula. Nothing discovered in O. megnini contradicted this conclusion. However, the general arrangement of the diverticula and their branches is more similar to the general diverticular arrangement of Ornithodoros species. This may indicate that O. megnini may be more closely related to the Ornithodoros genus than to Argas.

Foregut and Midgut and Hindgut. The foregut and midgut did not differ significantly from other argasid ticks that

have been described. In all species, the esophagus extends postero-laterally up through synganglion to join the antero-ventral region of the stomach. Likewise, the central stomach of *O. megnini* (minus the diverticula) did not differ in size or appearance from the other argasid species.

In all species, except *O. savignyi*, the rectal sac is a U-shaped, bilobed organ as in *O. megnini*. In *O. savignyi*, the rectal sac is a globular organ with no discernable lobes (Christophers 1906). True (1932) illustrated the rectal sac of *O. coriaceus* with an unusual third dorsal lobe. Balashov (1968) stated that when unfilled the rectal sac of ticks remain globular with poorly defined posterior lobes. This is clearly not the case in *O. megnini*. Even in the one nymph examined having an unfilled sac, the U-shape of the rectal sac was well preserved.

The attachment point of the Malpighian tubules in *O. megnini* did vary somewhat from that of other ticks in the Argasidae. Sonenshine and Gregson (1970) showed the Malpighian tubules of *O. kelleyi* emerging from the antero-lateral corners of the rectal sac. Balashov (1968) and True (1932) showed the tubules of *O. papillipes* and *O. coriaceus* emerging from the rectal sac on either side of the rectal tube. In *O. megnini*, the tubules emerge directly from the ventro-lateral sides of the rectal tube just anterior to its union with the rectal sac. The relative length and course the tubules follow through the body is comparable to other species. The tubules in *O. megnini* nymphs are larger in

diameter (by approximately 50%) than those of the adults because they contained more waste material. This was undoubtedly because the nymphs were actively feeding and metabolizing the bloodmeal. The adults do not feed, therefore their tubules were filled only with what little waste the tick generated from its daily metabolism.

Salivary Glands. The salivary glands of *O. megnini* are somewhat unique. Neither the nymphal or adult glands are similar to those of other argasid species. The nymph salivary gland exhibits two types of acini externally. Two acinar types (one larger and granular and one smaller, agranular type) have been reported for all species of the Argasidae studied except *O. savignyi*. Christophers (1906) described only one type of acinus (granular in nature) in species. The arrangement of the acini in *O. megnini* nymphs is identical to that of the other adult argasidae studied. The larger type B acini are arrayed along the entire length of the gland while the smaller type A acini are restricted to the mesial side of the anterior half of the gland. Of course, the nature of these acini could not be determined from the external anatomy alone. This was the same arrangement illustrated for all the species studied. The larger type B acini in *O. megnini* nymphs are more tightly clustered than has been shown for the large acini of other Argasidae. For all *Ornithodoros* and most *Argas* species, these acini are arranged in looser grape-like clusters. The smaller type A acini are present in the nymphs, but are much

smaller than the corresponding acini in other adult ticks including adult O. megnini. Functionally, these acini may not be as crucial in the nymphs.

The large type B acini in adult O. megnini are visibly shrunken when compared to those in the nymphs. The shrinkage also progresses as the adults age. In young adults (less than 1 month old), the type B acini are discernable as intact acini. Also, when live dissections had Mallory's Azure II Methylene Blue applied, a few cells in each acinus took up the stain and appeared a bright blue. This indicates that at least a few of the cells are still active in these acini. In older adults (9 months or older), the type B acini appeared to be completely degenerate. The outlines of very few acini could be detected. Also, the tissue did not react to stain as in the nymphs. These observations would seem to indicate that these acini play a role in feeding. The adults do not feed and would therefore have no use for the these acini. All of the other Argasidae feed as adults and all have the large acini as adults. In contrast, the small type A acini undergo an increase in size in the transition from nymph to adult. The size and number of these acini are comparable to that for the other Argasidae studied. Obviously, these acini increase in size because of increased demand for whatever product or function they produce or perform in the adults.

Coxal Glands. The coxal glands of species in the Argasidae have not been intensively studied. Lees (1946) described the coxal glands of *O. moubata* as consisting of an accessory organ and the coxal gland itself. Each gland was found to consist of an outer filtration chamber and an inner tubule system leading to the coxal pore. Sonenshine (1970) and Christophers (1906) described the coxal glands of *O. kelleyi* and *O. savignyi* as saccular or flask-shaped organs with no visible accessory glands.

The coxal glands of nymphal *O. megnini* resemble those of *O. moubata*. The gland itself is a simple tubule which is bent into a "U" shape. No obvious filtration membrane as present in *O. moubata* was observed. The small globules seen in the tubules of *O. megnini* nymphs are not reported in *O. moubata*. The nature and function of these globules is unknown, but they may play a role in filtration or fluid movement across the tubule wall. A large accessory gland similar in appearance to that of *O. moubata*, but comparatively much larger, was also observed. Robinson and Davidson (1914) reported a similar gland with large acini in *A. persicus*. The histology of the acini resembled that of the non-granular acini in the salivary glands. A small diverticulum was also found associated with the gland.

The muscles attaching to the walls of the tubule of *O. megnini* are as described for other argasid coxal glands. Lees (1946) proposed that these muscles were present to dilate the gland and draw in fluid. The positioning of the

muscles around the tubule in _O_. _megnini_ seems to suggest this purpose as well.

The coxal tubules of adult _O_. _megnini_ are much different in appearance from that of the nymphs. They are flaccid and resemble more the flattened sacs described as the coxal glands of the other argasidae discussed above. In addition, the globules are much reduced and virtually invisible. Furthermore, the accessory glands are not present in the adults. However, the muscles attached to the coxal tubule were present in the adults. The partial degeneration of the coxal gland from nymph to adult could be explained by its accepted function. The coxal gland is believed to be a organ of osmoregulation that helps the tick expel unwanted water and ions especially after feeding (Kaufman and Sauer 1982). Although adult _O_. _megnini_ do not feed and therefore do not need to rid themselves of excess water or ions taken in with the bloodmeal, their coxal glands may not be completely non-functional. Severely heat stressed adults have been observed to exude a small amount of fluid from the coxal pores. The prescence of the coxal muscles also suggests that the coxal tubule, although partially degenerate, can function minimally in adults.

The function of the accessory gland has not been elucidated for any argasid tick. Interestingly, Obenchain and Oliver (1974) and Binnington (1975) discovered a similar gland in the teneral and pharate individuals of several species of Ixodidae, although no species of the Ixodidae is

known to possess a coxal gland. Furthermore, Binnington (1975) described the degeneration of this gland in nymphal and adult Ixodidae after ecdysis. Since the accessory gland appeared to be most active during apolysis, Binnington (1975) suggested that this gland functions in the hardening of the newly formed cuticle. The presence of this gland in mature nymphs of _O. megnini_ and its absence in newly molted adults suggests that the accessory gland in this species may also function in the formation of the adult cuticle or other tissues. The temporary existence of this gland may also explain why it has not been identified in other adult ticks of the family, Argasidae.

Reproductive System

Male Reproductive Organs. The testis and vasa deferentia did not differ significantly from that of other argasid species. In all species examined, the testis was comprised of a narrow central portion which merged into swollen sections, laterally. In _A. vespertilionis_ (Roshdy 1961) the enlarged portions of the testis are convoluted to the point of appearing like a string of large beads. The enlarged testis of _O. megnini_ and _Argas_ species, other than _A. vespertilionis_ are tubular. Sonenshine (1970) stated that packets of spermatids were found only in the expanded lateral portions of the testis in _O. kelleyi_. Spermatozoa were found primarily in the middle regions of the expanded testis not in the seminal vesicle or vasa deferentia. The

positions of the spermatids and spermatozoa in the testis of O. megnini were not examined.

As in other argasidae, including O. megnini, the narrow vasa deferentia expands to some degree near the union with the accessory gland. In O. kelleyi this expansion is abrupt, but in O. megnini and other species, the expansion is gradual. There appears to be some disagreement regarding the function of the expanded portions of the vasa deferentia. Sonenshine (1970) referred to these regions as the postero-lateral lobes of the seminal vesicle. In the five species of Argas studied by Roshdy (1961, 1962, 1963, 1966 and Robinson and Davidson 1914) and O. savignyi (Christophers 1906) these expanded portions were deemed to be part of the vasa deferentia. In O. megnini, these regions appear to be continuous with the remainder of the vasa deferentia. The greatly enlarged terminal coils of the vasa deferentia in O. megnini are unusual, but not unique. Similar structures were identified in O. savignyi. In O. megnini at least, these terminal coils obviously serve as storage sites for spermatozoa. When these structures were punctured under saline, copious amounts of spermatozoa were released. When the spermatophores are formed in O. megnini, the spermatozoa packed into them probably originate from the terminal coils. The terminal coils are replenished with spermatozoa from the expanded regions of the vasa deferentia and testis. In other ticks, such as O. kelleyi and Argas sp., the storage function is apparently served by the

expanded regions of the vasa deferentia and the median seminal vesicle. Consequently, the seminal vesicle in O. megnini probably serves merely as a chamber where the spermatozoa and accessory gland components are mixed.

The accessory gland of O. megnini is typical for Argasidae. The tubular lobes of the gland are common to all species of Argasidae examined. The number and positioning of the lobes in O. megnini are also identical to that found in A. persicus and the other Argas species studied. As in O. megnini, the second lateral granular lobe is substantially smaller than other lobes in the Argas species and nearly unseen from a dorsal view. The second lateral granular lobe of O. kelleyi is also the smallest, but in that species, the third lateral granular lobe appears to be an outgrowth of the second lateral granular lobe. In O. megnini and the Argas species, the two lobes are distinctly separate. The antero-dorsal granular lobe appears to be the most variable among all the species studied. This lobe was quite complex in shape in O. kelleyi and A. vespertilionis (Roshdy 1961). In O. megnini and other argasid ticks, the antero-dorsal granular lobe appears to be the result of the fusion of two original lobes. In O. megnini, the indentation along the midline of the antero-dorsal lobe varies in appearance among individuals. In some individuals, the indentations clearly indicate the multilobular origin of this large dorsal lobe. This

indentation was present to some degree in the illustrations of all the argasids studied.

The anterior and posterior spongy lobes of _O_. _megnini_ are similar in shape and position to those of the four _Argas_ species studied in Roshdy 1961, 1962, 1963, and 1966. In all cases, both lobes are small with the anterior spongy lobes being the smaller of the two. Robinson and Davidson (1914) show these lobes in _A_. _persicus_ to be several times larger than the spongy lobes in other species, with the posterior spongy lobe looking suspiciously similar to the postero-dorsal granular lobe of _O_. _megnini_ and other _Argas_ ticks. The same authors refer to two small projections of the antero-dorsal lobe as the postero-dorsal granular lobes. All of the others authors consider these to be part of the antero-dorsal lobe. In _O_. _kelleyi_, only one spongy lobe was illustrated on the ventral side of the accessory gland. A granular lobe located ventrally was also found in _O_. _megnini_ and all the other species examined. This lobe was the smallest granular lobe in _O_. _megnini_ and the _Argas_ species, but was the largest granular lobe in _O_. _Kelleyi._

The various lobes of the accessory gland are known to produce components of the spermatophore. Tatchell (1962) determined which lobes produced the individual components of the spermatophore in _A_. _persicus_. What components the lobes produce in _O_. _megnini_ is unknown.

In all of the species examined, a pair of genital muscles inserted in the walls of the ejaculatory duct. The

inferior genital muscles attached to the ventro-lateral wall near the genital aperature, and the superior genital muscles attached to the lateral walls posterior to the genital aperture.

Female Reproductive Organs. The female reproductive organs were also similar to those in other argasidae. In all cases, the uterus was a large bilobed organ. The lobes were depicted as being wide as in O. megnini and A. boueti (Roshdy 1962) or long and narrow as in O. kelleyi and Argas transgariepinus White (Roshdy 1963). In Argas brumpti Neumann (Roshdy 1966) and O. savignyi, the lobes were virtually undetectable and the uterus appeared as a triangular shaped sac. The uterus serves as a seminal receptacle in argasidae. Robinson and Davidson (1914) and Sonenshine (1970) found as many as five to eight spermatophores in the posterior region of the uterus in A. persicus and O. kelleyi.

The ovaries in most of the ticks, including O. megnini, were continuous tubes of tissue covered with ova of varying size. The ovary of A. vespertilionis (Roshdy 1961) appeared as two lateral clumps of oocytes connected by a thin strand of tissue devoid of ova. Also, an area of ovarian tissue devoid of visible ova as in O. megnini was noted for most species. This area was usually located anteriorly or dorsally. In both O. megnini and O. savignyi, the ova were depicted as getting smaller near the ova free region of the ovary. The purpose of the ova free area is unknown. It may

be possible that the ova are present but too small to see with a stereomicroscope. Robinson and Davidson (1914) published a cross section of an A. persicus ovary that showed an area without oocytes. They concluded that the cells in this area were glandular and labelled the region the ovarian gland. It may be possible that the ova free regions of the ovaries in O. megnini and the other ticks correspond to the ovarian gland in A. persicus.

The oviducts of all species are long, coiled tubes similar to those of O. megnini. They expanded abruptly near the uterus in all species. Robinson and Davidson (1914) noted that the expanded oviducts in A. persicus were frequently filled with spermatozoa. It is probable that the expanded areas of the oviducts in argasid ticks serve as temporary holding areas for oocytes undergoing fertilization. The merger point of the oviducts with the uterus varied among species. The point of union in O. megnini and A. vespertilionis is approximately midway along the organ. In most of the other species, this point is near the distal ends of the uterine lobes.

The vagina in O. megnini and the other species examined consists of distinct vestibular and cervical regions. The distinction between the two regions is not as evident in O. megnini as in Argas species and O. kelleyi. Sonenshine (1970) considered the vestibular region in O. kelleyi to be histologically distinct from the vagina. Other authors considered this region to be part of the vagina.

Interestingly, the small tubular accessory glands in other argasidae are illustrated as emerging from the vestibular vagina near the union with the cervical portion. In *O. megnini*, the glands clearly appeared to originate from the cervical region, perhaps indicating that the two regions are not as distinct morphologically as in other ticks.

Gene's organ of *O. megnini* like that of other ticks consists of glandular and cuticular regions. The glandular region is sac-like not digitate as in *A. persicus*. In all of the other species, the glandular part is depicted as being sac-like. The bilobed cuticular sac in *O. megnini* is similar in design to that found in *A. persicus* but was not folded. The bifurcate lobes, however, are unique to *O. megnini*. In *O. megnini*, the retractor muscles are attached only to the inner bifurcations. In *A. persicus* and the other *Argas* species, the retractor muscles appears to attach to the apices of the lobes. In *O. kelleyi*, the retractors attach to the bases of the lobes laterally.

The glandular tissue of Gene's organ attaches to the lateral portions, including the lateral bifurcations, of the cuticular lobes in *O. megnini*. In the other species, the glandular tissue attaches to the lateral bases of the lobes. In the Gene's organs of *A. persicus* and *O. kelleyi*, hypodermal sacs were identified (Robinson and Davidson 1914 and Sonenshine 1970). These were not identified in *O. megnini*. Roshdy (1961, 1962, 1963, and 1966) did not mention whether or not the hypodermal sacs were present in

any of the *Argas* ticks studied. The cuticular sac found in *O. megnini* and *A. persicus* was not found by Sonenshine (1970) in *O. kelleyi*. However, the structure identified as the hypodermal sac of *O. kelleyi* is very similar in shape to the cuticular sac of *O. megnini*.

Lees and Beamont (1948) believed that Gene's organ in *O. moubata* was an outgrowth of the epidermis which was detached from the surrounding cuticle. It was also believed that the hypodermal sacs did not exist. In *O. megnini*, the cuticular sac also appears to be continuous with the cuticle aroung the slit-like opening through which Gene's organ protrudes when in use. Therefore, the cuticular sac could be considered to be an invagination of the anterior cuticle. The glandular tissue could then easily be visualized as a proliferation of the epidermal lining of the invaginated cuticle as hypothesized by Lees and Beamont (1948) for *O. moubata*.

Respiratory System

The tracheal system of most Argasidae is comprised of a few large trachae called trunks which arise from the atria. Smaller trachae then branched from the trunks. Sonenshine (1970) found eight tracheal trunks (five ventral and three dorsal) in *O. kelleyi*. Roshdy (1961,1962,1963, and 1966) found five trunks in *A. brumpti* and *A. transgariepinus* and three trunks in *A. vespertilionis*. Christophers (1906) found five major trunks in *O. savignyi*. *Otobius megnini*

does not exhibit a trunk arrangement. Instead numerous smaller trachae arranged in groups extend to all regions of the body directly from the atria. Argas boueti (Roshdy 1962) also exhibits grouped trachae, however only three groups are defined (anterior, dorsal, and posterior). In O. megnini, six groups have been defined (antero-lateral, antero-medial, medial, postero-medial, postero-lateral, and ventral). With the exception of the ventral group, the groups corresponded to the trunks of A. persicus.

A prominent feature of the tracheal system of O. megnini is the dorsal anastomosis, located directly above the stomach. Roshdy (1962) described a similar dorsal anastomosis to that of O. megnini in A. brumpti. Otobius megnini had an additional, less extensive ventral anastomosis located directly beneath the brain. A similar feature which Christophers (1906) labelled the tracheal ganglion was described for O. savignyi. Sonenshine (1970) shows only anterior and posterior cerebral anastomoses associated with the brain in O. kelleyi. No such structure was identified in O. megnini, and the brain was supplied by several small trachae that ramify into a network of fine tracheoles.

In general, it is difficult to trace trachae from the organs back to their origin at the atrium because trachae from different branches often supply the same organs. In those species with a trunk system, the trachae supplying an organ could be more easily traced back to its trunk. For

instance, the trachae supplying the legs in O. kelleyi all originate from a single trunk, but the corresponding trachae in O. megnini originate individually from two groups as well as from the ventral anastomosis.

The internal structure of the larger trachae of O. megnini resembles that of other Argasidae. The trachae are sheathed externally in an epithelium, which is supported by cuticle arranged spirally in taenidia. When these large trachae were cut, the taenidia could sometimes be pulled free of the epithelium. In this state, the taenidia resemble a tightly coiled spring.

In O. megnini, a spiracular muscle inserts into each atrium. A similar muscle is found in A. persicus. No such muscle was described for other argasid ticks. Balashov (1968) states that the purpose of these muscles is to dilate the atrium and allow more air into the tracheal system. This is may be the function of these muscles in O. megnini as well.

Muscular System

In general, the muscular system of O. megnini resembles that of O. kelleyi and A. persicus, but exhibits several unique features. Sonenshine (1970) states that the muscular system of O. kelleyi is similar to what Robinson and Davidson (1914) describe for A. persicus, but lacks the spiracular and superior sub-coxal muscles found in the latter species. Both of these muscle groups are present in

O. megnini. Ornithodoros kelleyi also has a pair of anterior dorso-ventral muscles, a group of preanal muscles, and a pair of pre-transversal and post transversal muscles which were all lacking in A. persicus. These muscles are present in O. megnini, with the exception of the preanal muscles. An additional group of posterior dorso-ventral muscles is also found in O. megnini.

The dorso-ventral genital muscles are present in all three species, but this muscle is two tiered only in O. megnini. The purpose of this arrangement is unclear. It seems that muscles extending from the dorsal cuticle to the ventral in one continuous group would be more efficient. Also, the attachment of the superior genital muscles to the sliver of cuticle uniting the dorso-ventral genital tiers is also unusual. It is possible that the dorso-ventral genital muscles are two-tiered to provide a attachment point for the superior genital muscles. In A. persicus and O. kelleyi, the superior genital muscles are attached to the endosternum. The lack of a pair of inferior genital muscles in the female is difficult to explain. The females of both of the other two species have these muscles. Most likely, these muscles in O. megnini females are more closely tied to the vagina and were thus simply overlooked.

The endosternum of O. megnini appears to play a more prominent role in the muscular system than in the other species examined. Five pairs of mesial intercoxal muscles, which extend from the endosternum to the coxal projections,

are present in _O_. _megnini_ and _A_. _persicus_. _Otobius megnini_ also has three pairs of lateral intercoxal muscles, but _A_. _persicus_ is shown with only two. _Ornithodoros kelleyi_ possesses four pairs of intercoxal muscles that extend from the endosternum to the lateral wall, but are not divided into lateral and mesial groups as in _O_. _megnini_ and _A_. _persicus_. These large, powerful intercoxal muscles are used to compress the tick laterally. Normally, an _O_. _megnini_ individual is somewhat flattened dorso-ventrally, but when one is placed on its back, it uses the intercoxal muscles to make its body more round. It can then rock back and forth in an effort to gain enough footing to right itself.

Another substantial difference is the absence of coxal adductor muscles in _O_. _megnini_. These muscles were found previously in both _A_. _persicus_ and _O_. _kelleyi_. _Otobius megnini_ has the four pairs of coxal abductors as in the other ticks. Although, _O_. _megnini_ does not have conventional coxal adductors, it possesses muscles that extend from the bases of the coxal projections to the endosternum. These muscles may perform similarly to the conventional adductors in the other species. The depressor muscles of the capitulum also differ in _O_. _megnini_. In the other two species, the muscles originate on the dorsal cuticle. However, the two groups of levators of the capitulum originate on the dorsal cuticle as in the other two species. The illustration of the musculature of

A. persicus presented by Robinson and Davidson (1914) is somewhat confusing. There appears to be two pairs of capitular depressor muscles, with one pair located slightly above the other. The upper pair of capitular depressors, located directly below the levators, could be analogous to one of the pair of levators in O. megnini and the lower depressors in A. persicus could be similar to the depressors in O. megnini.

The manner in which the extensor muscles of the trochanter are arranged was also unique in O. megnini. The extensors are attached both to the endosternum and coxal projections. The flexors of the trochantor, which attach only to the coxal projections, are conventionally arranged. The purpose of this split muscle is unclear. The long fibers to the endosternum may allow a more powerful foreward leg stroke.

One feature notably lacking in O. megnini are the marginal muscles. Argas persicus is illustrated with many marginal muscles around the entire periphery of the tick. Ornithodoros kelleyi is depicted with a line of marginal muscles above the supracoxal fold along the lateral body margins. Otobius megnini has only three pairs of marginal muscles. The pair found at the postero-lateral corners of the body are the most similar in arrangement to the marginal muscles of the other ticks. The other two pairs located at midbody and antero-laterally are somewhat unusual in their

positioning. The remainder of the dorso-ventral muscles are all conventional in position and design.

The endosternum itself is an interesting feature. When all the muscles are removed, the endosternum most nearly resembles a bird wishbone. Its wide base provides ample space for the mesial intercoxal muscles and posterior dorso-ventral to attach, and the endosternal arms provide attachment points for the anterior dorso-ventral muscles. The endosterna of _Argas_ _radiatus_ Railliet and _Ornithodoros_ _turicata_ Duges are in two halves, with the halves connected by muscles medially (Obenchain and Oliver 1976a). In _O_. _megnini_ and _Antricola_ _mexicanus_ Hoffman, the endosterna are fused medially. It is unknown how the endosternum of _O_. _megnini_ compares to the endosterna of other argasids. The endosternum, especially the cuticular arms, varies in size from individual to individual, but the basic form is retained. How the tick forms and enlarges this structure at each molt would be interesting to discover.

Although the mature nymphs possess all the muscle groups of the adults, some slight differences were noticed. The endosternum is not nearly as defined and the associated muscles did not seem as well organized. This is reasonable because the nymphs remain in the ears of the host throughout their development and thus do need to be as mobile. Therefore, the muscles associated with movement need not be as powerful. Conversely, those muscles needed for feeding,

such as the cheliceral retractors, are larger and probably more powerful in the nymphs.

Nervous System

The arrangement of nerves from the synganglion of O. megnini is more complex than what has been illustrated for some Argasidae. Sonenshine (1970) shows only nine pairs of nerves in O. kelleyi and Robinson and Davidson (1914) identify only seven pairs in A. persicus, but it was stated that there were several smaller nerves which were not named in A. persicus. Christophers (1906) did not state how many nerve pairs were present in O. savignyi, only that nerves extended to the legs and viscera along the esophagus. Obenchain and Oliver (1976a) found seven pairs of nerves, not including secondary branches, and one unpaired nerve in A. radiatus. Discounting secondary branches, there are 13 pairs of nerves visible in O. megnini and one unpaired nerve.

The positioning of the large pedal nerves is consistant for all species studied. However, in both A. radiatus and A. persicus, the pedal nerves subdivide as they progress towards the coxa. In O. megnini, the pedal nerves are not subdivided, but in some individuals, the hemal nerves are so close to the pedal nerves that they have the appearance of being a subdivided nerve. The pedal nerves of O. kelleyi likewise do not subdivide. The large postero-lateral trunk in O. megnini which gives rise to the genital nerve,

paraspiracular nerve, and others is identical in arrangement to that for A. radiatus. This trunk is also equivalent to the splanchnic nerve identified by Robinson and Davidson (1914) in A. persicus. In the corresponding position in O. kelleyi, Sonenshine (1970) shows only two pair of small nerves labelled visceral nerves. These visceral nerves were assumed to ennervate the mid-gut and other viscera, but in O. megnini and A. radiatus, these nerves innervate posterior body muscles and the reproductive organs, not the mid-gut.

The three species illustrated differ substantially in the anterior region of the synganglion. Sonenshine (1970) shows only a palpal nerve pair, cheliceral nerve pair, and pharyngeal nerve pair emerging directly from the anterior margin of the synganglion in O. kelleyi. For A. radiatus, Obenchain and Oliver (1976a) show only two pairs of nerves from the anterior region, a pedipalpal (palpal) nerve and a second nerve trunk which gives rise to the cheliceral and optic nerves. An additional unpaired stomodeal nerve arises dorso-medially. In O. megnini, there are three pairs of trunks which give rise to the palpal, salivary, and cheliceral nerves as well as others. There is also a pharyngeal nerve pair and a unpaired stomodeal nerve as in A. radiatus. These nerves were located within the tissue surrounding the esophagus and are very difficult to visualize clearly. For A. persicus, Robinson and Davidson (1914) show only a palpal and cheliceral nerve pair along with several smaller unidentified nerves are shown. The

cheliceral nerve in A. persicus emerges dorsally from the antero-lateral corners of the synganglion as did the gastric nerve trunk in O. megnini.

For A. radiatus, an extensive sympathetic plexus of many small nerves produced four nerve pairs that innervated the salivary gland. A similar plexus is present in O. megnini but is almost impossible to view in its entirety because it passes among the intercoxal muscles around the endosternum. However, what little of it that could be viewed is superficially similar to that of A. radiatus. Several small nerves could be seen connecting to the hemal nerves and to the coxal gland. There also appears to be at least two pairs of extremely fine nerves extending to the posterior regions of the salivary glands as in A. radiatus. No such plexus was described for O. kelleyi or A. persicus, and no nerve analogous to the optic nerve in A. radiatus was found in O megnini.

Circulatory System

The organs of the circulatory system in O. megnini are very difficult to observe because of their delicate nature. Sonenshine (1970) noted no circulatory system in O. kelleyi, but it was also acknowledged that these structures could have been inadvertently removed during dissection. In the few individuals examined with the circulatory system intact, the heart was found to be similar to that described for other argasidae. The triangular shape of the heart was

observed in A. persicus as well as O. turicata and A. radiatus (Obenchain and Oliver 1976b). The hearts of O. turicata and A. radiatus are suspended by dorso-lateral and ventro-lateral suspensory muscles within pericardial sinus (Obenchain and Oliver 1976b). These structures were not directly observed in O. megnini, but may have been part of the amorphous tissue that was observed surrounding the heart. The heart ostia found in other ticks were also not observed probably because of the surrounding tissue.

In all of the ticks examined, a dorsal aorta extended from the heart to the neurolemma of the brain. Robinson and Davidson (1913a) show the neurolemma of A. persicus forming a periganglionic sinus around the brain which extended along the larger nerves to form arteries. The periganglionic sinus continued anteriorly along the esophagus to form the paraesophageal sinus. This appeared to be the arrangement in O. megnini as well. In O. megnini, the pharyngeal and stomodeal nerves were within the paraesophageal sinus, and in some individuals, the aorta continued anteriorly to ensheath the retractor muscles of the chelicera. In A. radiatus and O. turicata, the tissue of the periganglionic sinus (neurolemma) was continuous with the tissue covering of parts of the endosterna. This seems to be the case in O. megnini as well. The brain was suspended directly beneath the endosternum by the neurolemma covering the postero-dorsal median region of the the brain.

The primary purpose of the circulatory system in _O_. _megnini_ and other ticks appears to be to supply the synganglion with a continual supply of fresh hemolymph. This is evidenced by the fact that the synganglion is the only organ directly connected to the heart via the aorta. In _O_. _megnini_ as well as _O_. _turicata_ and _A_. _radiatus_, the heart and surrounding tissue were oxygenated by an extensive tracheal network from the dorsal anastomosis, which indicates that these tissues are very active metabolically. Hemolymph is pumped down to the synganglion via the aorta and then drains into the arteries around the nerves and along the esophagus within the paraesophageal sinus to the mouthparts.

Water Vapor Uptake

The results of these experiments show that the mouthparts are the site of water vapor uptake in _O_. _megnini_. The ticks of both age groups with their mouthparts covered with wax failed to imbibe water vapor from the atmosphere and increase their average weight. The control ticks with mouthparts uncovered did show at least a minor increase in average weight due to water vapor uptake. However, only the 1 month control females managed to imbibe enough water to replace all that they lost during dehydration and exceed their average, initial weight. Similar results have been reported for several species of the Ixodidae (Lees 1974, Rudolph and Knulle 1974 and McMullen _et_. _al_ 1976).

Furthermore, a white crystalline solid, similar to that which Lees (1974) attributed to *A. variegatum*'s success at taking in water vapor, was observed around the mouthparts of some *O. megnini* individuals.

The reason why the 1 month control females were so successful at utilizing water vapor may have been that these females were the largest on average, and they lost the least amount of water weight during desiccation. In fact, the largest individuals lost less than 5% of their initial weight after 30 days at near zero percent R.H.. The smallest individuals also lost the most weight during dessication, sometimes 10% and more of their initial weight. The other groups could not imbibe enough water vapor to regain what they had lost, although the 9 month control females almost did. These females were the second largest on average. The difference in percent weight loss could have been the result of the larger ticks having a smaller surface to volume ratio which resulted in less evaporative water loss.

A similar situation has been observed in some ticks of the Ixodidae. Hair *et. al* (1975) observed that after 8 days of desiccation, *Amblyomma maculatum* Koch individuals lost 8% of their initial average weight, while *A. americanum*, one-third the size of *O. megnini*, lost 20%. The ability of the former species to withstand desiccation is lacking when compared to *O. megnini*. The *O. megnini* of smallest size were males that lost 10.8% of their initial average weight

after 30 days exposure to a relative humidity lower than that to which A. maculatum were exposed. However, the O. megnini weighed three times as much as the largest A. maculatum females.

Water losses in other argasid species have also been documented. Hafez et al. (1970) found that second instar nymphs of O. savignyi lost only 11.66% of their initial body weight after 30 days at 0% R.H.. Hefnawy et al. (1975) found that A. arboreus adults lost between 20 and 25% of their initial weight after 30 days at near zero percent R.H.. This was significantly greater than what O. megnini lost after 30 days. Balashov and Filippova (1964) tested several argasid species and found that the drought resistant species, A. lahorensis, A. persicus, and O. papillipes lost less than 10 to 20% of their initial body weights after 25 days at 20% relative humidity. The more hygrophilus species, Argas vulgaris Filippova, Ornithodoros tartakovskyi Olenov, and Ornithodoros capensis Neumann lost more than 20% after 25 days. Otobius megnini compares closely to the drought resistant species in their ability to resist desiccation. Balashov and Filippova (1964) also determined that body size enhances survival. In general, most drought resistant species they studied also happened to be the largest. The largest species studied, A. lahorensis, weighed 30 to 40 mg on average. This weight was only slightly larger than the smallest O. megnini studied. The authors also mentioned that permeability of the integument

also played a role in drought resistance. The integument of O. megnini is possibly quite resistant to water loss.

Balashov and Filippova (1964) also discovered that all species examined except, A. lahorensis, absorbed water vapor at relative humidities of 80% or greater, and after 10 days, recovered all or part of the weight lost after 10 days of desiccation. At 28 C and 100% R.H., the weight increases ranged form 28.8% to 9.5% of initial weight. These increases were substantially greater than the largest increase in O. megnini (5.2% in the 1 month control females after 10 days at 97% R.H.). The authors hypothesized that A. lahorensis did not sorb water because it was less stressed by the desiccation and sorption of water was unnecessary. This may have been the case in O. megnini as well.

Age influenced the water vapor uptake abilities of O. megnini insignificantly. After 30 days of desiccation, the 1 and 9 month ticks did not differ greatly in their average percent weight loss. This seems to indicate that the ability of O. megnini individuals to resist desiccation is not dramatically effected by age. Only the average, percent weight changes of the 1 and 9 month control females were significantly different at 15, 30, and 55 days of rehydration. During these times, the 1 month control females continued to exceed their initial average weight, but the increase in weight of the 9 month, control females was not as much. The rate of weight decrease was also

greater in the 9 month control and test males and test females than in the corresponding 1 month groups, but not significantly so. Apparently, the older ticks were unable to maintain their weight through water vapor uptake as well as the younger ticks. Gathering water vapor from the atmosphere probably requires substantial energy consumption. Possibly, the older ticks, with less internal energy reserves, were unable to expend as much of the required energy in water vapor uptake. However, _O_. _megnini_ also presents a unique physiological situation in the fact that the adults do not feed; therefore, they are constantly consuming internal food reserves gathered as nymphs and losing weight as a result. The 9 month ticks would have consumed more of their internal reserves and weighed less on average. This is what was observed in this experiment. The higher rates of weight loss in the 9 month old ticks may have been a result of their lower initial average weight and resulting higher surface to volume ratio. The effects of age and weight loss on water vapor uptake and consequent water balance may, therefore, be inseparable in _O_. _megnini_. The constant consumption of food reserves also made it virtually impossible to detect the true weight changes due to water vapor uptake and water loss.

Salivary Gland Ultrastructure

Salivary Ducts

The primary, secondary, and efferent ducts of adult and nymphal O. megnini salivary glands were basically identical in structure to those of other argasidae and ixodidae. The arrangement of the epicuticle and procuticle were similar to that of ixodid ticks (Balashov 1979). The structure of O. moubata salivary ducts, examined by El Shoura (1985), was also identical to that of O. megnini. An inner, thin layer (epicuticle of Balashov) and a thicker middle layer (procuticle of Balashov) as well as the spiral thickenings were observed in both O. moubata and O. megnini. Descriptions of salivary ducts for A. persicus (Robinson and Davidson 1913 and Rosdy and Coons 1975), A. arboreus (Guirgis 1971), and O. kelleyi (Sonenshine and Gregson 1970) were also virtually identical.

The epithelial cells surrounding the ducts in O. megnini were also similar to those described for A. arboreus and O. moubata. The tortuous lateral membranes and large nuclei of the epithelial cells of O. moubata (El Shoura 1985) were common in O. megnini epithelial cells as well. The abundant mitochondria and microtubules described for epithelial cells of A. arboreus (Roshdy and Coons 1975) were also common in O. megnini. However, the apical microvilli mentioned by Roshdy and Coons were not observed in.

Type A Acini

The type A, agranular acini examined in O. megnini adults were virtually identical in ultrastructure to those of other argasidae. The characteristic infoldings of the basal plasma membrane and abundant mitochondria were common to all the type A or type I, agranular acini of the argasidae (Dzhafarov 1965, Roshdy and Coons 1975, El Shoura 1985, and Needham et al. 1990). The ultrastructural characteristics of these acini were also similar to the type I acini of ixodidae (Kirkland 1971, Balashov 1968 and 1979, Coons and Roshdy 1973, Meredith and Kaufman 1973, Megaw and Beadle 1979, Krolak 1982, Barker et al. 1985, Walker et al. 1985, and Needham et al. 1990).

The large central cell and peripheral lamellate cells observed in O. megnini were found in O. moubata (El Shoura 1985) as well as A. arboreus (Roshdy and Coons 1975 and Needham et al. 1990). Roshdy (1972) noted that the cytoplasm near the center of the type A acini in A. persicus was much reduced, but did not describe a specific cell occupying a central postion in the acinus. Needham et al. (1990) noted that the central lamellate cell (=central cell) of O. moubata and A. arboreus exhibited basal infoldings that interdigitated with the infoldings of the peripheral lamellate cells. It is unclear whether a similar arrangement exists in O. megnini. However, low magnification, photomicrographs revealed that similar interdigitating infoldings may exist in O. megnini. The

circumlumenal and peritubular cells described for A. arboreus and O. moubata were not located in O. megnini. The lack of lipid inclusions in the cytoplasm of O. megnini lamellate cells was noticeable. Lamellate cells of other argasidae have also been observed to lack lipid inclusions (Needham et al. 1990).

It has been suspected for some time that the agranular acini in ixodidae and argasidae are involved in water vapor uptake. Many species of the Argasidae and Ixodidae are known to possess this ability (Lees 1946, Balshov and Filippova 1964, Hafez et al. 1970, and Sauer and Hair 1971). The ultrastructure of the lamellate cells in O. megnini and other ticks is characteristic of epithelia that transport salts and fluid (Berridge and Oschman 1972). The large central cell in both families of ticks probably functions to collect and store fluids from the peripheral lamellate cells and transport them to the lumen of the acinus (Coons and Roshdy 1973, Roshdy and Coons 1975, Krolak et al. 1982, Barker et al. 1984, and El Shoura 1985). Needham et al. (1990) found that the central lamellate cell was the only cell that extended from the basal membrane to the lumen and may thus act as a conduit for fluid movement. They also noted that the cytoplasm of the central cell appeared less dense and had a greater volume in actively rehydrating ticks, which indicated that fluid transport may be occuring.

Furthermore, the ultrastructure of the peripheral lamellate cells in A. americanum and I. ricinus has been

shown to change perceptively as rehydration occurs (Needham and Coons 1984, Kahl _et_ _al_. 1990, and Needham _et_ _al_. 1990). In these species, the mitochondria became more condensed, indicative of increased respiration, and the lipid inclusions were consumed. In argasid species glycogen, not lipids, appear to be the energy source for the process of fluid movement (Needham _et_ _al_. 1990). The presence of Na^+, K^+ - ATPase in the membranes of the lamellate cell basal infoldings is also evidence that the agranular acini can secrete salt solutions concentrated enough to absorb water from unsaturated atmospheres (Needham _et_ _al_. 1990).

The presence of these agranular acini in adult _O_. _megnini_, which do not feed, lends further credence to the assumption that these acini play a role in water vapor uptake. In fact, older, adult _O_. _megnini_ individuals have only the agranular acini, the granular acini having degenerated as the nymphs molted to the adult stage. The adults would have no need of the agranular acini if they were required for feeding. However, the adults would have definite need of these acini if they were involved in water vapor uptake. Likewise, the agranular acini in nymphal _O_. _megnini_ are much smaller and may or may not be as functional as in the adults. The nymphs of _O_. _megnini_ remain in the protected environment of the host's ears throughout their development. Within the ears, the nymphs detach only to molt or to find a new feeding site, therefore they would have little need for imbibing water vapor. Their

water needs would be entirely supplied from the host's blood.

Type B Acini

The granules of the type Ba cells in O. megnini resembled the type c granules of both A. persicus (Roshdy 1972) and A. arboreus (Roshdy and Coons 1975) and O. moubata (El Shoura 1985). As in O. megnini, the granules in all the above species consisted of a well-delineated, electron dense core surrounded by a shell of less dense material.
El Shoura (1985) found that the peripheral zones of these granules were polysaccharide positive and that the cores, but not the peripheral zones, were completely digested by pronase. Roshdy (1972) also discovered that these granules in A. persicus reacted positively for proteins and carbohydrates. After application of Mallory's, the central core of these granules in O. megnini appeared to have a reddish tint. This indicated the presence of negatively charged compounds which could be negatively charged proteins corresponding to the pronase digestible protein cores in O. moubata (El Shoura 1985). The peripheral zones stained a pale blue-violet, indicating the presence of compounds with a weak, postive charge, possibly carbohydrates of some type (Roshdy 1972). The small, dark granules observed within the less dense peripheral zones were undoubtedly composed of the same material as the cores. These small granules were either newly synthesized core material moving into the core

or core material moving out into the cell. The extensive rough endoplasmic reticulum with associated ribosomes also indicated that proteins constituted a large part of these granules in O. megnini.

Roshdy (1972) proposed that the staining properties of the double-layered c granules in A. persicus B acini indicated the prescence of a moderately sulfated anticoagulant. El Shoura (1985) also stated that the corresponding granules in O. moubata were depleted 5 minutes after attachment, which may also indicate an anticoagulatory function for these granules. Anticoagulants have been identified in some species of the Argasidae. Howell (1966) identified a anticoagulant in the saliva of O. savignyi. Anticoagulants have also been identified in some species of the Ixodidae.

The uniformly electron dense granules of the type Bb cells in O. megnini may correspond to the granules of the b cells in the type B acini of A. persicus (Roshdy 1972), A. arboreus (Roshdy and Coons 1975), and O. moubata (El Shoura 1985). Although these granules were uniformly electron dense, and stained an overall darker blue-violet with Mallory's, some reddish coloration was observed near the center of these granules. This indicates that both acidophilic and basophilic compounds (possibly carbohydrates and proteins) were present. The substantial amount of rough endoplasmic reticulum around these granules implies that proteins are a component of these granules. However,

El Shoura (1985) found that the b cell granules in O. moubata reacted positively for polysaccarides but negatively for proteins. Roshdy (1972) found that the b cell granules in A. persicus were non-metachromatic and reacted faintly for carbohydrates and only moderately for proteins. Roshdy proposed that these granules were responsible for producing a lytic salivary component in this species.

A third cell type has been identified in A. arboreus, A. persicus, and O. moubata. In A. persicus and A. arboreus, this cell type, labelled Ba, contained granules that reacted positively for phospholipids, and proteins rich in tryptophan, but not carbohydrates (Roshdy 1972). It was suggested that these granules contained pharmacologically active salivary components. In O. moubata, the third cell type, labelled Bc, contained granules which were faintly positive for polysaccharides and were partially digested by pronase. El Shoura (1985) also suggested the possibility that these granules contained unknown, pharmacologically active salivary components. Such components have been found in A. arboreus (Coons and Roshdy 1981).

A third granular cell type could not be positively identified in O. megnini. However, the dark staining granules of some of the Bb cells in O. megnini were somewhat larger than in other cells. This could imply a third, granular cell type. This could not be confirmed because the granules reacted identically to Mallory's. It is also

possible that the larger granules may simply be more developed versions of the smaller granules. In any case, more histochemical tests need to be performed to determine conclusively whether more than two cell types are present in the type B acini of O. megnini nymphs.

The interstitial epithelial cells of O. megnini type B acini were similar to those found in O. moubata. El Shoura (1985) found that these cells formed extensive canaliculi during feeding. It has been theorized that the function of these cells is to remove excess fluid taken in during feeding (Megaw and Beadle 1979). These cells have been known to transport fluids in other argasid species (Coons and Roshdy 1979 and 1981). It is conceivable that the epithelial cells with their canaliculi perform a similar function in O. megnini as well.

The three small cells observed at the apices of some type B acini in O. megnini nymphs may correspond to the cap cells observed in O. moubata. El Shoura (1985) suggested that these cells may help hold the fluid filled acinus together. Similar cells identified in some species of Ixodidae have been hypothesized to play role a in fluid transport (Meredith and Kaufman 1973), or have a myoepithelial function (Krolak et al. 1982). A more complete examination of the ultrastructure of these basally located cells in O. megnini may lead to a more precise determination of their function.

Salivary Gland Degeneration

The results presented here indicate that degeneration of the type B acini of the nymph salivary gland began within 2 days post-removal from the host. By this time, it was evident that the type Ba and Bb cell granules were in the process of being catabolized and absorbed. The larger Ba cell granules appeared to degenerate interiorly. The electron dense core material was first fractured into smaller granules that dispersed throughout the electron lucent shell of the granule. Not all of the Ba granules appeared to be undergoing this process at the same time, which indicated that the granules were broken down a few at a time. Most likely, the ticks could only catabolize limited amounts of granular material at a time. Residual bodies located within the autophagic vacuoles were probably the remains of rough endoplasmic reticulum, mitochondria, and other cell structures. Correspondingly little intact rough endoplasmic reticulum and mitochondria were observed indicating that new protein synthesis and cell construction had virtually ceased by 2 days post-removal.

The type Bb cell granules appeared to be reabsorbed from the outside. The irregular shape of the granules was due to portions of the periphery being dissolved by nearby vacuoles. Apparently, empty vacuoles were most likely what remained of a granule. The degeneration of organelles in the type Bb cells was more advanced than in the type Ba cells. Virtually no rough endoplasmic reticulum could be

recognized at 2 days post-removal. The large amount of debris in the cytoplasm was probably all that remained of the organelles. Judging from appearances, it is likely that the Bb cells degenerate before the Ba cells, or their substance is more easily catabolized by the tick during degeneration.

The canaliculi of the interstitial epithelial cells was still reasonably intact, but the cytoplasm showed evidence of debris and few intact mitochondria, which indicated that degeneration had been initiated. However, the intact canaliculi may indicate that these cells are still functioning at least minimally at 2 days post-removal.

At 5 days, cellular regression had progressed predictably. More Ba granules had been disassembled and autophagic vacuoles were larger and more abundant. In fact, some of the largest vacuoles may have been all that remained of an equally large Ba granule. Also, at 5 days post-removal, the whorled membrane structures began to appear. Barker et al. (1985) referred to similar whorled membrane structures in the degenerating central cells of type I acini of A. americanum as myelin bodies. It was also suggested that these structures were the remains of cytolysosomes that had consumed mitochondria and other cell structures. Fawcett (1981) interpreted the whorled membranes as hydrated phospholipid. In O. megnini, a few autophagic vacuoles were observed with whorled membrane surrounding them, which suggests that the interpretation of the above authors may

hold true for _O. megnini_ as well. The fact that these membrane whorls were not common at 2 days post-removal indicates that the autophagic vacuoles had not yet had time to produce them.

By 8 days post-removal, degeneration of the type B acini was virtually complete. The bulk of the B acini were composed of extracellular space that contained vacuoles, residual bodies, and clumps of granular material that may have been unabsorbed cytoplasm or granule material. The granule containing cells were compressed into a small area of each acinus. At this time, some Ba granules still remained, however, the presence of small autophagic vacuoles in the substance of the granules indicated that reabsorption was still actively occurring. No Bb cell granules were observed at all, which indicated that they had all been completely digested by 8 days. The nearly complete degeneration of the few remaining granular cells was evidenced by the cytoplasm which was either vacuous or completely filled with membrane whorls and other cell debris. The presence of a few interstitial cells with debris filled cytoplasm, but no canaliculi, showed that these cells, like the granular cells, do not degenerate at the same pace. Many had degenerated by 5 days post-removal, but a few still remained at 8 days. During dissection, at 8 days post-removal, it was immediately apparent that the type B acini of the nymphs were only 50 to 30% of their size in the actively feeding nymph. The B acini were still visible

in newly molted adults, but apparently continued to be assimilated into the glandular tissue as the adults aged. In adults older than 9 months, virtually no traces of the type B acini were visible in the amorphous tissue comprising the bulk of the adult salivary gland. The vacuolated appearance of the cytoplasm of the epithelial cells surrounding the secondary and efferent ducts of the B acini also revealed that associated ducts of the B acini had begun degeneration by 8 days post-removal.

Unfortunately, the ultrastructural changes occurring in the type A acini, if any, were not observed in detail. However, the cytoplasm of a few of the type A acini cells appeared to by highly vacuolated, implying that some ultrastructural changes are occurring. By the time nymphs had molted to adults, these acini appeared to be fully developed, at least externally.

Salivary gland degeneration has not been thoroughly studied in Argasidae. It has been studied in some species of Ixodidae. Till (1961) reported that histological disruption of salivary glands of R. appendiculatus engorged females occurred within a few days after host attachment. Kaufman (1976) showed that the isolated salivary glands from engorged D. andersoni females secreted at 25% the rate of glands from incompletely engorged females. This result was attributed to impending histological autolysis. A similar response was noted for A. hebraeum (Harris and Kaufman 1981). In this species, autophagic vacuoles were found in

the fluid secretory labyrinth of the type III acinus within 24 hours post-repletion. This is comparable to *O. megnini* nymphs in which autophagic vacuoles were found at 48 hours post-removal from the host. Kahl *et al*. (1990) found that the old agranular acini of *I. ricinus* degenerated during molting and new ones formed and were functional in teneral adults and nymphs. The granular acini of this species also degenerated post-repletion and disintegrated completely at apolysis. Presumably, they were reformed in the next feeding stage if necessary. This result is similar to *O. megnini* nymphs in which the granular acini disintegrate completely during molting to the adult, but since *O. megnini* does not feed as an adult, these acini are not regenerated. It is unknown to what extent the agranular type A acini degenerate in *O. megnini* nymphs during molting. However, some degeneration and regeneration would be logical because these acini must enlarge greatly to reach adult size.

The factor or factors involved in initiating salivary gland degeneration in *O. megnini* nymphs are unknown. In some ixodid species, several factors including weight, mating status, and hormonal and neural components are known to be involved. *Amblyomma hebraeum* females must reach an engorged weight of at least 400 mg and have been inseminated before complete salivary gland degeneration occurs (Harris and Kaufman 1984). A weight limit may also occur in *O. megnini*. *Otobius megnini* nymphs must reach a weight of approximately 26 mg before they can molt to adults

(Wanchinga 1983). This may also be the weight required for salivary gland degeneration to be initiated in _O_. _megnini_. Mating is not a factor in _O_. _megnini_ salivary gland degeneration because the type B acini have already degenerated by the time the adult female has mated.

In _A_. _hebraeum_, hormonal and neural factors are involved (Harris and Kaufman 1981 and 1984). The hormone which acts as the salivary gland degeneration factor in _A_. _hebraeum_ was identified as an ecdysteroid similar or identical in nature to 20-hydroxyecdysone (Harris and Kaufman 1985 and Kaufman 1988 and 1990). Ecdysteroids have also been implicated as the salivary gland degeneration factors in _A_. _americanum_ and _A_. _variegatum_ (Kaufman 1988 and 1991). Future studies could be performed to determine if similar salivary gland degeneration factors exist in _O_. _megnini_.

CHAPTER VI

SUMMARY AND CONCLUSIONS

Internal Anatomy

The internal anatomy of _O_. _megnini_, with a few exceptions, did not differ significantly from that of other argasidae. The reproductive, respiratory, circulatory and digestive systems were similar in organ design and arrangement to _Argas_ and _Ornithodoros_ species. The muscular system did differ from other species in a few details. The extensor muscles of the trochantor differed in arrangement as well as the depressors of the capitulum. Adductor muscles of the coxae similar to other argasid species were also absent in _O_. _megnini_. However, additional muscles connected to the endosternum may have performed a similar function. All other muscle groups were found in species of _Argas_ and _Ornithodoros_.

The anatomy of the salivary glands and coxal glands of adult _O_. _megnini_ differed significantly from that of _O_. _megnini_ nymphs and other argasidae. The salivary glands of the nymph was comprised of two types of acini labelled type A and type B. The type A acini were a fraction of the size of the type B acini and were restricted to the anterior, inward facing portion of the gland. Two types of

acini have also been identified in most other species of the Argasidae. In the adults, the type A acini were much enlarged and the type B acini were degenerated.

The coxal glands of *O. megnini* nymphs each consisted of a coxal tubule and an accessory gland. These organs were similar in appearance to those found in other argasidae. However, the coxal tubules of the nymphs displayed numerous globules on the external surface of the tubule. The function and internal composition of these globules is unknown. The histology and function of the accessory gland also remains unknown. The coxal tubules of the adults appeared partially degenerated with fewer, less prominent globules, and the accessory glands were absent.

The degeneration of the type B acini and coxal glands in the adult *O. megnini* can be attributed to the fact that they do not feed. The type B acini are most likely needed for feeding and are thus not required by the adult. The retainment and further development of the type A acini in the adults indicates that the function of this acinus, probably in water vapor uptake, is essential for adult survival. The coxal glands of argasidae are used primarily to eliminate excess water taken in with the bloodmeal during feeding. This function would also not be required by the non-feeding adults, and the coxal glands would be rendered superfluous.

Water Vapor Uptake

The water vapor uptake experiment clearly indicated that the mouthparts are the site of water vapor uptake in adult O. megnini. Ticks with mouthparts covered by wax did not show any weight increase due to intake of water vapor. Ticks with mouthparts free did show a weight increase which indicated water vapor was being taken in. The experiment also showed that O. megnini adults were highly resistant to desiccation. Compared to other argasidae tested, O. megnini individuals lost the least amount of water weight at relative humidities of less than 10%. The high resistance to desiccation of O. megnini is most likely due to their large size and correspondingly lower surface to volume ratio.

The ability of O. megnini to imbibe water vapor from unsaturated atmospheres (97% R.H.) was also less than has been measured in most other argasid species. The high resistance of O. megnini to desiccation most likely reduces the need for supplemental water uptake.

The ability of O. megnini adults to withstand desiccation did not differ significantly with age, indicating that the water retaining properties of the cuticle did not degrade substantially with age. However, the ability to imbibe water vapor did lesson somewhat with age, but not significantly in most cases. Nine month old individuals did show a reduced ability to imbibe water vapor when compared to 1 month old adults. This could have been

due to the probability that the older ticks were physiologically unable to expend as much of the required energy to initiate the process of water vapor uptake.

Salivary Gland Ultrastructure

Upon ultrastructural examination, the type A acini of adult O. megnini were observed to consist of a large central cell and several peripheral lamellate cells. The lamellate cells exhibited numerous infoldings of the basal plasma membrane and large numbers of associated mitochondria. These cells were identical in utrastructure to the agranular acini of other argasidae and ixodidae, and probably serve to transport ions and fluids.

The type B acini of O. megnini feeding nymphs contained at least two types of granular cells based on granule size and staining properties. These cells were labelled type Ba and type Bb. The Ba cell granules exhibited a dark red-violet core ,possibly of protein, surrounded by a pale blue-violet shell, possibly composed of carbohydrate containing compounds. The core was also electron dense and the outer shell less dense.

The Bb cell type contained smaller dark-violet staining granules, also possibly of protein. These granules were overall electron dense. Interstitial epithelial cells with canaliculi, possibly for fluid transport, and basal cells were also observed in the type B nymphal acini.

Salivary Gland Degeneration

After observing the degeneration of the nymphal type B acini at the ultrastructural level, the time course of the B acini degeneration appeared as follows: By day 2 post-removal from the host, the granules of the Ba and Bb cells were being broken up and reabsorbed, and autophagic vacuoles with inclusion bodies indicated that cytoplasmic material was also being reabsorbed. The lack of substantial, organized rough endoplasmic reticulum and other organelles indicates that new granule formation had probably ceased by day 2. The interstitial epithelial cells with their canaliculi also showed signs of disruption and autolysis.

By day 5, granule and cytoplasmic autolysis had progressed to the point where visible cell shrinkage had occurred and larger amounts of extracellular space existed between the cell bodies and basal membrane of the acinus. The cytoplasm of the granular cells began to fill with the remnants of cellular autolysis, residual bodies and membrane whorls. The canaliculi of most of the interstitial epithelial cells were almost completely absent the cytoplasm filled with residual bodies.

By day 8, granular cell shrinkage had increased to the point where the cells occupied a small portion of the acini. The enlarged extracellular space became filled with the remains of cellular degeneration including vacuoles, residual bodies, membrane whorls, and free granular material. Some intact granules remained, but most had been

reabsorbed or had coalesced into large, amorphous masses of granular material. The cytoplasm of the granular cells had almost completely filled with membrane whorls and other cellular debris. By day 8 post-removal, most of the interstitial epithelial cells had been completely reabsorbed, but a few with canaliculi still apparent remained. At 8 days, the epithelial cells around the secondary and efferent ducts also showed degenerative changes. Degeneration of the type B acini appeared to continue for some time after the nymphs had molted to adults until virtually all external traces of the B acini were removed. Throughout the process of the type B degeneration, the type A, agranular acini matured into the adult form, but the time course and ultrastructural changes involved were not examined.

REFERENCES

Allen, W.E. 1905. Internal morphology of the american cattle tick. Stud. from Zool. Lab. Univ. Nebraska, No. 4, pp.245-280, 4 pls.

Balashov, Yu.S. and N.A. Filappova. 1964. Water balance and critical temperature of the epicuticle in argasid ticks. Ent. Obozr. 42: 351-363.

Balashov, Yu.S. 1968. Blood sucking ticks (Ixodoidea) - vectors of diseases of man and animals. Zoo. Instit. USSR Acad Science Leningrad: Nauka Publ. (English translation: Misc. Publ. Entomol. Soc. Amer. (1972).

1979. An atlas of ixodid tick ultrastructure. Zoo. Instit. USSR Acad Science Leningrad: Nauka Publ. (English translation: Special Publ. Entomol. Soc. Amer. (1983).

Banks, H. 1912. New American Mites. Proc. Entomol. Soc. Wash. 14: 96-99.

Barker, D.M., C.L. Ownby, J.M. Krolak, P.L. Claypool, and J.R. Sauer. 1984. The effects of attachment, feeding, and mating on the morphology of the type I alveolus of salivary glands of the lone star tick, *Amblyomma americanum* (L.). J. Parasitol. 70: 99-113.

Beck, D.E. 1955. Some unusual distributional records of ticks in Utah. J. Parasitol. 41: 198-210.

Berridge, M.J. and J.T. Oschman. 1972. Transporting Epithelia. New York and London: Academic Press.

Binnington, K.C. 1975. Secretory coxal gland active during apolysis in ixodid and argasid ticks (Acarina). Int. J. Insect Morph. Embryol. 4: 184-192.

1978. Sequential changes in salivary gland structure during attachment and feeding of the cattle tick, *Boophilus microplus*. Int. J. Parasitol. 8: 97-115.

Bonnet, A. 1907. Recherches sur l'anatomie comparee et le developpement des Ixodides. Ann. Univ. Lyon. n.s. 1,20: 1-180.

Browning, T.O. 1954. Water balance in the tick Ornithodoros moubata Murray, with particular reference to the influence of carbon dioxide on the upatake and loss of water. J. Exp. Biol. 31: 331-340.

Chellappa, D.R. 1973. Note on spinose ear tick infestation in man and domestic animals in India and its control. Madras Agric. J. 60: 656-658.

Chinery, W.A. 1965. Studies on the various glands of the tick, Haemaphysalis spinigera Neumann 1897. Part III. The salivary glands. Acta Tropica 22: 321-349.

Christophers, S.R. 1906. The anatomy and histology of ticks. Sci. Mem. Med. Sanit. Dept. Govt. India n.s. 23: 55pp.

Cooley, R.A. and D.M. Kohls. 1944. The argasidae of North America, Central America, and Cuba. Am. Midland Natur. Monogr. 1: 1-52.

Coons, L.B. and M.A. Roshdy. 1973. Fine structure of the salivary glands of unfed male Dermacentor variabilis (Say) (Ixodoidea: Ixodidae). J. Parasitol. 59: 900-912.

1979. Functional morphology and cytochemical localization of chloride ions and oubain sensitive phosphatase activity in the salivary gland transport epithelia of four species of ticks. In: Recent Advances in Acarology, Vol. I. New York: Academic Press.

1981. Ultrastructure of granule secretion in salivary glands of Argas (Persicargas) arboreus during feeding. J. Parasitol. 65: 225-234.

Davis, W.D. 1934. The life-history and some bionomics of Ornithodoros megnini (Duges). M.S. Thesis, Oklahoma State University, Stillwater.

Douglas, J.R. 1943. The internal anatomy of Dermacentor andersoni Stiles. Univ. Calif. Pub. Entomol. 7: 207-272.

Duges, A. 1884. Turicata y garrapata de Guanajuato. La Naturaleza, Periodico Cientifico de la Sociedad Mexicana de historia natural, VI (1882-84). 195-198.

Dzhafarov, T.E. 1965. An electron microscope investigation of secretory alveoli of salivary glands of the tick Ornithodoros moubata. Tsitologiya 7: 233-236.

El Shoura, S.M. 1985. Ultrastructure of salivary glands of Ornithodoros (Ornithodoros) moubata (Ixodoidea: Argasidae). J. Morph. 186: 45-52.

Ewing, S.A., J.R. Harkess, K.M. Kocan, R.W. Barker, J.C. Fox, R.D. Tyler, R.L. Cowell, and R.B. Morton. 1990. Failure to transmit Ehrlichia canis (Rickettsiales: Ehrlichieae) with Otobius megnini (Acari: Argasidae). J. Med. Entomol. 27: 803-806.

Fawcett, D.W. 1981. The cell. Saunders. Philadelphia. 448 p.

Guirgis, S.S. 1971. The subgenus Persicargus (Argasidae, Argas). Histological studies on A. (P.) arboreus Kaiser, Hoogstraal and Kohls. J. Med. Entomol. 8: 648-667.

Hafez, M., S. El-Ziady, and T. Hefnawy. 1970. Biochemical and physiological studies of certain ticks (Ixodoidea). Uptake of water vapor by the different developmental stages of Hyalomma (H.) dromedarii Koch (Ixodidae) and Ornithodoros (O.) savignyi (Audouin) (Argasidae). J. Parasitol. 56: 354-361.

Hair, J.A., J.R. Sauer, and K.A. Durham. 1975. Water Balance and humidity preference in three species of ticks. J. Med. Entomol. 12(1): 37-47.

Harris, R.A. and W.R. Kaufman. 1981. Hormonal control of salivary gland degeneration in the ixodid tick Amblyomma hebraeum. J. Insect Physiol. 27: 241-248.

1984. Neural involvement in the control of salivary gland degeneration in the ixodid tick, Amblyomma hebraeum Koch. J. Exp. Biol. 109: 281-290.

1985. Ecdysteroids: possible candidates for the hormone which triggers salivary gland degeneration in the ixodid tick Amblyomma hebraeum. Experentia 41: 740-742

Hefnawy, T., S.L. Bishara, and T.T.M. Bassal. 1975. Biochemical and physiological studies of certain ticks (Ixodoidea): Effect of relative humidity and starvation on the water balance and behaviour of adult Argas (P.) arboreus (Argasidae). Expl. Parasitol. 38: 14-19.

Herms, W.B. 1917. Contribution to the life history and habits of Ornithodoros megnini. J. Econ. Entomol. 1: 344-351.

Hoogstraal, H. 1966. Ticks in relation to human diseases caused by viruses. Ann. Rev. Entomol. 11: 261-308. 1973. Ticks. In: Parasites of Laboratory Animals, R.J. Flynn (Ed.). Iowa State Univ. Press, Ames: 398-424.

Hooker, W.A. 1908. Life history, habits, and methods of study of the Ixodoidea. J. Econ. Entomol. 1: 34-51.

Hooker, W.A., F.C. Bishop and H.P. Wood. 1912. The life history and bionomics of some North American ticks. U.S. Dep. Agr., Bur. Entomol. Bull. 106: 239pp.

Hopla, C.E. 1955. Observations on the life history of a rabbit tick (Otobius lagophilus). J. Kan. Entomol. Soc. 28: 114-116.

Howell, D.E., G.W. Stiles, and L.H. Moe. 1943. The fowl tick (Argas persicus), a new vector of anaplasmosis. J. Vet. Res. 4(10): 73-75.

Jellison, W.L., E.J. Bell, R.J. Huebner, R.R. Parker, H.H. Welsh. 1948. Q-fever studies in Southern California. IV. Occurrence of Coxiella burnetti in the spinose ear tick, Otobius megnini. Publ. Health Repts. 63(46): 1483-1489.

Kahl, O., R. Hoff and W. Knulle. 1990. Gross morphological changes in the salivary glands of Ixodes ricinus (Acari: Ixodidae) between bloodmeals in relation to active uptake of atmospheric water vapour. Exp. and Appl. Acarology. 9: 239-258.

Kaufman, W. 1976. The influence of various factors on fluid secretion by in vitro salivary glands of ixodid ticks. J. Exp. Biol. 64: 727-742.

Kaufman, W. R. 1988. The effects of steroids and azadirachtin on salivary gland and ovary in ixodid ticks. J. Insect Physiol. 34: 721-723.

1990. Effect of 20-hydroxyecdysone on the salivary glands of the male tick, Amblyomma hebraeum. Exp. Appl. Acarol. 9: 87-95.

1991. Further investigations on the action of ecdysteroids on the salivary glands of the female tick Amblyomma americanum. Exp. Appl. Acarol. 10: 259-265.

Kaufman, W.R. and J.R. Sauer. 1982. Ion and water balance in feeding ticks. In: Physiology of ticks. Ed. F.D. Obenchain and R. Galun. Pergamon Press. New York.

Kirkland, W.L. 1971. Ultrastructural changes in the nymphal salivary glands of the rabbit tick, Haemaphysalis leporispalustris, during feeding. J. Insect Physiol. 17: 1933-1946.

Knulle, W. and T.L. Devine. 1972. Evidence for active and passive components of sorption of atmospheric water vapour by larvae of the tick Dermacentor variabilis. J. Insect Physiol. 18: 1653-1664.

Knulle, W. and D. Rudolph. 1982. Humidity relationships and water balance of ticks. In: Current themes in tropical science. Physiology of Ticks. Vol. I. Chapter 2. Pergamon press, Ltd. p. 43.

Koshy, T.J., G. Rajavelu and C.M. Lalitha. 1979. Studies on the life-cycle of Otobius megnini (Duges 1884) (ear tick, sheep). Cheiron 8(1): 52-55.

Krantz, G.W. 1978. A manual of acrarology. Oregon State University Book Stores Inc. Corvallis, Oregon.

Krolak, J.M., C.L. Ownby, and J.R. Sauer. 1982. Alveolar structure of salivary glands of the lone star tick, Amblyomma americanum (L.): Unfed females. J. Parasit. 68: 61-82.

Lees, A.D. 1946. The water balance in Ixodes ricinus L. and certain other species of ticks. Parasitol. 37: 1-20.

1974. Site and mechanism of water vapour uptake from the atmosphere in ixodid ticks. Nature 249: 84-85.

Lees, A.D. and J.W.L. Beament. 1948. An egg waxing organ in ticks. Quart. J. Micr. Sci. 89: 291-332.

Loomis, E.C. 1961. Life histories of ticks under laboratory conditions (Acarina: Ixodidae and Argasidae). J. Parasitol. 47: 91-99.

Machin, J. 1979. Atmospheric water absorption in arthropods. In: Advances in insect physiology. J.E. Treherne, M.J. Berridge, and V.B. Wigglesworth, Eds. Academic Press, New York, Vol 14: 1-48.

1983. Water vapor absorption in insects. Am. J. Physiol. 244(2): 187-192.

Machin, J., J. O'Donnell and P.A. Coutchie. 1982. Mechanisms of water vapor absorption in insects. J. Exp. Zool. 222: 309-320.

Marx, A. 1895. Obituary notice of his work. Proc. Entomol. Soc. Wash. 3: 195-201.

McMullen, H.L., J.R. Sauer and R.L. Burton. 1976. Possible role in uptake of water vapor by ixodid tick salivary glands. J. Insect Physiol. 22: 1281-1286.

Megaw, M.J. and D.J. Beadle. 1978. Structure and function of the salivary glands of the tick, Boophilus microplus Canestrini (Acarina: Ixodidae). Int. J. Insect Morph. and Embryol. 8: 67-83.

Meredith, J. and W.R. Kaufman. 1973. A proposed site of fluid secretion in salivary gland of the ixodid tick Dermacentor andersoni. Parasitology 67: 205-217.

Needham, G.R. and L.B. Coons. 1984. Ultrastructural changes in type I alveoli of the salivary glands from hydrating and desiccating lone star ticks. In: D.A. Griffiths and C.E. Bowman (Editors), Acarology VI. Vol. 1. Ellis Horwood, Chichester. p. 366-373.

Needham, G.R., R. Rosell, L. Greenwood, and L.B. Coons. 1990. Ultrastructure of type-I salivary-gland acini in four species of ticks and the influence of hydration states on the type-I acini of Amblyomma americanum. Exp. Appl. Acarol. 10: 83-104.

Noble-Nesbitt, J. 1970. Water uptake from unsaturated atmospheres: Its site in insects. Nature, Lond. 225: 753-754.

Nordenskiold, E. 1908. Zur anatomie und histologie von Ixodes reduvius. I. Zool. Jahrb., Abt. f. Anat. 25: 637-672.

1909. Zur anatomie und histologie von Ixodes reduvius. Ibid. 27: 449-464.

1911. Zur anatomie und histologie von Ixodes reduvius. Ibid. 32: 77-106.

Obenchain, F.D. and J.H. Oliver. 1974. Undescribed glandular tissues from Amblyomma tuberculatum (Arachnida; Parasitiformes; Ixodidae). Bull. ASB. 21(2): 72.

1976a. Peripheral nervous system of the ticks, Amblyomma tuberculatum Marx and Argus radiatus Railliet (Acari: Ixodoidea). J. parasitol. 62: 811-817.
1976b. The heart and arterial ciruculatory system of ticks(Acari: Ixodoidea). J. Arachnol. 3: 57-74.

Rich, G.B. 1957. The ear tick _Otobius megnini_ (Duges) (Acarina: Argasidae) and its record in British Columbia. Canada J. Comparat. Med. 21: 415-418.

Robinson, L.E. and J. Davidson. 1913. The anatomy of _Argas persicus_ (Oken) 1818. Part I. Parasitology 6: 20-48.

1913a. The anatomy of _Argas persicus_ (Oken), 1818. Part II. _Ibid_. 6: 217-256.

1914. The anatomy of _Argas persicus_ (Oken), 1818. Part III. _Ibid_. 6: 382-424.

Roshdy, M.A. 1961. Comparative internal morphology of subgenera of _Argus_ ticks (Ixodoidea, Argasidae) I. Subgenus _Carios_: _Argas vespertilionis_ (Latreille, 1802). J. Parasitol. 47(6): 987-994.

1962. Comparative internal morphology of subgenera of _Argas_ ticks (Ixodoidea, Argasidae). 2. Subgenus _Chiropterargas_: _Argas boueti_, Roubaud and Colas Belcour, 1933. _Ibid_. 48(4): 623-630.

1963. Comparative internal morphology of subgenera of _Argas_ ticks (Ixodoidea, Argasidae). 3. Subgenus _Secretargas_: _Argas transgariepinus_ White, 1846. _Ibid_. 49: 851-856.

1966. Comparative internal morphology of subgenera of _Argas_ ticks (Ixodoidea, Argasidae). 4. Subgenus _Ogadenus_: _Argas brumpti_ Neumann, 1907. _Ibid_. 52(4): 776-782.

1972. The subgenus _Persicargas_ (Ixodoidea: Argasidae: _Argas_). 15. Histology and histochemistry of the salivary glands of _A_. (_P_.) _persicus_ (Oken). J. Med. Entomol. 9: 143-148.

Roshdy, M.A. and L.B. Coons. 1975. The subgenus _Persicargus_ (Ixodoidea: Argasidae: _Argas_). 23. Fine structure of the salivary glands of unfed _A_. (_P_.) _arboreus_ Kaiser, Hoogstraal, and Kohls. J. Parasitol. 61(4): 743-752.

Rudolph, D. 1982. Site, process and mechanism of active uptake of water from the atmosphere in the Psocoptera. J. Insect Physiol. 28(3): 205-212.

Rudolph, D. and W. Knulle. 1974. Site and mechanism of water vapour uptake from the atmosphere in ixodid ticks. Nature 249: 84-85.

1982. Novel uptake systems for atmospheric water vapor among insects. J. Exp. Zool. 222(3): 205-212.

Saito, Y. 1960. Studies on ixodid ticks. Part IV. The internal anatomy in each stage of *Haemaphysalis flava* Neumann, 1897. Acta Med. Biol. Niigata 8: 189-239.

Samson, K. 1909. Zur antomie und biologie von *Ixodes ricinus* L. Zeit. f. wiss. Zool. 93: 185-236.

Sauer, J.R. and J.A. Hair. 1971. Water balance in the lone star tick (Acarina: Ixodidae): The effect of the relative humidity and temperature on weight changes and total water content. J. Med. Entomol. 8(5): 479-485.

Sigal, M.D., J. Machin, and G.R. Needham. 1991. Hyperosmotic oral fluid secretion during active water absorption and during desiccation-induced storage-excretion by the unfed female tick *Amblyomma americanum*. J. Exp. Biol. 157: 585.

Sonenshine, D.E. and J.D. Gregson. 1970. A contribution to the internal anatomy and histology of the bat tick *Ornithodoros kelleyi* Cooley and Kohls, 1941. I. The alimentary system, with notes on the food channel in *Ornithodoros denmarki* Kohls, Sonenshine, and Clifford, 1965. J. Med. Entomol. 7(1): 46-64.

Sonenshine, D.E. 1970. A contribution to the internal anatomy and histology of the bat tick *Ornithodoros kelleyi* Cooley and Kohls, 1941. II. The reproductive, muscular, respiratory, excretory, and nervous systems. J. Med. Entomol. 7(3): 289-312.

Silva-Goytia, R. and A. Elizondo. 1952. Estudios sobre fiebre manchada en Mexico, 2. Parasitos hematofagos encontrados naturalmente infectados. Rev. Med. 32: 278-282.

Steele, R.G.D. and J.H. Torrie. 1960. Principles and procedures of statistics. McGraw-Hill Book Co. New York.

Stiles, G.W. 1944. Isolation of the *Bacillus anthracis* from the spinose ear tick *Ornithodoros megnini*. J. Vet. Res. 5(17): 318-319.

Stricker, K.W. 1990. Chemical, physical, and biological aspects of spinose ear tick courtship behavior. Master's Thesis. Oklahoma State University, Stillwater.

Tatchell, R.J. 1962. Studies on the male accessory reproductive glands and the spermatophore of the tick, *Argus persicus* Oken. Parasitology 52(1,2): 133-142.

Theiler, G. and L.E. Salisbury. 1958. Zoological survey of the Union of South Africa. Tick survey part XI. The distribution of Otobius megnini, the spinose ear tick. Onderstepoort J. Vet. Res. 27(4): 605-610.

Till, W.M. 1961. A contribution to the anatomy and histology of the brown dog tick, Rhipicephalus appendiculatus (Neumann). Mem. Entomol. Soc. Sth. Afr. 6: 1-124.

Townsend, C.H.T. 1893. Ticks in the ears of horses. J. New York Entomol. Soc. 1(2): 49-52.

True, G.H. 1932. Studies on the anatomy of the pajorello tick, Ornithodoros coriaceus Koch. I. The alimentary canal. Univ. Calif. Pub. Entomol. 6: 21-48.

Venable, J.H. and R. Coggelshall. 1965. A simplified lead citrate stain for use in electron microscopy. J. Cell Biol. 24: 407-408.

Walker, A.R., J.D. Fletcher, and H.S. Gill. 1985. Structural and histochemical changes in the salivary glands of Rhipicephalus appendiculatus during feeding. Int. J. Parasitol. 15: 81-100.

Wanchinga, D.M. 1983. The external ultrastructure and biology of the spinose ear tick, Otobius megnini Duges, 1884 (Acarina: Argasidae). Ph.D. Dissertation, Oklahoma State University, Stillwater.

Wanchinga, D.M. and R.W. Barker. 1986. Colonization and laboratory development of Otobius megnini (Acari: Argasidae). J. Econ. Entomol. 79: 999-1002.

Williams, S.R. 1905. The anatomy of Boophilu annulatus Say. Proc. Boston Soc. Nat. Hist. 32: 313-334.

Winston, P.W. and D.H. Bates. 1960. Saturated solutions for the control of humidity in biological research. Ecology 41: 232-237.

Zebrowski, G. 1926. A preliminary report on the morphology of the american dog tick, Trans. Am. Entomol. Soc. 51: 331-369.

APPENDIXES

APPENDIX A

FIXATION PROCEDURE USED TO PREPARE SALIVARY GLAND TISSUE FOR ELECTRON AND LIGHT MICROSCOPIC EXAMINATION

1. Primary fixation in 2% gluteraldehyde buffered with 0.1M sodium cacodylate for two hours.

2. Three 20 minute washes in 0.1M cacodylate buffer.

3. Secondary fixation in 2% osmium tetroxide mixed 1:1 with 0.2M cacodylate buffer for two hours.

4. Three 20 minute washes in 0.1M cacodylate buffer.

5. Dehydration with ethyl alcohol as follows:
    50% ETOH for 20 minutes
    70% ETOH for 20 minutes
    90% ETOH for 20 minutes
    95% ETOH for 20 minutes
    100% ETHOH three times for 20 minutes each.
    Two hours and 20 minutes total.

6. Three 20 minute rinses in propylene oxide.

7. Twelve hours or overnight in 1:1 propylene oxide and Poly/Bed 812 followed by and additional seven to 24 hours in vacuum desiccator.

8. Embedding in pure Poly/Bed and setting in an oven at 60°C for a minimum of 48 hours.

9. For electron microscope observation, sections post-stained for 4 minutes in 5% uranyl acetate in methanol, rinsed in methanol, and dryed. Sections then stained for an additional 4 minutes with lead citrate (prepared by adding 0.03g lead citrate to 10 ml CO_2 free, nucleopore filtered, water and 3 drops of 50% NaOH) in a CO_2 free atmosphere created by moistening NaOH pellets in a petri dish (Venable and Coggelshell 1965).

APPENDIX B

ABBREVIATIONS FOR INTERNAL ANATOMY STUDY

ac. A	type A acinus
ac. B	type B acinus
ad. g. l.	antero-dorsal granular lobe
a. g.	accessory gland
a. lat. div.	antero-lateral diverticulum
a. med. div.	antero-median diverticulum
an.	anus
an. f.	anal fold
ant. d. v. f.	anterior dorso-ventral fold
ant. lat. grp.	antero-lateral tracheal group
ant. med. grp.	antero-median tracheal group
an-myN	anal myosomal nerve
ao.	aorta
a. s. l.	anterior spongy lobe
at.	atrium
b. c.	basis capituli
br.	brain
cam.	camerostome
cc. al.	alimentary caecae
c. f.	capitular foramen
ch.	chelicerae

ch.N	cheliceral nerve
ct. s.	cuticular sac of Gene's organ
c. v.	cervical vagina
cx. 1-4	coxae 1-4
cx. f.	coxal fold
cx. pt. 1-4	coxal projections 1-4
dct.	coxal duct
dor. anast.	dorsal anastomosis
d. ov.	oviduct
ej. dct.	ejaculatory duct
end.	endosternum
end. arm	arm of endosternum
end. bs.	base of endosternum
gen. ap.	genital aperature
gen.' f.	dorso-ventral genital fold
gen. h.	genital hood
gen.N	genital nerve
Gen. org.	Gene's organ
gl.	globules of coxal tubule
gl. cxl.	coxal gland
gl. sal.	salivary gland
gN	gastric nerve
hN 1-4	hemal nerve 1-4
ht.	heart
lat. d. v.	lateral diverticulum
lat. g. l. 1-3	lateral granular lobe 1-3
m. abd. cx. 1-4	abductor muscle of coxae 1-4

m. ant. d. v.	anterior dorso-ventral muscles
m. an.	anal muscle
m. ant.N	nerve to anterior muscles
m. b. c.	muscles of basis capituli
m. cam.	muscles of the camerostome
m. cxl. gl. d. v.	dorso-ventral muscles of coxal gland
m. cxl. gl. lat.	lateral muscles of coxal gland
m. d. c.	depressor muscles of capitulum
med. grp.	median tracheal group
m. e. tch. 1-4	extensor muscles of trochantor 1-4
m. f. tch. 1-4	flexor muscles of trochantor 1-4
m. g. l.	median granular lobe
m. gen.'	dorso-ventral genital muscles
m. gen. i.	inferior genital muscle
m. gen.'N	nerve to dorso-ventral genital muscle
m. gen. s.	superior genital muscle
m. l. c.	levator muscle of capitulum
m. l. c.N	nerve to levator muscle of capitulum
m. l. int. cx. 1-3	lateral intercoxal muscles 1-3
m. m. int. cx. 1-5	mesial intercoxal muscles 1-5
m. mg.	marginal muscle
m. p. a.	post anal muscle
m. p. m.	postero-median muscle
m. post. d. v.	posterior dorso-ventral muscle
m. prtrs. i.	inferior pretransversal muscle
m. prtrs. s.	superior pretransversal muscle
m. p. trs.	post transversal muscle

m. r. ch.	retractor muscle of chelicerae
m. r. ch.N	nerve to cheliceral retractor muscle
m. r. Gen. org	retractor muscle of Gene's organ
m. sp.	spiracular muscle
m. sub. cx. i.	inferior sub-coxal muscle
m. sub. cx. s.	superior sub-coxal muscle
NL	neurolemma
oes.	esophagus
ov.	ovary
p. a. f.	post anal fold
pd. g. l.	postero-dorsal granular lobe
phN	pharyngeal nerve
p. lat. div.	postero-lateral diverticulum
p. lat. grp.	postero-lateral tracheal group
pl-myN	postero-lateral myosomal nerve
p. m. f.	post median fold
p. med. div.	postero-median diverticulum
p. med. grp	postero-median tracheal group
pN 1-4	pedal nerves 1-4
p. s. l.	posterior spongy lobe
pspN	paraspiracular nerve
sal. dct.	salivary duct
sal.N	salivary nerve
s. cx. f.	supracoxal fold
sp.	spiracle
stN	stomodeal nerve
stom.	stomach

s. r.	rectal sac
syn.	synganglion
t. cxl.	coxal tubule
tes.	testis
t. mpg.	malpighian tubule
tN	nerve trunk
t. r.	rectal tube
trans. p. a. g.	transverse post anal groove
tr.	trachae
tr. 1-4	trachae to legs 1-4
tr. br.	trachae to brain
tr. dor. anast.	trachae to dorsal anastomosis
tr. Gen. org.	trachae to Gene's organ
tr. gl. cxl.	trachae to coxal gland
tr. gl. sal.	trachae to salivary gland
tr. or.	oral trachae
tr. ped. 1-3	pedal trachae 1-3
ut.	uterus
v. d.	vas deferens
v. d. c.	terminal coil of vas deferens
v. v.	vestibular vagina

APPENDIX C

ABBREVIATIONS FOR SALIVARY GLAND ULTRASTRUCTURE STUDY

Ac A	Type A acinus
Ac B	Type B acinus
Ba	Type a cell of B acinus
Ba Gr	Type Ba cell granule
Bb	Type b cell of B acinus
BC	Basal cell of type B acinus
Bf	Basal infoldings of cell membrane
BM	Basement membrane
C	Core of a type B cell granule
CC	Central cell
Cn	Canaliculi of interstitial cell
EC	Epithelial cell
ED	Efferent duct
Epc	Epicuticle
Ex	Extracellular space
Gn	Glycogen granule
Hd	Hemidesmosome
IE	Interstitial epithelial cell
LD	Lipid droplet
Lu	Lumen

M	Mitochondria
MD	Main salivary duct
Mv	Microvilli
N	Nerve
Nu	Cell nucleus
PC	Peripheral lamellate cell
PL	Peripheral layer of a type B cell granule
Prc	Procuticle
RER	Rough endoplasmic reticulum
ST	Spiral thread of procuticle
Tr	Trachae

APPENDIX D

ABBREVIATIONS FOR SALIVARY GLAND DEGENERATION STUDY

Ac B	Type B acinus
AV	Autophagic vacuole
Ba	Type Ba cell
Ba Gr	Type Ba cell granule
Bb	Type Bb cell
Bb Gr	Type Bb cell granule
BM	Basement membrane
Cn	Canaliculi of interstitial epithelial cell
CR	Cellular remnant
Ex	Extracellular space
FGr	Free granular material
IB	Inclusion body
IE	Interstitial epithelial cell
MW	Membrane whorl
PL	Primary lysosome
RB	Residual body
SR	Secondary salivary duct

VITA

Kerry Wade Stricker

Candidate for the degree of

Doctor of Philosophy

Thesis: INTERNAL ANATOMY AND SALIVARY GLAND ULTRASTRUCTURE OF THE SPINOSE EAR TICK (OTOBIUS MEGNINI DUGES) WITH NOTES ON WATER VAPOR UPTAKE AND SALIVARY GLAND DEGENERATION

Major Field: Entomology

Biographical:

Personal Data: Born in Wichita, Kansas on March 27, 1965, the son of Judith C. Stricker and Thomas D. Stricker.

Education: Graduated from Woodward High School, Woodward, OK 1983; attended Northwestern Oklahoma State University at Alva, OK from August 1983 to May 1984; Bachelor of Agriculture degree in Entomology from Oklahoma State University, May 1988; Master of Science degree in Entomology from Oklahoma State University, December 1990: completed requirements for the degree of Doctor of Philosophy at Oklahoma State University in December, 1993.

Professional Experience: Graduate research assistant, Department of Entomology, Oklahoma State University, 1988-1993.

Professional Organization: Entomological Society of America.