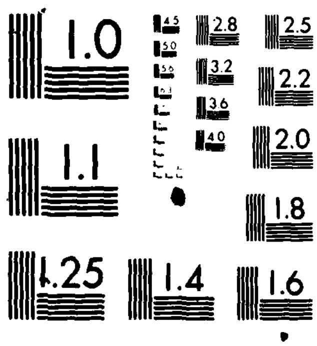
AD-A114 890   OLD DOMINION UNIV NORFOLK VA DEPT OF BIOLOGICAL SCIENCES   F/G 6/1
HORMONAL INTERFERENCE WITH PHEROMONE SYSTEMS IN PARASITIC ACARI--ETC(U)
MAY 82 D E SONENSHINE, P J HOMSHER   N00014-80-C-0546

UNCLASSIFIED   NL

1 of 2

1489



OFFICE OF NAVAL RESEARCH

Contract No. N00014-80-C-0546

Task No. NR 205-039

ANNUAL TECHNICAL REPORT NUMBER 2

HORMONAL INTERFERENCE WITH PHEROMONE SYSTEMS IN PARASITIC ACARINES, ESPECIALLY IXODID TICKS

By

Daniel E. Sonenshine, Ph.D., Principal Investigator
Professor, Department of Biological Sciences
Old Dominion University, Norfolk, Virginia 23508

and

Dr. James H. Oliver, Jr., Professor, Co-Investigator
Acarology Group, Department of Biology
Georgia Southern College
Statesboro, Georgia 30460

and

Dr. Paul J. Homsher, Professor, Co-Investigator
Department of Biological Sciences
Old Dominion University
Norfolk, Virginia 23508

This report was supported in part by the Office of Naval Research,
Microbiology Program, Naval Biology Project,
under Contract No. N00014-80-C-0546, NR 205-039.

OFFICE OF NAVAL RESEARCH

Contract No. N00014-80-C-0546

Task No. NR 205-039

ANNUAL TECHNICAL REPORT NUMBER 2

HORMONAL INTERFERENCE WITH PHEROMONE SYSTEMS IN PARASITIC ACARINES, ESPECIALLY IXODID TICKS

By

Daniel E. Sonenshine, Ph.D., Principal Investigator
Professor, Department of Biological Sciences
Old Dominion University, Norfolk, Virginia 23508

and

Dr. James H. Oliver, Jr., Professor, Co-Investigator
Acarology Group, Department of Biology
Georgia Southern College
Statesboro, Georgia 30460

and

Dr. Paul J. Homsher, Professor, Co-Investigacor
Department of Biological Sciences
Old Dominion University
Norfolk, Virginia 23508

This report was supported in part by the Office of Naval Research, Microbiology Program, Naval Biology Project, under Contract No. N00014-80-C-0546, NR 205-039.

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE		READ INSTRUCTIONS BEFORE COMPLETING FORM
1. REPORT NUMBER 2	2. GOVT ACCESSION NO. AD-A114	3. RECIPIENT'S CATALOG NUMBER 890
4. TITLE (and Subtitle) Hormonal Interference with Pheromone Systems in Parasitic Acarines, Especially Ixodid Ticks		5. TYPE OF REPORT & PERIOD COVERED Annual, May 1, 1981 to April 30, 1982
		6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s) Daniel E. Sonenshine, Principal Investigator, Drs. P.J. Homsher & J.H. Oliver, Co-Investigators		8. CONTRACT OR GRANT NUMBER(s) N00014-80-C-0546
9. PERFORMING ORGANIZATION NAME AND ADDRESS Department of Biological Sciences Old Dominion University, Norfolk, VA 23508		10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS Naval Biology NR 205-039
11. CONTROLLING OFFICE NAME AND ADDRESS Microbiology, Naval Biology Office of Naval Research Arlington, VA 22217		12. REPORT DATE May , 1982
		13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office) ONR Resident Representative George Washington University, 2216 G St. N.W. Washington, D.C. 20037		15. SECURITY CLASS. (of this report) Unclassified
		15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

This document has been approved for public release; its distribution is unlimited.

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

18. SUPPLEMENTARY NOTES

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

Hormones; Edcysteroids; Pheromones; Genital Pheromone(s); Precocene-2; Hyalomma dromedarii; Dermacentor variabilis; Radioimmunoassay

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

Evidence of the existence of (an) ecdysteroid(s) in the ticks Dermacentor variabilis (Say) and Hyalomma dromedarii Koch is presented. Radioimmunoassay demonstrated ecdysteroids in unfed adults and different physiological states of the immatures. X-ray microanalysis demonstrated a 3.6 fold greater amount of chlorine in the pheromone glands of ecdysone treated than untreated H. dromedarii females. Gas chromatographic studies demonstrated a 3.8 fold greater accumulation of 2,6-dichlorophenol in ecdysone treated than untreated feeding H. dromedarii females. In contrast, no difference in 2,6-dichlorophenol content was

Unclassified
SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. Abstract

was observed between ecdysone treated and untreated feeding *D. variabilis* females. Studies to determine whether sex pheromone activity in *D. variabilis* can be affected by administration of ecdysteroids are being continued, but with lower doses.

Study of the ultrastructure of the pheromone glands of developing, mature, feeding and replete female *H. dromedarii* were completed. Comparisons were also made with males.

Studies on the chemical and biological regulation of mating in the genera *Hyalomma* and *Dermacentor* were also completed. Male *Hyalomma dromedarii* and *H. anatolicum excavatum* respond to different DCP concentrations proportional to amounts present in conspecific females. Male *Dermacentor variabilis* and *D. andersoni* are attracted to feeding females of either species but recognition occurs only when copulation is attempted. These findings suggest the presence of a specific genital pheromone serving as a conspecific aphrodisiac to stimulate copulation. Recognition of the female gonopore in the 4 tick species is facilitated by a contact sex pheromone on the ventral body surface, which may be similar or identical to the genital pheromone. These findings suggest that mating in hard ticks may be regulated by more than one pheromone. In the 4 species studied in this project, mating apparently involves 2,6-dichlorophenol and other more specialized mechanisms, including other pheromones, for sexual recognition and copulation

Studies to assess the effects of Precocene-2 on *D. variabilis* were also completed. In addition to the toxic effects reported previously, administration of Precocene-2 to feeding females was found to antagonize feeding and virtually eliminate oviposition. However, unfed females, replete females, and males were unaffected.

Studies to determine the presence and probable physiological role of gonadotrophic hormone in ticks were done. Precocene (P-2) reversal experiments were done by application of exogenous insect juvenile hormone (JH). JH did not reverse the effects of P-2 in these trials. Fumigation of males with Precocene-2 had no observable effect on male reproductive functions.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure		Page

I. Introduction

The overall objectives of this study are concerned with the nature of the presumed hormonal regulation of mating behavior in ixodid ticks. The substantial evidence for hormonal regulation of reproductive processes in insects, including mating behavior, was described in the original project proposal and need not be repeated here. However, no direct evidence of a similar role for hormones in ticks has been reported. Indeed, little evidence concerning the identity and regulatory role of any acarine hormones has been obtained. Consequently, this project was designed to investigate these phenomena, with special emphasis on the role of tick hormones in regulating sex pheromone activity. Specifically, the project aimed to determine (1) whether the sex pheromone system is disrupted by artificially introduced hormones, hormone analogues, or antagonists, (2) whether male mating behavior is altered by these same treatments, (3) whether biosynthesis and/or release of sex pheromone(s) is disrupted or stimulated, and, finally, (4) to develop a data base describing the interrelationships among pheromones and selected, highly active hormones and/or hormone analogues or antagonists which would aid in tick control.

The project was designed and has progressed as a collaborative effort among the participating laboratories: (1) the Medical Zoology Department, US NAMRU-3, Cairo, Egypt, (2) the Department of Biological Sciences, Old Dominion University, Norfolk, Virginia, and (3) the Department of Biology, Georgia Southern College, Statesboro, Georgia. In addition, assistance with work on pheromone chemistry was provided by the Department of Chemistry, State University of New York, Syracuse, New York. Throughout this report, an effort is made to give due credit to the work done at the various collaborating laboratories.

This report is concerned almost exclusively with the results of studies done at Old Dominion University and Georgia Southern College. However, in order to present a coherent report, reference is made to findings done at NAMRU-3, which will be reported in detail separately.

Part I of the report describes evidence of the existence of ecdysteroids in the ticks H. dromedarii and D. variabilis. This report also reviews our findings that indicate that sex pheromone activity in H. dromedarii is stimulated by increased concentrations of one or more ecdysteroids. Part II of the report presents a comprehensive overview of the development, ultrastructure, and physiological processes of the pheromone glands of H. dromedarii. This is the most extensive study of the sequences involved in the development of sex pheromone activity ever reported, beginning with the gland primordium in the nymph and ending with the mated, nearly replete female. Comparisons were also made with various physiological states as well as between males and females. Part III briefly summarizes previously reported evidence of the toxicity of Precocene-2 for development stages of D. variabilis, and describes new evidence indicating an anti-feedant and possible anti-gonadotrophic effect on attached female ticks. Part IV describes evidence of the existence of "genital pheromones" (i.e., mating-stimulant pheromones that guide the sexually excited males to the female gonopore and excite copulation). Different mechanisms for discriminating conspecific and heterospecific mates in the two genera are described and their implications for understanding the regulation of mating in the Ixodidae are discussed. Finally Part V summarizes the results of a program of studies relating to the possible role of juvenile hormone in D. variabilis adults, using Precocene-2 as an antagonist of gonadal activity.

II. Hormone - Pheromone Interactions in Ticks. The Role of Ecdysteroids in Stimulating Sex Pheromone Activity in *Hyalomma dromedarii* With Evidence of the Existence of Ecdysteroids in *H. dromedarii* and *Dermacentor variabilis*.

Introduction

Zooecdysteroids, of which at least 17 are known (Hetru and Horn, 1980), are a group of steroid hormones that probably occur in all arthropods. Similar compounds--phytoecdysteroids--also occur in plants. Best known for their role in regulating molting, they have also been found to regulate vitellogenesis (Borovsky, 1981; Hagedorn et al., 1975), diapause termination (Bradfield and Denliger, 1980), as well as a variety of developmental processes in internal tissues (Ryerse, 1980). Much less is known about ecdysteroids in ticks. The first demonstration of natural ecdysteroids in ticks was that reported by Delbecque et al. (1978) who found both alpha-ecdysone and 20-hydroxyecdysone in an extract of nymphal *Amblyomma hebraeum* Koch. Other evidence has shown that ticks' response to exogenous ecdysteroids includes accelerated molting of nymphs and supermolting of adults (Mango, 1978; Mango et al., 1976) and termination of diapause (Wright, 1969; Sannasi and Subramoniam, 1972).

Ecdysteroids may also be implicated in the regulation of mating behavior. Hormonal regulation of this phenomenon has been reported in diverse insects (Barth, 1961, 1962; Endo, 1973) and some form of hormonal or neuroendocrine triggering of sex pheromone activity is probably essential in all arthropods. In females of several species, it appears to involve the ovaries (Endo, 1973), although direct stimulation of sex pheromone activity by juvenile hormones (JH) from the corpora allata is also known. Ovarian production of alpha-ecdysone, first described by Hagedorn et al. (1975) for mosquitoes, has now been shown to occur in numerous other species (Riddiford and Truman, 1978). Endo (1973) alluded to ovarian stimulation of female mating behavior, presumably mediated by some ovarian hormone. In light of more recent discoveries, this hormone may be an ecdysteroid. Little additional evidence of ecdysone regulation of mating behavior in insects is available. In crabs, however, Kittredge and Takahashi (1972) noted that males seize and hold pre-molt females for subsequent copulation when stimulated by crustecdysone.

This study was done to determine whether sex pheromone activity is affected by ecdysteroids in the camel tick, *Hyalomma dromedarii* Koch, and the American dog tick, *Dermacentor variabilis* (Say). We also wanted to determine whether ecdysteroids occur in these ticks, and whether changes in the ecdysteroid titer might be correlated with physiological events in the pheromone glands.

Materials and Methods

Ticks. The American dog tick, *D. variabilis*, was colonized and reared as described previously (Sonenshine et al., 1977). The camel tick, *H. dromedarii*, was colonized with specimens from the Medical Zoology Department, U.S. NAMRU-3, Cairo, Egypt (HH# 59,723). Specimens from this source (U.S. APHIS permit no. 9433) were allowed to feed on laboratory rabbits. Ticks (both species) were held in an Aminco Climate Lab® environmental chamber at 27 ± 0.5° C and 90 ± 2% RH during their non-parasitic periods.

Bioassays. These were done as described by Sonenshine et al. (1976) and Khalil et al. (1981), but modified as described below. Briefly, sexually active (SA) males of each species attached to hosts for at least 8 days were detached and tested for their ability to respond to confirmed attractive virgin (CAV) females of the same species. Males with deformities or which failed to respond to females were discarded. Unfed females which received injections of ecdysone as nymphs and untreated controls were restrained by embedding their mouthparts in paraffin and assayed with SA males. Each male was allowed 3 trials and each female was assayed with 10 separate males. The bioassays were also done on rabbits after the females had fed 7 days, as described in the previously cited publications.

Tick Extracts. The materials used for radioimmunoassay (RIA) are listed in Tables 1 and 2. Extracts of each population were prepared by

Table 1. Sex pheromone, 2, 6-dichlorophenol[1], in the ticks *H. dromedarii* and *D. variabilis*, in relation to treatment with beta-ecdysone[2].

Species and Life Stage	Physiological State	No. Ticks in Sample	Treatment	Amount DCP ng/tick
H. dromedarii				
nymphs	15 days post-engorgement	80	none	0.00
females	unfed, 2-3 wks. post-eclosion	100	none	3.46
females	"	50	10 ng ecd	16.00
females	fed 7 days	65	1% saline	5.57
females	fed 7 days	75	10 ng ecd	21.30
females	fed 7 days	21	100 ng ecd	4.47
males	unfed, 2-3 wks. post-eclosion	70	none	0.00
males	"	106	10 ng ecd	3.90
D. variabilis				
females	unfed, 2-3 wks. post-eclosion	117	none	1.87
females	"	78	10 ng ecd	1.68

[1] Amounts determined by gas liquid chromatography (see text for details).

[2] Beta-ecdysone or 1% saline administered to engorged nymphs on the day of drop off (see text for details).

Table 2. Chlorine content of microscopic areas of the foveal glands of H. dromedarii as determined by X-ray microanalysis (scanning electron microscope).

Chlorine Content (Percent of Osmium or Gold)

Unfed Females				Fed Females		Unfed Males			
Spec. No.	B' Ecd.	Spec. No.	Control	Spec. No.	Control	Spec. No.	B' Ecd.	Spec. No.	Control
1	4.40	1	1.18	1	1.11	--	--	--	--
2	3.50	2	1.55	"	0.80	--	--	--	--
3	5.59	--	--	"	0.73	--	--	--	--
4	5.46	--	--	--	--	--	--	--	--
5	7.02	--	--	--	--	--	--	--	--
6	3.37	3	1.80	2	1.50	1	0.32	1	0.02
7	4.06	"	1.38	3	0.21	"	0.31	--	--
8	2.85	4	0.49	--	--	2	0.00	--	--
9	3.84	5	1.06	--	--	--	--	--	--
$\overline{X}$ ±	4.45		1.24		0.87		0.21		--
S.D. ±	1.33		± 0.45		± 0.48		± 0.18		

homogenizing the tick material with a Ten Broeck glass homogenizer (American Scientific Products, Columbia, MD) in methanol and water (65:35, v/v). The mixture was freeze-thawed, centrifuged, and washed 2 X with this same solvent. The precipitate was discarded, the supernatant evaporated, and the dried sediment re-extracted 3 X with 100% reagent-grade methanol (J.T. Baker Co., Phillipsburg, NJ) with vigorous shaking. The methanol extract was centrifuged to remove extraneous material, dried, re-extracted 4 X with 50% methanol/benzene, again with 25% methanol/benzene, and finally, reconstituted in 100% ethanol. The final extract was stored (-20° C) until needed for assay. Samples of these extracts were also assayed by High Performance Liquid Chromatography as described below, the putative ecdysteroids were collected, and the materials were assayed by RIA.

The tick material used for gas liquid chromatography studies is listed in Table 3. The tick material was extracted in double-distilled hexane Omni-solv (Krackler Chemical Co., Albany, NY) as described previously (Sonenshine et al., 1977; Sonenshine et al., in press:a).

Radioimmunoassay (RIA). The Horn I-1 anti-ecdysone antiserum used in these tests was obtained (as a lyophilized powder) from Dr. J.D. O'Connor, University of California, Los Angeles, was reconstituted in distilled water, and stored frozen (-65° C) until needed. Aliquots of the tick extracts (methanol) described above were dried under nitrogen and reconstituted in 100 µl borate buffer. These were mixed with 50 µl aliquots (also in borate buffer) of tritiated alpha-ecdysone (16,600 DPM) (New England Nuclear Corp., Boston, MA., 80 Ci/mmol) and 50 µl of antiserum. The mixture was incubated for 24 hours at room temperature. After cooling (4° C), 200 µl of a saturated ammonium sulfate (SAS) solution was added to each tube. The proteins were allowed to precipitate overnight and the solution was vortexed and centrifuged at 2500 rpm. After centrifugation, the supernatant was removed, the precipitate resuspended in fresh borate buffer:SAS solution (1:1), and the mixture was centrifuged again. The supernatant was removed and the precipitate dispersed in 25 µl 100% ethanol plus 600 µl RIA Fluor

(New England Nuclear Corp., Boston, MA) in minivials. Duplicate samples were prepared for each extract and each dilution of that extract. Standards were prepared in a similar manner using known quantitites ranging from 25 pg/100 μl to 1000 pg/100 μl of authentic alpha-ecdysone (Sigma Chemical Co., St. Louis, MO). Radioassay was done with a Beckman Model LS 250 liquid scintillation counter. Counting efficiency after quench correction (external standard method) was 29%.

The purity of the ^3H alpha-ecdysone was confirmed by thin-layer chromatography (TLC) before use in the various tests (see below).

Table 3. Ecdysteroid concentrations in *Hyalomma dromedarii* adults during different periods of adult activity.

Age Post-Emergence and Physiological State	No. in Sample	Ecdysteroid Content ng/tick	Wt. (mg)
day 0, unfed female	20	1.34 ± 0.21	0.06 ± 0.009
day 15-20, unfed female	20	1.20 ± 0.10	0.08 ± 0.007
day 15-20, unfed female from B'-ecdysone nymph	10	42.94 ± 9.25	2.87 ± 0.62
day 21, partially fed female	10	1.45 ± 0.44	0.015 ± 0.005
day 0, unfed male	10	0.87 ± 0.39	0.04 ± 0.019
day 15-20, unfed male	10	0.48 ± 0.14	0.03 ± 0.010
day 15-20, unfed male Beta-ecdysone nymph	200	2.03 ± 0.51	0.14 ± 0.035

Electron Microscopy and Elemental Analysis. Following inoculation of 10 ng beta-ecdysone into engorged nymphs, the foveal glands of the unfed females were excised following ecdysis (age, 5-7 days) and fixed in cold 4% glutaraldehyde (with 0.1 M s-collidine buffer). The identity of the foveal glands was verified with the aid of a Wild dissecting microscope, and fixation continued for two or three hours. Fixation was followed by three 15-minute washes in 0.1 M s-collidine buffer at 15-minute intervals. Tissues were post-fixed for two hours in 1% OsO_4 in 0.1 M s-collidine buffer (4° C, pH 7.4). After the three washes in s-collidine buffer, the foveal glands were dehydrated through a graded series of ethanol solutions to pure ethanol, cleared in propylene oxide, infiltrated overnight in 1:1 propylene oxide:Epon 812, and embedded in Epon. Following hardening, thick and thin sections were cut on an LKB Ultramicrotome III (LKB Instruments, Inc., Rockville, MD). Thick sections were stained for 30 seconds in a modified azure-methylene blue stain (Dawes, 1971). Thin sections (0.1 um) were mounted on uncoated copper grids, and stained with saturated uranyl-acetate and lead citrate. Controls consisted of unfed females, never previously treated with ecdysteroids, prepared in a similar manner. The stained sections of both experimentally treated and control specimens were viewed with a Hitachi HU-11B Transmission Electron Microscope. Measurements and estimates of secretory droplet content were made with an Electronics Graphics Calculator (Numonics Corp., Philadelphia, PA).

For elemental analysis, excised foveal glands from treated and untreated ticks were excised, fixed, and dehydrated as described above, dried in a Denton DCP-1 critical point dryer (Denton Vacuum Systems, Cherry Hill, NJ) and coated with gold-palladium in a Technics Hummer V Sputter Coater. The coated specimens were mounted on metal stubs and examined with a Cambridge Stereoscan Model 150 scanning electron microscope. Following verification of the identity of the pheromone glands, elemental analysis for chlorine was done with an Edax energy-dispersive analyzer Model 707B and Texas Instruments Silent ASR data terminal for intervals of 100 seconds. Other specimens were examined with a JOEL Co. JSM U-3 scanning electron microscope and Edax model 9100 analyzer, also for intervals of 100 seconds.

Autoradiography. Foveal glands, brain, heart, salivary glands, reproductive organs, midgut, integument, and haemolymph samples were collected from adult males and females (26 of each) previously injected (as engorged nymphs) with 0.06 uCi 3H α-ecdysone (80 mCi/mol). Frozen sections were prepared from these tissues as described previously (Sonenshine et al., 1981), and coated with Kodak NTB-3 nuclear emulsion. After 21 days of exposure at 4° C, the coated sections were developed, stained with haematoxylin and eosin, and examined with a Nikon Optiphot microscope for evidence of silver grain accumulations. Photographs were taken with the Model HFM-35A camera attached to the microscope.

Chemistry. To determine the amounts of sex pheromone in the ticks, hexane extracts of tick material were passed through pre-cleaned Sep Paks® (Waters and Associates, Inc., Milford, MA) containing Florasil® to separate the 2,6-dichlorophenol from other organic materials. The Sep Paks were rinsed with fresh hexane (3 X) to release residual non-polar molecules, then rinsed with a mixture of 1:1, diethyl ether:petroleum ether to release the phenol. Further extraction with diethyl ether partitioned the phenol into the ether phase. Recovery with this technique is approximately 100%, as confirmed by tests with known standards. Aliquots of the final extract were injected into a Perkin-Elmer Model Sigma 3 gas chromatograph (Rockville, MD) using Hamilton Model 701-RN microliter syringes with Chaney adapter and 26 S gauge needles. The gas chromatograph was equipped with a constant-current electron capture (EC) detector with a ^{63}Ni foil to detect halogenated compounds. Detector and injection port temperatures were 250° C, the column was 200° C, and the flow rate for the carrier gas (N_2) was 75 ml/min. The column was a 0.6 cm O. D. × 56 cm, long, curved glass tube packed with Tenax 80/100 absorbent. Recordings and quantification of compounds were made with a Shimadzu C-RIA Chromatopac Multiprocessor (Rockville, MD) connected to the gas chromatograph.

Identification of 2,6-dichlorophenol in the extracts was done by comparing sample compound and authentic 2,6-dichlorophenol retention times, and by coinjection of the authentic standard and observing that it co-chromatographed with the sample compound.

The chemical characteristics of the ecdysteroids in the tick extracts were determined by TLC and high pressure liquid chromatography (HPLC). TLC was done on IB-2 thin-layer silica gel plates (Baker Chemical Co., Phillipsburg, NJ) in chloroform:methanol (4:1, v/v). HPLC was done with a Waters System comprising a Model 6000 and M45 pumps, model 720 system controller, 730 data module, Model u6K injector, and a model 440 uv absorbence detector. The column was a model 100 RCM, 8 mm I.D., packed with 10 μm MicroBondapax® C-18. The solvent system was methanol:water:acetic acid (65:35:1). Flow rate was 1.5 mil/min. The ecdysteroids in the tick extracts were identified by comparison of their retention times with authentic insect ecdysones and by radioimmunoassay of the specific isolates. Samples consisted of 500 μl of an original 1.0 ml extract of 1000 nymphs, dried, and reconstituted in reagent grade methanol; 20 μl aliquots were assayed.

Statistical Tests. Differences between the percentages of treated- and control-tick behavioral responses were tested by the t-test with Arcsin transformation. Differences in other parameters (e.g., molting time, chlorine content) were evaluated by the t-test (Sokal and Rohlf, 1969).

Results

Effect of Beta-Ecdysone on Molting. Molting of beta-ecdysone-inoculated nymphs was not accelerated when compared with controls. The molting interval (in days) for the 10 ng- and 100 ng-treated *H. dromedarii* nymphs was 20.28 ± 2.28 (n = 152) and 19.41 ± 1.73 (n = 91), respectively, while that of the untreated controls was 20.36 ± 2.14 (n = 65); controls injected with 1% saline were 21.67 ± 2.16 (n = 230). These differences are not significant ($p > 0.05$). Similarly, molting of *D. variabilis* nymphs was not accelerated by the 10 ng beta-ecdysone injections. The molting interval (in days) for the ecdysone treated nymphs was 16.35 ± 2.15 (n = 200) as compared to 16.84 ± 1.55 (n = 55) for the saline-treated controls and 16.34 ± 1.97 (n = 180) for untreated controls. Again, these differences are not significant ($p > 0.05$).

Effect of Beta-Ecdysone on Sex Attractant Behavior. Mature unfed female *H. dromedarii* that emerged from 10 ng beta-ecdysone-injected nymphs attracted SA males significantly more often (23.3%) than untreated controls ($p > 0.01$); 9 of the females were attractive to at least 1 of the males, and 5 were attractive to 3 or more test males. The attractive females responded to the courting males by elevating their opisthosomas. Males were allowed to remain in copula while these females deposited spermatophores. When allowed to feed on rabbits, the beta-ecdysone treated females fed normally and attracted SA males as readily as the untreated controls. However, when the tests were repeated with unfed females that received 100 ng beta-ecdysone/nymph, no difference in sex attractant activity was found between the treated females and the untreated controls (4.7% and 3.3%, respectively; $p > 0.05$).

Mature unfed female *D. variabilis* that emerged from beta-ecdysone treated nymphs were not significantly more attractive to SA males than the untreated controls (8.0% and 8.7%, respectively; $p > 0.05$).

Effect of Beta-Ecdysone Treatment on Sex Pheromone Content. The sex pheromone content of *H. dromedarii* appears to have been increased significantly by the 10 ng treatment but not by the 100 ng treatment. Table 1 summarizes the amounts of 2,6-dichlorophenol (DCP) found in treated and control specimens. The amount found in the 10 ng beta-ecdysone treated unfed females was 4.6 X that of the untreated controls. Similarly, the amount found in the beta-ecdysone treated part of fed females was 3.8 X the amount found in the saline-treated part of fed female controls. However, the amount found in the ticks that received 100 ng beta-ecdysone/tick was approximately the same as in the controls. Another interesting finding is the presence of substantial amounts of DCP in the 10 ng beta-ecdysone treated males. However, no DCP was found in the untreated males. No difference in DCP content was found between 10 ng beta-ecdysone-injected and untreated control *D. variabilis* females.

Evidence of increased sex pheromone content in beta-ecdysone treated *H. dromedarii* was also found when the chlorine content of their pheromone glands was assayed by X-ray microanalysis (Table 2). In addition, to the greatly increased chlorine content of the treated females as compared to the controls, it is noteworthy that significant chlorine accumulations were also found in one of the two beta-ecdysone treated males.

Other Evidence of Beta-Ecdysone Effects on the Pheromone Glands. The results of autoradiographic analysis revealed exceptionally intense accumulations of silver grains, too numerous to count, over sections of the foveal glands (Figure 1) of both unfed female and unfed male *H. dromedarii* that emerged from 3H α-ecdysone treated nymphs. No significant accumulations of silver grains were observed with any other organ or tissue, including the heart or hemolymph where such accumulations might be expected, except for occasional grain clusters over cells of the midgut. When examined with the electron microscope, the pheromone gland cells of unfed female *H. dromedarii* that emerged from 10 ng beta-ecdysone treated nymphs exhibited extensive vesicular disruption and numerous free neutral lipid secretory droplets; extensive vacuolation and droplet-free areas were noted (Figure 2). In contrast, the pheromone gland cells of unfed female controls exhibited normal vesiculation and membrane-bound secretory droplets (Figure 3). The pheromone glands of beta-ecdysone treated female *D. variabilis* also exhibited vesicular disruption but the abundance of neutral lipid secretory droplets did not appear to have been reduced (Figure 4) when compared with untreated controls (Figure 5).

Ecdysteroid Titers in D. variabilis and H. dromedarii. Radioimmunoassay revealed evidence of ecdysteroids in all life stages and/or physiological states of the ticks that were examined (Tables 3 and 4). In *D. variabilis*, the lowest titers were found in the unfed larvae, and unfed nymphs ranging from 1.15 pg/larva to 1.71 pg/nymph. A tremendous increase in ecdysteroid titer was found in the nymphs, more than 40 times greater than that found at any stage in larval development. The ecdysteroid titer declined precipitously in the unfed adult, to only 37.0 pg/female and only 38.0 pg/male (Table 4).

Natural ecdysteroid titers were much higher in *H. dromedarii* than in comparable life stages of *D. variabilis* (Table 3). Moreover, the titer for 2- to $2^{1}/2$-week-old unfed females, 1.24 ng/tick, is approximately 2.6 X greater than in the unfed males of the same age. Much larger amounts of

Table 4. Ecdysteroid concentrations in *Dermacentor variabilis* during different periods of tick development.

Life Stage	Days Post-Ovip./Eclosion/ or Feeding	No. in Sample	Ecdysteroid Conc. pg/tick	Ecdysteroid Conc. pg/mg Tick Tissue
embryos	0	14,659	5.22 ± 2.51	68.63 ± 32.99
"	7	15,077	8.66 ± 5.46	113.84 ± 71.78
"	14	15,797	81.16 ± 13.64	1066.89 ± 0.89
unfed larvae	21	111,423	1.15 ± 0.31	34.66 ± 9.34
engorged larvae	0	4,000	12.56 ± 3.68	24.64 ± 7.22
" "	2	4,000	23.52 ± 8.92	49.49 ± 18.77
" "	5	4,000	19.33 ± 4.94	44.05 ± 11.26
" "	8	4,000	13.19 ± 4.79	32.56 ± 11.83
unfed nymphs	21	7,595	1.71 ± 1.20	11.70 ± 8.21
engorged nymphs	0	100	21.32 ± 7.02	1.41 ± 0.46
" "	5	100	394.74 ± 156.56	34.57 ± 10.33
" "	10	100	973.50 ± 297.69	85.25 ± 26.07
" "	15	100	822.93 ± 296.41	77.42 ± 27.88
unfed females	0		to be done	to be done
" females	15	20	37.00 ± 0.013	"
" males	0		to be done	"
" males	15	30	38.00 ± 0.003	"

ecdysteroid were found in the beta-ecdysone treated adults than the untreated controls. The increase in the beta-ecdysone treated unfed female is especially significant since only 10 ng was injected. Consequently, most of the titer observed appears to represent ecdysteroid synthesized by the ticks in response to these injections. The amount present in the beta-ecdysone treated unfed males is also higher than in the unfed controls, but much less than the 10 ng originally injected.

Assay of the D. variabilis extracts (10 days post-engorgement) by HPLC revealed the presence of at least 3 ecdysteroids, which reacted in the RIA system. These compounds had retention times of 2.52, 2.93, and 4.21 minutes. All 3 compounds were distinctly different from either α or B-ecdysone, which had retention times of 3.11 and 3.85 minutes. Three other putative steroids were also observed, but these failed to react in the RIA.

Thin-layer radiochromatograms of extracts of hemolymph from H. dromedarii adults that emerged from ^{3}H α-ecdysone treated nymphs revealed peaks of radioactivity corresponding to unknown fractions at the origin, α-ecdysone, and a small peak corresponding to B'ecdysone. Synganglion, ovary, hemolymph, and foveal glands exhibited the highest accumulations of ecdysteroids (α and B only), respectively, in the female; hemolymph, synganglion, and accessory glands had the highest accumulations in the male. Radiochromatograms of extracts of these tissues revealed substantial peaks at approximately Rf 0.55 and 0.95, neither of which corresponded to α or B-ecdysone.

Discussion

Ecdysterone (20-hydroxyecdysone, or beta-ecdysone), when administered in physiological doses, appears capable of stimulating sex attractant pheromone activity in H. dromedarii. The dominant effect appears to be increased pheromone production, as demonstrated by the great increase in DCP content in treated ticks. DCP even accumulated in the males, which normally lack this compound or exhibit only trace amounts (Sonenshine, unpublished). The ecdysteroid concentration also increased greatly, and this increase may have induced onset of DCP secretion in unfed H. dromedarii females. Female (unfed) attractiveness to SA males increased three-fold, and changes characteristic of pheromone-secreting ticks were evident in the foveal glands (Figure 2).

The frequency of these events is much greater and more consistent than the occasional instances of sex attractant activity (Khalil et al., 1981) that may occur in very large unfed females of H. dromedarii. These effects were observed only with the 10 ng (2.2×10^{-5} M) doses; much higher doses (i.e., 100 ng) resulted in abnormally high mortality. Presumably, the neurohumoral range or "window" in which ecdysteroids may affect physiological events may be very limited.

Other evidence of sex pheromone responsiveness to ecdysteroids is suggested by the autoradiographs indicating accumulation of tritium-labelled substances on or in these glands. One possible explanation of these findings is that the foveal glands have specific receptors which bind ecdysteroids. However, the subsequent events that might translate the ecdysteroid accumulation into sex pheromone activity are unknown.

Treatment of D. variabilis nymphs with beta-ecdysone did not induce an increase in DCP content in the adults, and unfed females emerging from treated nymphs remained unattractive to SA males. However, the ultrastructure of the pheromone glands of treated females was more characteristic of feeding individuals (Figure 4) than of fasting females (Figure 5). Thus, although the results are ambiguous, the possibility that the beta-ecdysone treatment did affect pheromone gland activity cannot be excluded. It is possible that the 10 ng dose used with this species was excessive, and the effects could not be expressed (note the relatively low natural ecdysteroid concentrations in the adults of this species).

The results of the radioimmunoassays indicate the presence of ecdysteroid material in H. dromedarii and D. variabilis. This does not necessarily imply that the ecdysteroid material found in the tick represents alpha or beta-ecdysone, or that it represents a single molecule. In fact, the radiochromatograms suggest the presence of one or more ecdysteroid molecules or metabolites and by-products of ecdysteroid synthesis. The latter may affect the RIA if they are very abundant. Other evidence of the presence of different ecdysteroids in these ticks was indicated by HPLC. Three steroids, none of which co-chromatographed with the alpha or beta-ecdysone standards, reacted in the RIA system. Competitive binding of other, nonecdyeroid steroids is unlikely (Borst and O'Connor, 1972), though some binding of ecdysteroid metabolites may occur (Reum and Koolman, 1979). However, the Horn antiserum used in this study exhibited the highest specificity for ecdysone of 3 different antisera evaluated, as well as being the most sensitive (Reum and Koolman, 1979). The former finding is especially important, increasing the probability that the RIA-sensitive material in the tick extracts is ecdysteroid in nature.

Considerable caution is required when interpreting the results of the radioimmunoassay. The amounts of ecdysteroids reported in this study must be regarded as crude estimates. Precise values can only be obtained with the homologous antigen in a radioimmunoassay, or by use of cross-reaction factors with other known ecdysteroids (Reum and Koolman, 1979). In the present case, the identity of the ecdysteroid(s) is unknown. Moreover, ecdysteroid metabolites may exhibit considerable affinity for the antibodies and lead to some over-estimation of total content (Borst and O'Connor, 1972). Consequently, the results can only be represented as the sum of all ecdysteroids that may be present.

Summary

Evidence of the existence of (an) ecdysteroid(s) in the ticks *Dermacentor variabilis* (Say) and *Hyalomma dromedarii* Koch is presented. Radioimmunoassay demonstrated ecdysteroids in unfed adults and different physiological states of the immatures. Ecdysone inoculated into engorged nymphs remained after molting in amounts exceeding that of untreated controls. Evidently, the inoculated ecdysone was not totally degraded. When nymphs were injected with ^{3}H-ecdysone, autoradiography demonstrated unusual accumulations of silver grains over adult foveal glands, but not over other organs and tissues. Following molting, a significantly higher percentage of ecdysone treated unfed *H. dromedarii* females attracted sexually active males than did the untreated females. X-ray microanalysis demonstrated a 3.6-fold greater amount of chlorine in the pheromone glands of ecdysone treated than untreated *H. dromedarii* females. Gas chromatographic studies demonstrated a 3.8-fold greater accumulation of 2,6-dichlorophenol in ecdysone treated than untreated feeding *H. dromedarii* females. In contrast, no difference in 2,6-dichlorophenol content was observed between ecdysone treated and untreated feeding *D. variabilis* females. Ultrastructural studies revealed substantial disruption of secretory vesicles and release of secretory droplets in the cytoplasm of the pheromone glands of ecdysone treated unfed *H. dromedarii* females.

III. The Development, Ultrastructure, and Activity of the Foveal Glands and Foveae Dorsales of the Camel Tick, Hyalomma dromedarii Koch.

The paired, lobulated foveal glands were reported to serve as the female sex pheromone glands in Dermacentor variabilis (Say) and Dermacentor andersoni Stiles (Sonenshine et al., 1977). Foveae and, presumably, foveal glands also occur in other species of metastriate Ixodidae. However, little is known of their structure or function in most of these species. Foveal glands, similar in appearance to those described in Dermacentor spp., were also reported in both males and famales of Amblyomma maculatum Koch (Axtell and LeFurgey, 1979), even though the sex attractant, 2,6-dichlorophenol, is produced only by the females (Kellum and Berger, 1977).

The camel tick, Hyalomma dromedarii Koch, is widely distributed in desert, semi-desert, steppe, and savanna biotopes of the Paleartic, Oriental, and Ethiopian Faunal Regions. This large, hardy acarine is an opportunistic parasite that frequently infests camels, cattle, horses, sheep, goats, dogs, and other domestic animals as well as various burrow-inhabitating mammals and other wildlife (Hoogstraal, 1956). It is a vector of important arboviruses (e.g., Crimean-Congo hemorrhagic fever, Dera Ghazi Khan, Dhori, and Tettnang) (Hoogstraal, 1973), rickettsiae (Rickettsia conorii and Coxiella burnetti) (Hoogstraal, 1967), and protozoan pathogens (Theileria annulata, T. dispar, and Ehrlichia bovis) (Hoogstraal, 1956; Uilenberg, 1976; Barnett, 1977; and Smith and Ristic, 1977).

Little is known about the regulation of mating in H. dromedarii. Khalil et al. (1981) demonstrated the role of a sex pheromone, possibly 2,6-dichlorophenol, which they associated with the foveae dorsales. However, the presence of 2,6-dichlorophenol or foveal glands in the ticks themselves was not determined.

The morphology of the sex pheromone glands (foveal glands) has been described in detail in only one species, D. variabilis (Sonenshine, et al., 1981), although somewhat similar glands occur in D. andersoni (Vernick et al., 1978). It is not clear whether these findings are applicable to H.

dromedarii (i.e., whether they are representative of all ixodid ticks). Virtually nothing is known of the cycle of development or physiological changes in the sex pheromone glands of ixodid ticks.

This study describes the morphology of the foveae dorsales and foveal glands (sex pheromone glands) of H. dromedarii females, including changes that occur during development, maturation, feeding, and mating. Comparisons between the glands in males and females are also described. The occurrence of the general ixodid sex pheromone, 2,6-dichlorophenol, in specific physiological states of these ticks is reported. Finally, contrasts between the sex pheromone systems in insects and ticks are compared.

Materials and Methods

Ticks. Hyalomma dromedarii originating from engorged females collected from camels in the Imbaba camel market, Giza, Egypt (HH 59,723) were colonized in the NAMRU-3 Medical Zoology Laboratories t 28°-29° C and 75% RH. Ticks were confined in metal capsules affixed to laboratory rabbits, Oryctalagus cuniculi, for feeding. Other ticks from the same source were transferred to the Department of Biological Sciences, Old Dominion University (U.S. APHIS permit no. 9433), fed on laboratory rabbits as described above, and held in an Aminco Aire Climate Lab environmental chamber (American Instrument Co., Silver Spring, MD) between feedings. The ticks used in this study included unfed larvae (of undetermined age); unfed nymphs (removed from rabbits before they could reattach); engorged nymphs of varying ages post-drop; unfed females collected on the day of eclosion, hereafter termed young females; unfed males and females collected 2 to 4 weeks post-eclosion, hereafter termed mature adults; partially fed virgin males and females forcibly removed from rabbits 7 days post-attachment; and mated females forcibly removed from rabbits 2 days post-insemination during the period of rapid engorgement (9 days of continuous attachment).

Histology. Replete nymphs, 5 days post-drop, were fixed in 10% neutral buffered formalin, dehydrated, embedded in paraffin (MP 56°-58° C), sectioned at 10 um, and stained with Mayer's haematoxylin and Eosin Y, in accordance with routine histologic procedures (Humason, 1972). Stained sections containing primordia of the foveal glands were viewed with a Nikon Optiphot compound microscope and photographed with a Nikon HFM 35-mm automated camera attached to the microscope. Drawings were made directly from the photographic image.

Ultrastructure. For examination of the foveae dorsales (external view) with the scanning electron microscope (SEM), unfed larvae, unfed nymphs, and the dorsal body cuticle of males and females were preserved in 70% ethanol, dried, and mounted on aluminum stubs (see below). For examination of the foveal glands (internal view) with the SEM, the tissues were removed from mature unfed males and females. Before dissection, each tick was perfused with cold (4° C) 4% glutaraldehyde buffered in 0.1 M s-collidine, pH 7.4, by injection using a 1-cc Tuberculin syringe and 27-gauge needle. The excised tissues were transferred to fixative for 2-3 hours, washed 3 X in 0.1 M s-collidine buffer, and post-fixed for 2 hours in 1% OsO_4 in 0.1 M s-collidine buffer (4° C, pH 7.4). After 2 washes of 5 minutes each in s-collidine buffer the tissues were dehydrated through a graded series of ethanol solutions to pure ethanol, and dehydrated further in a Denton DCP-1 critical point dryer (Denton Vacuum Systems, Cherry Hill, NJ). The dehydrated whole ticks and dehydrated excised tissues were mounted on aluminum stubs with carbon conductive paint (E.F. Jeld, Burlington, MA), coated with 100-200 Å of gold-palladium in a Technics Hummer V sputter-type vacuum evaporator (Technics Co., Alexandria, VA) and viewed with a JOEL Co., Model JSM-35 scanning electron microscope.

For examination of the foveal glands with the transmission electron microscope (TEM), the tissues were removed from the ticks as described above, and the presence of glands verified microscopically. Fixation and osmication were also carried out as described above. Following dehydration in ethanol, the tissues were cleared in propylene oxide, infiltrated overnight with 1:1 propylene oxide:Epon 812, and embedded in Epon. Following hardening, thick (1 um) and thin (600 Å) sections were cut using an LKB Ultramicrotome III (LKB Instruments, Inc., Rockville, MD). Thick sections were stained for 3 seconds in a modified azure-methylene blue stain (Dawes, 1971), viewed and photographed using the Nikon Optiphot light microscope and camera attachment. Thin sections were mounted on uncoated copper grids, and stained with saturated uranyl-acetate and lead citrate. The stained sections were viewed using a Hitachi HU-11B transmission and electron microscope and a Phillips Model 301 Transmission Electron Microscope. Measurements of ultrastructural components were made with an Electronics Graphics Calculator (Numonics Corp., Philadelphia, PA).

Elemental Analysis for Chlorine. To determine the presence of chlorine accumulations, the foveal glands of unfed mature females, partially fed virgin females, and unfed, mature males were excised in cold 4% glutaraldehyde buffered with 0.1 M s-collidine, and processed as described above for SEM studies. Following dehydration in the critical point dryer, mounting, and coating, the foveal glands were viewed with the scanning electron microscope. Most specimens were examined with a Cambridge Stereoscan Model 150 SEM at an operating voltage of 15 KV. X-ray microanalysis for chlorine was done with an EDAX energy dispersive X-ray analyzer Model 707B and Texas Instrument Co. Silent ASR data terminal for intervals of 100 seconds. Other specimens were examined with a JOEL Co. JSM U-3 scanning electron microscope and EDAX model 9100 X-ray analyzer, also for intervals of 100 seconds.

Gas Chromatography. To determine the amounts of 2,6-dichlorophenol (hereafter termed DCP) present in the different tick life stages, a sample of 100 engorged nymphs (15 days post-dropping), 100 unfed young females, 100 unfed, mature females, 100 unfed, mature males, and 100 virgin, feeding females were placed in cold (-30° C) reagent-grade, double-distilled hexane (Omnisolv, Krackler Chemical Co., Albany, NY). The samples were held in flame-sealed glass ampoules. The sealed samples were subjected to repeated cycles of disruption with ultrasonic sound (Branson Sonic Power Co., Danbury, CT; power setting no. 9), freezing in liquid nitrogen, and thawing. The ampoules were opened, and the liquid contents extracted with Flourasil® containing Sep Paks (Waters & Associates, Inc., Milford, MA) to separate the DCP from other organic materials. The Sep Paks were rinsed with fresh hexane (3 X) to release residual non-polar molecules, and then rinsed with a mixture of 1:1 diethyl ether:petroleum ether to release the trapped DCP.

Further extraction with diethyl ether partitioned the phenol into the ether phase. Recovery with this technique was estimated at approximately 100%, based on tests with an authentic standard (Aldrich Chemical Co., Milwaukee, WI). Aliquots of the final extract were injected into a Perkin-Elmer Model Sigma 3 gas chromatography (Rockville, MD) using Hamilton Model 701-RN microliter syringes with Chaney adapter and 26-S gauge needles. The gas chromatograph was equipped with a constant-current electron capture (EC) detector with a ^{63}Ni foil to detect halogenated compounds. Detector and injection port temperatures were 250° C, the column temperature was 200° C, and the flow rate for the carrier gas (N_2) was 75 ml/min. The column was a 0.6-cm o.d × 56-cm long, curved glass tube packed with Tenak 80/100 mesh absorbent. Recordings and quantification of compounds were made with a Shimadzu C-RIA Chromatopac Multiprocessor (Rockville, MD) connected to the gas chromatograph.

Results

Larva. No evidence of foveal primordia was found on the external body surface in this stage when specimens were examined with the SEM.

Nymph. Small, paired pore clusters were observed on the alloscutal surface of unfed nymphs (Figure 6). Each pore cluster, 18.3 um in diameter, contains only 3 pores. Histologic study of nymphs fixed 5 days post-engorgement revealed the presence of a small, multicellular gland primordium, with at least 3 large cells connected by ducts to the 3 pores of the foveal primordium (Figure 7). No DCP was found in a sample of 100 engorged nymphs collected 15 days post-feeding when assayed by gas chromatography.

Adult. The paired sex attractant pheromone glands and associated foveae dorsales of both males and females are located in the dorsal midsection of the body, ca. 0.5 to 1.0 mm posterior to the scutum. Each gland consists of the (1) fovea dorsalis (FD), a cluster of minute pores, (2) the duct zone (DZ), with numerous minute ducts connecting the foveal pores to the secretory areas of the gland, and (3) the secretory lobes (SL) of the gland, which contain the secretory or storage cells.

a. Fovea dorsalis and foveal pores. This pair of pore clusters provides the outlets of the pheromone glands. The surface of the female fovea appears as a slightly elevated subcircular area, 67.4 to 75.5 um in diameter (N = 3), with from 20 to 28 slit-like pores (Figure 8). No difference was found between unfed and feeding females regarding the appearance of these structures. The male fovea resembles that of the female, but it is much smaller, only 35.3 to 47.4 um in diameter (N = 3), and contains only 15 to 18 slit-like pores (Figure 9). When the fovea of the female is examined with the TEM, each of the many pores of both the unfed and partially fed virgin female is found to contain a pore tube (pt) with a mean lumen diameter of 2.3 um ± 1.36 (N = 5) (Figures 10 and 11). Within the region of the cuticle, each tube consists of several interdigitating cells separated from one another by septate desmosomes (sds). The cells in at least some specimens contain abundant accumulations of granules resembling glycogen (gly). The luminal surface of the cells is lined with a non-laminar coating of varying electron dense material. An amorphous bean-shaped deposit, often with an extremely dense core, occurs on one side of the open tube lumen. Often, in other pores, the tube lumen is completely closed (Figure 12). The tube cells in these pore tubes appear much more highly vacuolated than the cells of the open pore tubes. Beyond the cuticle, toward the interior of the tick body, the pore tube cells have disappeared. The tube lumen appears unchanged (2.45 um, N = 1). However, the tube wall is also similar in thickness, but the intraluminal deposit is lacking the electron dense core seen in the cuticular zone. The pore tube appears to lie in a cavity (cav) filled in part by structural microtubules. Support cells, with extensive membranous infoldings and abundant rough endoplasmic reticulum occur between the pore tubes and constitute most of the tissue mass at this level (Figure 13). Extending further into the body, the pore tube is connected to a cone-shaped ampulla (Figure 14). The ampulla (from 4.16 to 5.23 um at its widest dimension; N = 2) is formed of a moderately electron-dense, amorphous outer wall similar in appearance to the lining of the pore tube and continuous with it. A duct (d) is inserted into the concave inner surface of the ampulla. The wall of the duct is formed of numerous structural microtubules, seen in cross-section in Figure 14, while the lining is formed of a double-walled membrane, with a brush border (bb). The ampulla is surrounded by a cavity (cav). This cavity is filled with a mass of minute microfibrils near the interior, and loosely organized granules, microtubules, and vesicles near its exterior portion.

b. Duct zone. Further toward the interior of the tick body, ducts replace the pore tubes and ampulla as the dominant feature of this zone of the pheromone gland. The ducts, of varying size (4.49 to 5.14 um, mean = 4.8, N = 3), are lined with numerous microvilli except near the insertion into the ampulla, where the brush border replaces the microvilli. Groups of ducts are separated by amorphous partitions, or fasicles (not shown in the figures). In the partially fed virgin female, vesicles of varying size surround the ducts, and many vesicles or vesicle remnants appear inside the lumen (Figure 15). Occasionally, electron-dense droplets occur in the ducts or in vesicles adjacent to them (Figure 16). In the unfed females, numerous vesicles also occur around the ducts and, occasionally, some appear within the lumen. Rarely, minute electron-dense particles are seen free in the ducts, but no electron-dense droplets typical of secretory-lobe neutral lipid droplets were found (Figure 17). Occasionally, vesicles containing dense masses of fine fibrils (not shown in the figures) also occur in the ducts of the feeding female, but not in the unfed female. The ducts of the emerging female (day zero) resemble those of the unfed, mature females.

Evidence of nervous innervation of the pheromone gland was found. Branches of the nerve, hereafter termed the foveal nerve (fn), occur in the fasicles between the lobes of the pheromone gland (Figure 18). The outer covering of amorphous material consists of the fasicles (f) and is continuous with the fasicles that separate clusters of ducts. The fasicles appear to enclose fiber bundles, as well as the entire nerve. At the lateral edges of the nerve, and at junctions between fibers bundles, the fasicular material forms large areas filled with moderately electron-dense amorphous material. Underlying the fasicles, and separated from them by a narrow space, is the neurilemma (nl), composed of non-laminar material similar to that of the fasicles. The neurilemma also encloses fiber bundles. In addition, it anastomoses to surround individual nerve fibers. Glial cells (gl), often with large nuclei, fill the areas between the nerve fibers. Some of the nerve fibers (presumably axons) contain minute vesicles resembling neurosecretory vesicles (nsv) (Figure 19). In the emerging young female, small branches of foveal nerve and individual fibers (arrows) are seen among the support cells of the duct zone and, evidently, provide innervation for the tissues of this region (Figure 20). Neurosecretory vesicles, some electron-dense, others electron-lucent, are visible in those nerve fibers. Electron-dense, osmiophilic neurosecretory vesicles appear to predominate in some fibers, while both electron-lucent and electron-dense vesicles occur in others (Figures 20 and 21). Electron-lucent vesicles were not observed in foveal nerves of mature unfed female ticks.

c. Secretory lobes. Within the tick body, the pheromone gland expands into large, bulbous lobes which contain the secretory cells. When examined with the SEM, each female foveal gland appears as a rosette-like cluster of 15 to 21 bulbous lobes of varying size. Both lobe clusters are widely separated. Individual lobes range in size from 53.0 to 86.7 um, mean = 65.5 um ± 11.1 um (N = 5) across their widest areas (Figures 22 and 23). Overall, the total width of the entire gland across its widest part averages 245.3 ± 36.0 um (N = 5). No difference in appearance was found between the glands of unfed and feeding females. In the male, the two clusters of lobes occur adjacent to one another and appear as a single gland; the width of the paired gland combination at its greatest width was 237.1 um (N = 1). Only 17 or 18 small lobes occur in the entire paired gland. The largest lobe in one specimen measured 67.6 um (Figure 24).

Transmission electron micrographs of the secretory lobes of the emerging young females reveal large cells with large nuclei filled with numerous, membranous vesicles of varying size (Figures 25 and 26). Some vesicles are filled with finely granular material distributed sparsely within the enclosure; others are filled with a homogeneous lipid-like material (Figure 26). Intensely osmiophilic droplets were extremely rare in these very young females. Those that were found were very small (less than 0.10 um). These very young females were also found to lack DCP. No evidence of this sex pheromone was found when an extract of 100 individuals was examined by gas chromatography. In the mature, unfed female, the large secretory lobe cells are also filled with numerous vesicles of varying size. Most of the intensely osmiophilic droplets within these vesicles were more or less uniform in size, with a mean diameter of 0.35 ± 0.04 um (range from 0.32 to 0.40 um, N = 5). Moreover, many of the vesicles contain one or more intensely osmiophilic neutral lipid droplets (nld) (Figure 27). Virtually all vesicles are found to be intact, and all of the neutral lipid secretory droplets were found within vesicles. Occasionally, small areas of one or more lobes were found with partial disintegration of some vesicles. The mean diameter of representative nuclei of these cells was 11.3 cm (N = 2). X-ray microanalysis of the secretory lobes of 5 specimens of mature, unfed females revealed abundant accumulations of chlorine, presumably the chlorine of DCP fixed in the tissues; expressed as a ratio of osmium to chlorine, the amounts averaged 100:1.24 ± 0.45 (N = 8) (Table 5). Analysis of an extract of 100 mature, unfed females by gas chromatography revealed the presence of 3.46 nanograms DCP/tick. In the feeding virgin female, in contrast to preceding types, the cells are filled with vesicle remnants, as well as areas free of membranes or vesicles. Numerous osmiophilic secretory droplets occur throughout the cells of the secretory lobes, mostly free in the cytoplasm (Figure 28). Few intact vesicles with secretory droplets were found. X-ray microanalysis of the secretory lobes of 5 specimens of feeding females also revealed abundant accumulations of chlorine, 100.0:0.87 ± 0.48 osmium to chlorine (N = 7). Analysis of an extract of 65 virgin, partly-fed females revealed the presence of 5.57 nanograms of DCP/tick. In the mated, repleting female, the secretory lobes appear intact and generally similar to those of unmated females. However, droplet content appears to be greatly reduced, especially in the more central lobes of the pheromone gland. Study of representative areas of the secretory lobes revealed cells and, occasionally, entire lobes without discernable secretory droplets. When examined with the TEM, clusters of scattered, irregularly shaped electron-dense material are found frequently in the secretory cells, in addition to discrete secretory droplets (Figure 29). Huge vacuoles, virtually empty except for a few secretory droplets and vesicles, occur in several of the secretory lobes. The cytoplasm of such cells is also virtually devoid of secretory droplets and the nuclei exhibit margin indentations (Figure 30). In other cells, the chromatus is concentrated near the nuclear membranes, the nucleoli are absent, and the nuclei are indented greatly.

Table 5. Chlorine content of microscopic areas of the foveal glands of H. dromedarii as determined by X-ray microanalysis (SEM).

Spec. No.	Unfed Females	Partially-fed Virgin Females	Unfed Males
1a	1.18	1.11	0.02
1b	----	0.80	----
1c	----	0.73	----
2	1.55	1.50	0.00
3a	1.80	0.21	----
3b	1.38	----	----
4	0.49	----	----
5	1.06	----	----
$\overline{X}$ ± S.D.	1.24 ± 0.45	0.87 ± 0.48	----

Transmission electron micrographs of the secretory lobes of the mature, male foveal glands revealed a cellular structure resembling that of the mature, unfed female. However, the highly vesiculated lobe cells contain irregularly shaped osmiophilic particles, occasionally grouped into clusters, and almost always within vesicles (Figures 31 and 32). The number of osmiophilic particles in representative secretory lobe cells--0.093 ± 0.049 particles/um^2 (N = 8)--is considerably less than the number of osmiophilic secretory droplets in comparable secretory lobe cells of the mature unfed female--0.172 ± 0.021 droplets/um (N = 5); or in the feeding, virgin female--.174 ± 0.059 droplets/um^2 (N = 8). Some lobes of the male gland appear virtually devoid of osmiophilic particles. Occasionally, vesicle disruption occurs in some secretory lobe cells.

Only trace amounts of chlorine were observed when the foveal glands of two specimens of mature, unfed males were examined by X-ray microanalysis. Only trace amounts of DCP (< 0.01 ng/tick) were found in an extract of 70 unfed, mature males examined by gas chromatography.

No evidence of symbiotic micro-organisms was found in any of the pheromone gland specimens examined in this study.

Discussion

The foveae dorsales of _H. dromedarii_ resembles similar structures that have been described in other species of ticks. The 20-28 slit-like pores visible in surface views of the foveae of _H. dromedarii_ are similar to the number of "slits," presumably slit-like pores, determined by Axtell and LeFurgey (1979) in surface views of _Amblyomma maculatum_ Koch (26), _Dermacentor andersoni_ Stiles (26), and _D. variabilis_ (Say) (22); only 15 slit-like pores were found by these authors in _A. americanum_ L. Cross-sections through the fovea may reveal larger number of pores than those visible on the surface. Sonenshine et al. (1981) observed 45-50 pores in a cross-section through the fovea of a female _D. variabilis_.

The foveal gland and pore system in _H. dromedarii_ resemble similar gland structures described in _D. variabilis_ by Sonenshine et al. (1981). However, there are several noteworthy differences. In the secretory lobes, the lipid secretory droplets found in the cells of unfed female _H. dromedarii_ are relatively small (ca. 0.35 um diameter) and more or less uniform in size (0.32 to 0.40 um); frequently, they appear clustered in groups of 2, 3, or even more droplets in each vesicle. In contrast, the secretory lobe cells of D. variabilis contain lipid droplets that exhibit extreme variation in size, with some as large as 3.5 um in diameter; however, each secretory droplet occurs in its own vesicle. No Wohlbachia-like symbionts such as those found in the pheromone gland ducts of D. variabilis were observed in any of the pheromone gland sections of H. dromedarii. Finally, the most important difference between the glands of the 2 species is the evidence indicating onset of limited secretory activity in unfed H. dromedarii females. Cells of some secretory lobes exhibit partial breakdown of vesicle organization, while the ducts contain abundant vesicle accumulations and even (occasionally) minute, electron-dense droplets. These characteristics are consistent with secretory activity, as exhibited by the secretory lobes of the glands of feeding females. Khalil et al. (1981) noted that "relatively large-size unfed female H. dromedarii weighing 13 mg or greater may induce a copulatory response by SA (sexually active) males." Hoogstraal (1956) also noted mating of male H. dromedarii with unfed females. Bioassays in our laboratory demonstrated that SA males would copulate with ca. 10% of unfed female H. dromedarii (Sonenshine, unpublished), an event never observed with unfed female D. variabilis.

The sex pheromone glands of H. dromedarii complete a cycle of growth and development consistent with the maturing, feeding, and reproductive cycle of these ticks. The simple 3-cell gland and pore primordium that develops in the nymphal stage is, apparently, nonfunctional since no sex-attractant pheromone occurs in the engorged nymph. Subsequently, this is replaced by the paired, multi-lobed foveal gland when the adult integument is formed. However, the secretory lobes of these glands are still immature, with little or no lipid accumulations. The numerous vesicles of the very young females contain only a fine granular substance or, occasionally, lipids characteristic of esters of saturated fatty acids. The very young females also lack the sex pheromone, DCP. As the female matures, the foveal glands accumulate neutral lipids rich in polyunsaturated fatty acids which condense into electron-dense secretory droplets. The mature females contain DCP. Feeding alters these relationships, inducing disruption of the vesicles and release of secretory droplets into the cytoplasm and migration of these materials to the ducts and pores. Control of secretory activity appears to be regulated by the pore tubes that exhibit an opening and closing mechanism influenced by turgor pressure of the gland cells. Little difference can be found between the chlorine content and, presumably, the DCP content of the unfed, mature female and the feeding, virgin female (Table 1). The slightly lower pheromone content found in the glands of the feeding female vs. the unfed female may reflect differences in gland physiology in the two different physiological states. Sonenshine et al. (in press/b) hypothesized that sex attractant is released by the virgin, feeding females more or less continuously, whereas it accumulates in the unfed female. Finally, in the mated, repleting female, the secretory lobe cells deteriorate; vesicles disintegrate even further, while osmiophilic secretory droplets are depleted. Presumably, no new synthesis occurs to replenish secreted pheromone in these exhausted glands, though further study is needed to confirm this. These findings do not support the hypothesis that the sex pheromone content following mating may actually increase as a deterrant against predators. Rather, our evidence suggests that the foveal glands serve only as sex pheromone glands, and cease to function when this is no longer needed.

The foveal glands of male H. dromedarii resemble those of the female, differing mainly in the smaller size of the dorsal foveae, smaller number of slit-like pores, smaller number of secretory lobes, and, most importantly, the absence of DCP. The secretory lobe cells contain fewer osmiophilic secretory droplets than the female glands, though their staining characteristics suggest a similar chemical content. Presumably, these glands are capable of some secretory function but its nature, if any, is unknown. Most likely, the male foveal glands in H. dromedarii are vestigial structures. However, relatively large, multi-lobed foveal glands occur in Amblyomma maculatum (Sonenshine, unpublished) and substantial quantities of DCP can be found (Kellum and Berger, 1977).

The sex pheromone glands of H. dromedarii appear to be related to the dermal glands of ticks. Evidence for this hypothesis was described in detail by Sonenshine et al. (1981). Additional evidence in support of this derivation of the foveal glands is suggested by the 3-cell gland and pore primordium found in the nymphs which resembles the type II dermal glands described by Balashov (1972). In contrast, insect sex pheromone glands typically contain acini or tubules emptying to the exterior via a common duct and pore instead of a porose fovea. In the noctuid moth *Trichoplusia ni* (Hubner), the sex pheromone gland is an eversible sac of glandular epithelium composed of tall columnar cells filled with "vacuoles" containing secretion droplets (Jefferson et al., 1966). The epithelium is covered by a thin layer of cuticle through which the pheromone is thought to pass, since no secretory ducts were found. In the red-banded leaf roller moth *Argyrotaenia velutinana* (Walker), the sex pheromone gland consists of convoluted tubules joined to a common duct which opens to the exterior. The tubular epithelium consists of columnar cells rich in electron-lucent lipid droplets (Feng and Roelofs, 1977). In the parasitic wasp, *Apanteles melanoscelus* (Ratzeburg), the sex pheromone gland is a small patch of columnar epithelial cells rich in non-osmiophilic lipid droplets (Weseloh, 1980).

The mode of sex pheromone secretion by ixodid ticks is also somewhat like that of insects. In the insect examples described above, secretion is accomplished by everting the glandular sac to allow pheromone evaporation (Jefferson et al., 1966), or oozing waxy pheromone containing secretions through the simple opening of the gland, coating the adjacent cuticle and allowing gradual vaporization of the volatile pheromone. In many Lepidoptera, males produce sex pheromone from scent glands located under specialized wing scales (androconia) to be dispersed from the frilled scale margins or tufts of setae (Chapman, 1971). Ixodid ticks, however, have evolved the fovea with its many pores, providing an efficient mechanism to disperse the oily secretions over a wide area, thereby enhancing vaporization of the volatile sex pheromone.

IV. Toxicity of Precocene-2 for the American Dog Tick, *Dermacentor variabilis* (Say)

Introduction

Study of the effects of the anti-allatotropics Precocene-2 were reported in the first annual report. To avoid unnecessary duplication, this chapter will be limited to a summary of previous data and a brief review of new data. The complete study is scheduled for publication in the Journal of Medical Entomology.

Summary

The dominant effect observed following treatment of *Demacentor variabilis* immatures with Precocene-2 was toxicity. Treatment (contact method) with doses of 1.0 ug/cm^2 was lethal for most unfed larvae and nymphs. When exposed to lesser doses, many more surviving larvae failed to feed than larvae exposed only to acetone or no treatment. Treatment of engorged larvae (contact method) resulted in ecdysial delay (0.015-0.50 ug/cm^2), ecdysial mortality (0.5 ug/cm^2), or death without molting (0.50 ug/cm^2). Treatment of engorged nymphs resulted in ecdysial mortality without ecdysial postponement (1.0 ug/cm^2), or it induced a slow death syndrome. In the latter case, adultiform characters developed normally but the treated nymphs failed to molt, and many remained in the nymphal skin for weeks or even months before eventual death. An inverse relationship was found between the developmental state of the immature ticks and sensitivity to Precocene. Nymphs were least sensitive to Precocene on the day of repletion. The LD_{50} for engorged nymphs (0.87 ug/cm^2) was only 2.1 X that for engorged larvae (0.42 ug/cm^2), even though the mean weight of an engorged nymph was more than 20 times that of an engorged larva.

In contrast to the immature ticks, adults were relatively insensitive to Precocene. Sex attractant activity was not inhibited, and insemination frequency was only slightly inhibited by treatment with this compound. Feeding and, possibly, oviposition, were impaired following topical treatment of partially fed females.

New Findings

Although unfed adults and replete females were insensitive to Precocene-2 (P-2), engorging virgin females were susceptible to this compound.

Experiments were done with (1) part-fed females that were forcibly detached, treated topically with 1 mg P-2 in DMSO:acetone (spectra-analyzed DMSO; Fisher Scientific Co., Fairlawn, NJ), and allowed to reattach, and with (2) part-fed females that were treated with the same dose of P-2 while attached and feeding. In the first experiment, 81.5% of the forcibly detached females treated with 1 mg P-2 in DMSO:acetone failed to reattach. All 4 that did reattach mated within 1-2 days when exposed to SA males, but these females failed to replete fully during the next 7 days. Recorded weights after 15 days of attachment were 285.5, 281.6, 131.9, and 142.9 mg, respectively; recorded egg production by these same females was 5.1, 101.5, 45.6, and 0.0 mg, respectively. If only the 3 egg-laying females are considered, egg production was 18.1% of replete female body weight. Among the controls treated only with solvent (DMSO:acetone), 61.5% reattached, mated, and engorged readily; recorded mean repletion and egg production weights were 363.8 ± 181.9 mg and 138.8 ± 126.9 mg, respectively. Hatching exceeded 95% in both experimental and control groups. In the second experiment, virgin females treated with 1 mg P-2 in DMSO:acetone while attached to a rabbit (fed 7 days) did not replete normally following insemination by SA males. Observation of ectospermatophores and the presence of spermatozoa in the uterus (examined after oviposition was completed) provided confirmation that mating had occurred. When examined 8 days after treatment, 2 females were dead and 14 of the 16 live females had detached. The mean weight of all living females on that day, 157.3 ± 80.2 mg, was much less than that of the solvent controls, 325.6 ± 115.8 mg (or only 48.3% of the control repletion weight). Mean egg weight production for the treated females was only 18.3 ± 5.5 mg (only 5 females oviposited), 11.6% of the mean weight of the ovipositing females, and only 0.7% of all females. In contrast, mean egg weight production by the control females was 117.5 ± 77.8 mg. Hatching exceeded 98% in both populations.

The results indicate both anti-feedant and anti-gonadotrophic effects, but only in the partially fed virgin females. It is not clear whether the partially fed females were actually more susceptible to P-2, or whether P-2 was able to penetrate the cuticle more readily during this physiological state.

V. Mating Regulation by Pheromones in the Genera *Hyalomma* and *Dermacentor*: Evidence for the Existence of Genital Sex Pheromones and Species-Specific Isolating Mechanisms.

Introduction

This study presents evidence indicating the existence of a previously undescribed sex pheromone (or pheromones) in the ticks *Dermacentor variabilis* (Say), *D. andersoni* Stiles, *Hyalomma dromedarii* Koch, and *H. anatolicum excavatum* Koch. In addition to DCP, which attracts mate-seeking males, a pheromone released on the cuticle of the female genital area enables the sexually excited male to locate the gonopore. A pheromone located in the vulva, perhaps the same as that found on the surface, enables males to identify conspecific females.

Studies investigating the possible existence and role of other sex pheromones in ticks arose as a result of observations that challenged the hypothetical role of DCP as the sole sex pheromone in these ticks (Sonenshine et al., 1976). Though not universal, the common occurrence of DCP in so many species with diverse taxonomic relationships raised doubts as to the validity of this hypothesis. This interpretation, though plausible, is probably misleading. In nature, multiple infestations of the same host by sympatric species are common. If mating behavior was mediated solely by the same sex attractant in all cases, numerous opportunities for inter-specific pairing would occur. In laboratory studies, most interspecific mating attempts are aborted before copulation. Those matings that do occur are unsuccessful; the few living progeny of such parents prove to be sterile hybrids (Oliver et al., 1973). Evidently, some means of species recognition has evolved that minimizes these wasteful interspecific sexual contacts in species that share common hosts. Indeed, the existence of a multi-component language guiding the mate-finding process, with specific chemical and behavioral elements to be discovered, must be considered in any explanation of tick mating behavior.

The hypothesis that other chemicals may regulate aspects of the mating process in ixodid ticks led us to investigate the mate selection process, as well as behavioral processes leading to copulation. The Old World species *H. dromedarii* and *H. anatolicum excavatum* sympatric on domestic livestock, and the New World ticks *D. variabilis* and *D. andersoni*, common on dogs or wild mammals, provided the experimental material. Studies with the *Hyalomma* species were done at NAMRU-3, under the supervision of Dr. G.M. Khalil. Though comparisons will refer to the findings made with these ticks, detailed reporting of the work on *H. dromedarii* and *H. anatolicum excavatum* will be made by Dr. Khalil. This report will concentrate on the findings with *D. variabilis* and *D. andersoni*. This report will present a summary with highlights of the major findings. Complete descriptions of all results are given by Sonenshine et al. (Experimental Parasitology, in press/c) and Khalil et al. (Journal of Medical Entomology, unpublished manuscript).

Materials and Methods

Ticks. *D. variabilis* was colonized from wild specimens caught near Richmond, Virginia USA, and *D. andersoni* Stiles from specimens from the U.S. Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana USA. Both species were reared in the Old Dominion University Department of Biological Sciences in accordance with previously described techniques (Sonenshine et al., 1976). *H. dromedarii* and *H. anatolicum excavatum* were colonized from engorged females collected from camels in the Imbaba camel market, Giza Governorate, Arab Republic of Egypt (HH No. 59,723) and reared as described by Khalil et al. (1981). All animal care and manipulations were done in accordance with the Animal Welfare Act Amendment of 1976 (PL 94-279) with subsequent amendments.

Bioassays. These were performed using female ticks naturally attached to the shaved backs of rabbits tranquilized with Acepromazine maleate® (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa USA), as described by Sonenshine et al. (1976) and Khalil et al. (1981). Sexually active (SA) males were allowed to feed for at least 8 or 9 days, then were forcibly detached and were found to respond to conspecific, confirmed attractive virgin (CAV) females that had been feeding for at least 5 days. Male responses were categorized as the following: Orientation (O)--movement toward the female; mounting (M)--climbing onto the female and palpating its body (omitted in some of the tests); positioning--probing the gonopore with its mouthparts (P); and copulation (C)--deposition of a spermatophore. Each male was allowed 3 trials, unless the trials were terminated following copulation or attachment. Spermatophore transfer was verified microscopically.

The responses of SA males to conspecific and transspecific CAV females were tested, both by evaluating their responses in a bioassay and by studying oviposition and hatching of females of mixed populations confined on a host.

Experimental Treatments. These included (1) washing the genital area surface of CAV females with saline, hexane, or acetone; (2) excision of the genital cuticle after washing (hexane); (3) severing the vagina and vestibule and detaching them from the genital surface cuticle after washing (hexane); and (4) obstructing the genital aperture and/or surrounding cuticle with lacquer, with or without washing, or introducing obstructions in the vulva.

Other experiments were done to determine whether SA males would copulate with heterospecific females in which the vulvas were mechanically manipulated or treated with DCP (Aldrich Chemical Co., Milwaukee, WI). CAV females of *D. variabilis* and *D. andersoni* were immobilized and 2 ul of a hexane solution containing 4 ug DCP/ul were introduced into the vulva of each female. Other females (*D. andersoni* only) were subjected to mechanical treatment of the vulva using a micropipette and a Leitz micromanipulator (Bunton Instrument Co., Rockville, MD). Controls (both species) received 2 ul of hexane in and around the vulva, or no treatment. Following the various treatments, the females were bioassayed in a petri dish with SA males. Subsequently, females of each species were confined with transspecific SA males on a host and allowed to reattach and mate, thereby providing unlimited opportunities for mating. Surviving fed females were dissected and examined for evidence of spermatozoa in the reproductive tract and mature eggs in the oviduct.

Electron Microscopy. The mouthparts of unfed *H. dromedarii* males, 2-4 weeks post-eclosion, were excised after inoculation of cold (4° C) 4% glutaraldehyde buffered in 0.1 M s-collidine, pH 7.4. Fixation, dehydration, embedding (Epon 812), sectioning, and staining were done in accordance with standard electron microscopy techniques (Dawes, 1971). Stained sections were viewed and photographed with Nikon/Apophot light microscope and a Hitachi HU-11B transmission electron microscope.

Results and Discussion

Evidence of a Species-Specific Mating Pheromone. Previous studies, reported in the first annual progress report, described evidence of conspecific mate recognition in *D. variabilis* and *D. andersoni*. When SA males of these species were exposed to heterospecific females, courtship behavior was similar to that observed with conspecific females, indicating equal attraction by DCP. This is not unexpected, in view of the responsiveness of the males of both species to a wide range of DCP concentrations (Sonenshine et al., 1976). However, the males failed to copulate. Most males probed the genital orifices of the females before they withdrew; often, they returned to the same female and repeated the process 2 or 3 X. When SA males were exposed to a mixture of heterospecific and conspecific females, the males mounted and probed the gonopores of the prospective mates until they encountered a conspecific female, whereupon copulation took place. Clearly, the decision point is reached only when the male contacts the female vulva. Cross-generic mating also failed when males of the two *Dermacentor* spp. were exposed to female *H. dromedarii*. Again, courtship behavior was unaffected and the males withdrew only after probing the female gonopores. These studies implicated a chemical stimulus, rather than perception of physical differences in the shape of the female genital aperture, as the factor necessary to complete copulation. It was noted that various physical obstructions placed in or over the female gonopore failed to deter copulation by the sexually excited males. Such males readily punctured lacquer barriers and even ignored glass fragments in order to implant their spermatophores. However, simply scraping and washing the female genital surface with hexane or acetone greatly reduced the frequency of male copulatory responses for at least 45 minutes (Tables 6 and 7). Sealing the female gonopore with lacquer after the scraping and washing treatments virtually eliminated the male copulatory response (Tables 8 and 9). Other evidence indicating chemical rather than physical stimuli as the factor regulating copulation behavior was obtained by severing the vestibule and vagina from the gonopore of CAV females. Performance of this operation left only small marginal wound scars, with the gonopore intact. Males exposed to CAV females subjected to the surgery after dewaxing of the external genital surface exhibited normal courtship behavior, but withdrew without copulating. However, if the surgery was performed on CAV females without prior cleansing of the genital surface, mating culminated in copulation in almost all instances. Presumably, the female gonopore is the source of some stable, persistent chemical(s) that guide(s) the male during the process of probing and implanting its spermatophore.

Studies with H. dromedarii and H. anatolicum excavatum by Khalil et al. (separate progress report) revealed similar courtship behavior, but with several notable differences. Many SA males of these species failed to mount the heterospecific CAV females, and few probed the female gonopores; again, none of the males copulated. In contrast, cross-generic matings were remarkably successful. Evidently, the mechanism that enables male H. dromedarii to discriminate between females of the two sympatric Hyalomma species does not enable them to exclude all other tick species, at least not the allopatric Dermacentor species. Clearly, the mechanisms regulating mating in the two genera are not identical, though there are many similarities.

Chemical Stimulants and Repellants Affecting Mating. Although experimental evidence implicates an important regulatory role for other pheromones and/or other chemicals in addition to DCP, their chemical nature and identity is unknown. Khalil et al. (separate progress report) observed that H. anatolicum excavatum males are deterred by concentrations of DCP that are attractive to H. dromedarii males. This finding is consistent with differences in the natural concentration of DCP in the two species (Silverstein, unpublished) and may account for at least part of the species isolating

Table 6. Responses of sexually active males to chemical alteration of the female genital area of *Dermacentor variabilis*.

Type of Treatment Solvent/Period	No. Males	No. Females	No. Trials	% Responses[1] O	P	C	WD	A	% Females Exposed That Copulated
Gen. area scraped/rubbed and washed with									
1. Saline									
<15 min.	17	17	21	100.0	81.0	47.6	19.1	33.3	58.8
<30 min., >15 min.	10	10	18	100.0	52.9	27.8	5.9	61.1	50.0
<45 min., >30 min.	12	12	18	100.0	94.4	38.9	50.0	27.8	58.3
>45 min.	11	11	14	100.0	92.9	87.5	14.3	28.6	100.0
2. Hexane									
<15 min.	15	15	42	100.0	52.2	0.0	72.7	24.2	0.0
<30 min., >15 min.	11	11	24	100.0	47.5	4.2	50.0	25.0	9.1
<45 min., >30 min.	15	15	35	100.0	80.0	5.7	62.9	25.7	13.3
>45 min.	10	10	14	100.0	85.7	42.9	14.3	35.7	60.0
3. Acetone									
<15 min.	14	14	24	100.0	66.7	8.3	50.0	37.5	14.3
<30 min., >15 min.	11	11	20	100.0	54.2	25.0	10.0	65.0	45.5
<45 min., >30 min.	10	9	15	100.0	86.7	33.3	33.3	33.3	55.6
>45 min.	7	6	9	100.0	77.8	33.3	22.2	44.4	50.0
4. Controls[2]									
Saline	5	5	6	100.0	100.0	83.3	0.0	16.7	100.0
Hexane	6	5	7	100.0	100.0	71.4	0.0	14.3	100.0
Acetone	5	4	5	100.0	100.0	60.0	20.0	20.0	75.0

[1]Definitions of abbreviations: O = orientation; P = positioning for copulation; C = copulation; WD = withdrawal after positioning; A = attached under or beside female.

[2]100 ul of solvent deposited on host skin adjacent to female serving as control.

Table 7. Responses of sexually active males to chemical alteration of the female genital area of *Dermacentor andersoni*.

Type of Treatment Solvent/Period	No. Males	No. Females	No. Trials	% Responses[1] O	P	C	WD	A	% Females Exposed That Copulated
Gen. area scraped/rubbed and washed with									
1. Saline									
<15 min.	13	12	22	100.0	90.9	27.3	40.9	31.8	50.0
<30 min., >15 min.	11	11	16	100.0	87.5	31.3	25.0	43.8	45.5
<45 min., >30 min.	10	10	17	100.0	100.0	23.5	58.8	11.8	40.0
>45 min.	10	10	15	100.0	93.3	40.0	33.3	26.7	60.0
2. Hexane									
<15 min.	10	10	34	100.0	45.8	0.0	62.5	33.3	0.0
<30 min., >15 min.	10	10	17	100.0	66.7	5.9	47.1	41.2	10.0
<45 min., >30 min.	10	10	18	100.0	68.1	11.1	72.2	16.7	20.0
>45 min.	13	13	23	100.0	92.6	12.1	78.8	9.1	30.8
3. Acetone									
<15 min.	11	11	22	100.0	72.7	18.2	18.2	59.1	36.4
<30 min., >15 min.	11	11	25	100.0	76.0	8.0	64.0	24.0	18.2
<45 min., >30 min.	10	10	22	100.0	76.2	9.5	28.6	61.9	22.2
>45 min.	11	11	21	100.0	81.3	12.5	12.5	68.8	25.0
4. Controls[2]									
Saline	7	7	7	100.0	100.0	100.0	0.0	0.0	100.0
Hexane	9	9	12	100.0	100.0	75.0	25.0	0.0	85.7
Acetone	8	8	8	100.0	100.0	100.0	0.0	0.0	100.0

[1]See footnote i, Table 6.

[2]100 ul of solvent deposited on host skin adjacent to female serving as control.

Table 8. Response of sexually active males to physical alteration of the female genital area of *Dermacentor variabilis*.

Type of Treatment Solvent/Period	No. Males	No. Females	No. Trials	% Responses[1]					% Females Exposed That Copulated
				O	P	C	WD	A	
1. Gen. aperture excised	48	10	117	98.3	88.9	0.0	75.2	22.2	0.0
2. Gen. aperture detached from vulva after scrape/wash[2]	32	10	49	100.0	67.8	0.0	55.1	44.9	0.0
3. Gen. aperture totally obstructed[3]	24	10	53	100.0	100.0[4]	3.8	71.1	24.5	20.0
4. Gen. aperture sealed; adjacent area exposed[3]	19	14	22	100.0	100.0[4]	63.6	13.6	22.7	100.0
5. Gen. aperture sealed after scrape/wash; adjacent area scrape/wash[2,3]	59	14	119	100.0	95.0[4]	1.7	70.0	26.1	7.1
6. Gen. area exposed; adjacent area obstructed[3]	21	9	36	100.0	97.2	13.9	47.2	36.1	55.6
7. Oil or glass in vulva[2]	5	5	5	100.0	100.0	100.0	0.0	0.0	100.0
8. Controls (no treatment)	32	27	32	100.0	100.0	71.7	21.9	21.9	85.2

[1]See footnote 1, Table 6.

[2]See text for details of treatment.

[3]Coated with lacquer or acrylic paint.

[4]Prepositioning only (see text for details).

Table 9. Response of sexually active males to physical alteration of the female genital area of *Dermacentor variabilis*.

Type of Treatment Solvent/Period	No. Males	No. Females	No. Trials	% Responses[1] O	P	C	WD	A	% Females Exposed That Copulated
1. Gen. aperture excised	42	9	103	100.0	97.1	0.0	79.0	21.0	0.0
2. Gen. aperture detached from vulva after scrape/wash[2]	35	9	61	100.0	55.8	0.0	54.1	45.9	0.0
3. Gen. aperture totally obstructed[3]	36	11	73	98.6	95.6[4]	0.0	75.3	23.3	0.0
4. Gen. aperture sealed; adjacent area exposed[3]	35	13	50	100.0	94.0[4]	22.0	40.0	38.0	84.6
5. Gen. aperture sealed after scrape/wash; adjacent area scrape/wash[2,3]	38	14	67	100.0	97.0[4]	1.5	65.7	32.8	7.1
6. Gen. area exposed; adjacent area obstructed[3]	21	10	37	100.0	91.9	13.5	51.4	35.1	50.0
7. Controls (no treatment)	30	24	31	100.0	100.0	70.0	3.3	26.7	87.5

[1,2,3,4]See footnotes, Table 8.

process in these arthropods. The ability of arthropods to discriminate different concentrations of specific pheromonal chemicals is well known as a means of selective attraction of their mates (Roelofs, 1978). Khalil et al. (unpublished manuscript) also reported evidence of specific repellants. Thus, all sexual responses were markedly reduced when _H. dromedarii_ SA males were exposed to conspecific females treated with _H. anatolicum excavatum_ extract; only 1.9% of the trials resulted in copulation. Similar drastic reductions were observed with the reciprocal cross.

Evidence that a contact sex pheromone, perhaps different from the mating stimulant pheromone, is necessary for mating is described in these studies with _Dermacentor_ and _Hyalomma_ species. This material appears to be essential to guide the SA male to the gonopore. Removal or obstruction of this surface component of the mating partner renders the male incapable of locating the female's genital aperture. The fact that all 4 species respond in a similar manner to these disruptive changes suggests the existence of a common chemical used by these ticks to guide the mating process. The use of surface chemicals as mating sex pheromones is well known in insects; e.g., olefins in the cuticular lipids of flies of the genus _Fannia_ (Uebel et al., 1978).

The existence of a specific mating sex pheromone (in contrast to the general sex attractant pheromone, DCP) does not necessarily imply the use of different chemicals for sexual communication. DCP may also be present in the anterior reproductive tract, and provide the essential stimulus for copulation. However, chemical tests of the anterior reproductive tract of CAV _D. variabilis_ females did not reveal any DCP (Silverstein, unpublished). In experiments with _D. variabilis_ and _D. andersoni_ (petri dish assay), sexually active males would not mate with heterospecific CAV females even when the females were treated with DCP in the gonopore and vestibule (Table 10). Mechanical stimulation or hexane flushing of the vestibular region also failed to stimulate interspecific mating. Only 3 females (2 _D. andersoni_ and 1 _D. variabilis_) were found with sperm after all 59 females were confined on hosts with heterospecific males, despite unlimited opportunities for mating. In experiments with _H. dromedarii_ and _H._

Table 10. Evaluation of transspecific mating attempts between D. variabilis and D. andersoni following treatment with 2,6-dichlorophenol (DCP) or mechanical stimulation of the female gonopore.[1]

Treatment	Species and No. Tested		Species and No. Tested		No. Trials	In Vitro Assay[2] % Males Responding			In Vivo Assay[3]	
						O	P	C	No. With Sperms in Uterus/Ovid.	No. Oviposi- ting or With Mature Eggs in Oviduct
DCP	D. and.	8	D. var.	24	64	32.8	29.7	1.6	1	2
Mechanical	D. and.	6	D. var.	6	36	36.1	19.4	0.0	1	0
Control hexane	D. and.	18	D. var.	42	144	45.1	31.2	0.0	0	0
Control untreated	D. and.	6	D. var.	6	36	77.8	72.2	0.0	0	0
DCP	D. var.	10	D. and.	30	90	1.1	1.1	0.0	1	8
Control hexane	D. var.	11	D. and.	33	99	15.2	7.1	0.0	0	1
Control	D. and.		D. and.		To be furnished				Not done	Not done
Control	D. var.	12	D. var.		40	77.5	52.5	27.5[4]	Not done	Not done

[1]Injected with 2 ul of hexane solution containing 4 ug DCP/ul directly into the vestibule with a glass micropipette (see text for details); mechanical stimulation performed with the same micropipette.

[2]Females immobilized and test performed in a 9-cm-diameter glass Petri dish.

[3]Treated females and fed males confined on a rabbit and monitored daily for mating and repletion; females which failed to deattach naturally after 30 days were removed.

[4]91.7% of all females were inseminated.

anatolicum excavatum, SA males would not copulate with dead, attached females treated with DCP on the dorsum and in the genital area, even though the concentrations of DCP used induced all other components of mating behavior (Khalil et al., separate progress report). Clearly, the results of these experiments and chemical tests do not support the role of DCP as a mating stimulant or aphrodisiac pheromone. However, other phenolic compounds (e.g., phenol, various cresols, or salicylaldehyde) might also be considered as possible mating stimulant pheromones, in view of the report of Wood et al. (1975) implicating these phenols in sexual communication in ixodid ticks.

Perception of ixodid sex pheromones. Detection of tick pheromones by sensillae on the palps and first leg tarsi has been reported (Leahy et al., 1975; Rechav et al., 1977; Leahy and Booth, 1978). DCP is perceived by Haller's organ in metastriate ticks (Leahy and Booth, 1978; Chow, 1974; Haggart and Davis, 1981). Cresols are also perceived by neurons associated with this same organ in *Amblyomma americanum* L. (Haggart and Davis, 1981). Perception of the female gonopore and, presumably, the specific identity of the female mating partner appears to be associated with sensillae on the cheliceral digits (Table 11). Excision of articles 3 and 4 of the right or left palp of *D. variabilis* SA males did not reduce their ability to copulate. Removal of both palps (entire appendage) did not alter the ability of these males to locate the gonopore, but copulation was reduced. Excision of the cheliceral digits, even if the excision was limited to one appendage, drastically reduced copulation. Many of the treated males were unable to locate the female gonopore, although most did eventually probe this structure. Study of the ultrastructure of the cheliceral digits revealed the presence of nerve-like structures in the cavity of the internal digit (Figure 33). Presumably, these apparent nervous components of the cheliceral digits are associated with sensillae on the digits, though none were found. No sensillae were found on the cheliceral sheaths. Moreover, a male in which the right cheliceral sheath, but not the digits, had been excised was able to copulate. These findings implicate the cheliceral digits in the perception of the mating sex pheromone. However, the chelicerae are also used by the male to grasp the spermatophore and implant it in the vulva of the female. Consequently, considerable caution must be exercised in interpreting the experimental findings.

Table 11. Mating responses of sexually active *D. variabilis* males following excision of cheliceral digits or palpal segments.

Parts Of Appendages Excised	No. Males Treated	No. Females	No. Trials	% Male Response (Of No. Trials) O	P	C	% That Copulated
Right palp (articles 3 & 4)	4	4	4	100.0	100.0	100.0	100.0
Left palp (articles 3 & 4)	8	8	8	87.5	87.5	87.5	87.5
Both palps (entire palp)	9	13	22	100.0	100.0	22.7	55.6
Right chelicera (digits & sheath)[1]	4	6	15	100.0	33.0	6.7	25.0
Left chelicera (digits & sheath)[1]	13	14	49	100.0	83.7	1.7[2]	7.7[2]
Both chelicera (digits & sheath)[1]	10	10	10	100.0	100.0	0.0	0.0

[1]Digits, anterior end of cheliceral shaft, and anterior portion of cheliceral sheaths removed.

[2]Examination of this male after copulation revealed excision of the right cheliceral sheath, but the cheliceral digits were intact.

Summary

Diverse chemicals and behavior regulate sexual communication in the Ixodidae. Assembly pheromones, but not sex pheromones, are known to induce clustering of unfed adults in *Ixodes ricinus* L. (Graf, 1975), *I. holocyclus*, *Aponomma concolor* (Treverrow et al., 1977), *A. hydrosauri*, and *Amblyomma limbatum* (Petney and Bull, 1981). Presumably, the assembly pheromone facilitates meeting of the sexes, but the signals used for sexual recognition, if any, are unknown. In most other ixodids, volatile phenols, especially DCP, serve as sex attractant pheromones, exciting feeding males to detach and search for the pheromone-emitting females. It is now evident that other, more specialized chemical signals influence other phases of the mating process and facilitate recognition of conspecific mates. Moreover, in the genera *Hyalomma* and *Dermacentor* there are important differences not previously recognized in how sexually excited males respond to differences in the surface chemistry of the females. Thus, additional sex pheromones, allomones, and even calibration of the male tick sensory system to minute changes in pheromone concentration suggest the existence of a substantial chemical language and subtle diversity in the processes by which mating is regulated in the Ixodidae.

VI. Comparative Effects of the Anti-Allatotropin Precocene-2 (P2) on 3 Acarine Species Representing 3 Reproductive Strategies

This section provides a semi-annual progress report on work completed at Georgia Southern College on ONR project "Hormonal-Pheromonal Interrelationships in Ticks and Parasitic Mites" (Contract N00014-80-0546; James H. Oliver Jr., co-principal investigator, subcontractor).

Experiments are underway and/or planned to determine effects of P2 on the following: (1) Precocity of molting as evidenced by stadium (larval, nymphal) deletion, (2) antigonadotrophic activity, (3) diapause induction, (4) ovicidal activity, and (5) blockage of molting. If anti-JH effects are noted, precocene reversal experiments will be attempted by application of exogenous insect juvenile hormone.

Experiments were continued on the effects of (P2) on Dermacentor variabilis. This progress report deals with reproduction in females that were treated as adults with P2 and juvenile hormone III (JH III), and effects of P2 on development and function of the testes and male genital accessory glands.

The last progress report indicated that significant differences ($P = 0.01$ or 0.005) in some reproductive events existed between control ticks (untreated and acetone solvent controls) and P2-treated adults (1, 2, 4, and 6 mg P2). The differences noted were in the percentage egg hatch, the percentage of females ovipositing eggs that hatched, and the mean number of mature eggs retained in the reproductive system for 30 days after initiation of oviposition. In attempts to determine whether these effects were due to a disruption of JH gonadotrophic activity, we attempted to reverse the effects of P2 by topically applying JH III to the venter of females previously treated with 2.0 mg P2. This experiment involved 8 groups of ticks of 6 or 7 each. These groups consisted of a control and 7 groups treated as follows: (1) 2.0 mg P2, (2) 0.1 μg JH, (3) 2.0 mg P2 + 0.1 μg JH, (4) 1 μg JH, (5) 2.0 mg P2 + 1 μg JH, (6) 10 μg JH, and (7) 2.0 mg P2 + 10 μg JH. Two females from each group were dissected on the second and sixth day after drop from the host to ascertain ovarian and egg development; others in each group were allowed to mate and oviposit. There were no observable differences at day 2 post-drop in ovarian development between the treatment groups and the control groups. All ovaries contained many stage-3 oocytes with a few entering stage 4. Dissection on day 6 post-drop showed a reduction of oocytes in the treated ticks; viz., no mature eggs in the oviducts, but ovaries appeared normal. The ovipositional parameters that were measured were similar to those recorded in earlier experiments; viz., significant differences between control specimens and P2-treated ticks regarding: (a) Percentage of egg hatch, (b) percentage of females ovipositing eggs that hatched, and (c) mean number of mature eggs retained in the reproductive system for 30 days after initiation of oviposition. Although JH was applied to the P2-treated ticks, it did not reverse the effects of P2. These results do not necessarily mean that JH does not function gonadotrophically in D. variabilis as there may be various explanations for the failure of JH to restore normal reproduction under these particular experimental conditions. The most probable explanation for failure is that the JH was not applied until quite late during the gonadotrophic cycle of this ixodid species. Interestingly, the 2 lower doses of JH (0.1 μg, 1.0 μg) had no measured detrimental effect when applied without P2, but the 10 μg dose of JH applied alone affected the 3 parameters cited above.

Effects of Precocene on the Development and Function of the Testes and Accessory Glands

To determine if precocene has an effect on spermatogenesis and/or the development of the accessory gland and hence on the male's fertility, virgin males were treated with precocene at two different points in their gonadotrophic cycle and for two lengths of exposure. One mg precocene topically applied in 4 microliters of either DMSO or acetone to the males prior to attachment resulted in 100% mortality in less than 24 hours. For this reason, we chose fumigation as an alternative treatment method. Fumigation of D. variabilis with 2 mg of precocene for 48 hours before the initial attachment or after being attached for 3 days did not cause the males to die or prevent them from attaching or reattaching to the rabbit.

The stage of spermatogenesis was determined by both gross observation of the testes upon dissection and by squashes of testes in 2% lacto-aceto-orcein. The state of the accessory gland was determined by gross observation only. Fertility was assayed by allowing one-half of the treated males in each group to mate with untreated females and allowing those females to oviposit. Embryogenesis of the eggs was considered the assay for normal spermatogenesis and fertility, and rapid engorgement by the female was the assay for normal function of the male's accessory gland.

Virgin males were divided into four treatment groups of 20 each and placed in two-dram vials. Treatments were carried out immediately prior to confinement on the rabbit's ear. Two groups acted as experimental controls. One control group (UC) was not fumigated but merely sealed in the glass vial for 48 hours. The other control group (AC) was sealed in the glass vial with an acetone-treated filter paper strip for 48 hours. Two groups were fumigated with 2 mg of precocene in acetone. One group was treated for 24 hours and the other group for 48 hours.

After the treatment period each group was exposed to a rabbit ear. Ten females were added to each group two days after the males were attached to the host. Ten males were removed from each group on the sixth day after attachment and dissected to determine the condition of the testes and accessory glands. One testis was removed from each male and squashed in 2% lacto-aceto-orcein. The squash preparation was then observed with a compound microscope at 100X and 430X. The ten males in each group that remained on the rabbit were allowed to mate with untreated females. The females were collected upon drop and allowed to oviposit.

Observations. Upon dissection, none of the treatment groups showed any difference in the size or physical condition of the accessory gland compared to both control groups. Squashes of the testes revealed no difference in the rate of spermatogenesis.

Females, which mated with the treated males, rapidly engorged and oviposited fertile eggs. There were no statistical differences in the amount of engorgement, number of eggs oviposited, or percentage of eggs that successfully hatched between those females which mated with treated males and those which mated with control (UC and AC) males.

Effects of Precocene on Males Treated Three Days After Attachment

Eighty virgin males were confined in an ear bag on a rabbit and allowed to attach and feed for 3 days. All ticks were removed from the rabbit and divided into four groups of 20 males each and placed in two-dram glass vials. Two groups served as controls. One control group (UC) was sealed for 48 hours. The other control group (AC) was sealed in a glass vial with an acetone-treated strip of filter paper for 48 hours.

The remaining two groups (P-3-24) and (P-3-48) were fumigated for 24 and 48 hours respectively with 2 mg of precocene in acetone. Each of the 4 groups of ticks was then confined to a separate ear bag on the host. Ten females were included in each bag.

Seven days after the initial attachment of males, ten from each group were removed and dissected to determine the condition of the testes and accessory glands by gross observation. One testis of each male was removed and squashed in 2% lacto-aceto-orcein stain to determine if spermatogenesis had been affected.

Ten males of each group were allowed to remain on their respective rabbit ears to copulate with the females. The replete females were collected and allowed to oviposit.

Observations. As in the previous experiment, the dissections showed no observable difference in either the accessory gland or the testes between the precocene-treated males and the controls. Likewise, the squashes of the testes disclosed no differences in the rate of spermatogenesis.

The females, which were mated with the treated males, engorged as fully as those mated with controls and produced egg masses of comparable size and viability. These results are the same as those reported for the previous experiment.

VII. Summary

Collaborative investigations on hormone-pheromone interactions were continued. Studies continued to emphasize the camel tick, *Hyalomma dromedarii*, the species of primary interest in the project, and the American dog tick, *Dermacentor variabilis*.

Treatment of *H. dromedarii* engorged nymphs with artifically introduced beta-ecdysone excited sex pheromone activity in unfed females following their emergence. Greater than normal increases in sex pheromone DCP were found in unfed treated females, as well as much greater (than controls) increases in ecdysteroid content. Autoradiographs revealed evidence of ecdysteroid accumulation on or in the pheromone glands following inoculation of engaged nymphs with ^3H α-ecdysone. Sex pheromone, DCP, was also found in males that emerged from beta-ecdysone treated nymphs. These and other findings described in detail in the text indicate strongly that ecdysteroid, probably similar (or identical) to beta-ecdysone, stimulates biosynthesis of sex pheromone in the young adult *H. dromedarii*.

Radioimmunoassay was used to determine the presence of ecdysteroids in various developmental stages of *D. variabilis*. Ecdysteroid was found in all stages, including embryos. Peak ecdysteroid content was found in the engorged nymphs. Increases in ecdysteroid content occurred following engorgement, with a peak on day 5 in the larval stage and day 10 in the nymphal stage. Studies to determine changes in ecdysteroid content in different physiological states of the adults are still in progress. High Pressure Liquid Chromatography demonstrated the presence of 6 steroid fractions, all with different retention times than α- or beta-ecdysone. When assayed by RIA, 3 of the fractions reacted, indicating that they were ecdysteroids (or metabolites). Further study to characterize these compounds is in progress.

Studies of the development, maturation, and ultrastructure of the pheromone glands of *H. dromedarii* were continued during the second year of the project. The existence of a pheromone gland primordium in the nymphal stage was determined. Following emergence of the females, changes in the physiological states of the pheromone glands was described. The appearance of pheromone, DCP, and changes in the lipid content of the glands needed to facilitate storage of DCP were described. Comparisons between male and female glands and ch..ges in gland physiology and ultrastructure following feeding and mating are also reported.

Studies using Precocene-2 to disrupt development and antagonize reproductive activity were completed. The primary and, probably, the only effect observed with the immatures was toxicity. A strong anti-feedant effect was observed when Precocene-2 was administered to females during feeding, but not at other periods. Anti-gonadotrophic effects were observed also.

The existence of "genital" pheromones, acting as secondary pheromones to guide the male to the female gonopore and excite copulation, are also described. Similar mechanisms exist in both genera studied (i.e., Hyalomma and Dermacentor) to the extent that the genital area pheromone is necessary to guide the male to the gonopore. In D. variabilis and D. andersoni, copulation follows only when the male probes the gonopore of a conspecific female. In H. dromedarii and H. anatolicum excavatum, differences in DCP content and the presence of a repellent deters heterospecific males. Evidence implicating the use of a sensillum on the cheliceral digits for determining the mating sex pheromone is described.

The effects of P-2 on reproduction in ticks was studied by project personnel at Georgia Southern College. Treatment of females with P-2 resulted in significant reductions in oocyte number of days post-drop. However, attempts to rescue gonadal development in the P-2 treated ticks with various concentrations of juvenile hormone were unsuccessful. Treatment of males with P-2 had no measurable effect on spermatogenesis.

VIII. Future Plans

Studies will continue to determine the precise mechanism of hormonal regulation of sex pheromone activity in ixodid ticks. The role of ecdysteroids will be explored in greater depth, with efforts to determine changes in ecdysteroid content that might be correlated with onset of sex pheromone activity. Artificially administered ecdysteroids will continue to be used as a means of assessing the response of the pheromone system to elevated concentrations of this group of hormones.

Although evidence of natural ecdysteroids in the various tick life stages has been obtained by radioimmunoassay and other means, efforts to chemically characterize these compounds are necessary. We hope to collect sufficient extracts to facilitate precise chemical identification by mass spectrometry and related sophisticated chemical methods. The different fractions will be isolated High Pressure Liquid Chromatography, their ecdysteroid character verified by RIA, and the active, purified fractions assayed by mass spectrometry to determine their molecular structure.

The interaction of juvenile hormone, acting as a gonadotrophic hormone, ecdysteroid synthesis, and the onset of sex pheromone biosynthesis will be investigated. Studies will be directed to determine whether increases in JH initiate fresh ecdysteroid biosynthesis in adults (or suppress it in immatures) and whether the resultant increase in ecdysteroid content results in pheromone biosynthesis. The current knowledge of ecdysteroid and sex pheromone activity in normal, untreated ticks will provide a basis for comparison, though concurrent controls will be included. A concomitant study will be concerned with identifying the source of ecdysteroid biosynthesis in the adult tick. All of these studies will be done at Old Dominion University or at ODU in collaboration with work done at NAMRU-3.

Studies to determine the existence and location of JH-secreting tissues (analogous to the corpora allata) in ticks will continue to be done at Georgia Southern College. Investigations of the role of JH or JH-like compounds on the gonotrophic cycle in ticks will be continued. Precocene will be used as the compound of choice because of its ability to selectively block different aspects of tick reproductive activity, presumably by reducing or eliminating natural JH, so as to facilitate an understanding of this aspect of endocrine processes in ticks.

IX. Publications and Manuscripts

1. Sonenshine, D.E., P.J. Homsher, J.S. VandeBerg, and D. Dawson. Fine structure of the foveal glands and foveae dorsales of the American dog tick, Dermacentor variabilis (Say). J. Parasitol. 67(5):627-646.

2. Hayes, M.J. and J.H. Oliver, Jr. Immediate and latent effects induced by Precocene-2 on embryonic Dermacentor variabilis (Say). J. Parasitol., in press.

3. Dees, W.H., D.E. Sonenshine, E. Breidling, and G.M. Khalil. Toxicity of Precocene-2 for developmental stages of the American dog tick, Dermacentor variabilis (Say). Submitted to J. Med. Entomol., January 11, 1982.

4. Sonenshine, D.E., P.J. Homsher, G.M. Khalil, and S.N. Mason. Dermacentor andersoni and Dermacentor variabilis: Evidence for the existence of genital sex pheromones. Manuscript submitted to Experimental Parasitology, October 6, 1981.

5. Khalil, G.M., D.E. Sonenshine, O.A. Sallam, and P.J. Homsher. Hyalomma dromedarii and Hyalomma anatolicum excavatum: Mating regulation and reproductive isolation. Completed manuscript, submitted to J. Med. Entomol. March 15, 1982.

6. Khalil, G.M., D.E. Sonenshine, W.H. Dees, and P.J. Homsher. Development, maturation, and fine structure of the pheromone glands of Hyalomma dromedarii. Completed manuscript forwarded to Dr. G.M. Khalil, USNAMRU-3, Cairo, Egypt for editing and technical approval. Submission to J. Med. Entomol. is planned for July 15, 1982.

7. Sonenshine, D.E. Pheromones of Acari and their potential use in biological strategies. Invited paper for presentation at the Symposium on Chemical and Biological Control of Acari, VIth International Congress of Acarology, Edinburgh, Scotland, September 5-11, 1982 and publication in the Proceedings (12 pp.).

8. Dees, W.H., D.E. Sonenshine, and E. Breidling. Ecdysteroids in Hyalomma dromedarii and Dermacentor variabilis and their effect on sex pheromone activity. For presentation in the section on Physiology and Biochemistry of Acari (section 6), IBID (also scheduled for publication in the Proceedings, 12 pp.).

9. Khalil, G.M., D.E. Sonenshine, R.M. Silverstein, P.J. Homsher, and H. Hoogstraal. Sex pheromones of Ixodidae. IBID (also scheduled for publication in the Proceedings, 12 pp.).

10. Sonenshine, D.E. Tick pheromones and other semicochemicals. Chapter in the three-volume book "Current Topics in Pathogen-Vector Host Relationships." Praeger Scientific, NY (completed chapter scheduled for September 1, 1982).

11. Hayes, M.J. and J.H. Oliver, Jr. 1981. Immediate and latent effects induced by the antiallatotropin Precocene-2 (P-2) on embryonic Dermacentor variabilis (Say) (Acari:Ixodidae). J. Parasitol. 67:923-927.

12. Oliver, J.H. Jr. and M.J. Hayes. Effects of Precocene-2 on adult Dermacentor variabilis (Say) (Acari:Ixodidae). Manuscript in preparation.

X. Literature Cited

Axtell, R.C. and A. LeFurgey. 1979. Comparisons of the foveae dorsales in male and female ixodid ticks Amblyomma americanum, A. maculatum, Dermacentor andersoni, and D. variabilis (Acari:Ixodidae). J. Med. Entomol. 16 (3): 173-179.

Balashov, Y.S. 1972. Bloodsucking ticks (Ixodoidea): Vectors of diseases of man and animals. Misc. Publ. Entomol. Soc. Amer. 8 : 1-376.

Barnett, S.F. 1977. Theileria. In: Parasitic Protozoa. J.P. Kreier (ed.). Academic Press , New York, NY. 4: 77-113.

Barth, R.H. Jr. 1961. Hormonal control of sex attractant production in the Cuban cockroach. Science 133: 1598-1599.

Barth, R.H. Jr. 1962. The endoerine control of mating behavior in the cockroach, Bryostria fumigata (Guêrin). Gen. Comp. Endocrinol. 2: 53-69.

Borovsky, D. 1981. In vitro stimulation of vitellogenesis in Aedes aegysti with juvenile hormone, juvenile hormone analogue (ZR515) and 20-hydroxy-ecdysone. J. Insect. Physiol. 27 (6): 371-378.

Borst, D.W. and J.D. O'Connor. 1972. Trace analysis of ecdysones by gas-liquid chromatography, radioimmunoassay and bioassay. Steroids 24 : 637-655.

Bradfield, J.Y. IV and D.L. Denliger. 1980. Diapause development in the tobacco hornworm: A role for ecdysone or juvenile hormone. Gen. Comp. Endocrinol. 41 : 101-107.

Chapman, R.F. 1971. The insects. Structure and Function. Elsevier Press, Inc., New York, NY, 819 pp.

Chow, Y.S. 1974. Electro-olfactory potential of Ixodes ticks to 2,6-dichlorophenol. In: Proc. 4th Internatl. Congr. Acarology, Hungarian Acad. Sci. Press, Budapest. pp. 501-505.

Dawes, C.J. 1971. Biological techniques in electron microscopy. Harper and Rowe, New York, NY. 193 pp.

Delbecque, J.P., P.A. Diehl and J.D. O'Connor. 1978. Presence of ecdysone and ecdysterone in the tick Amblyomma hebraeum Koch. Experientia 34: 379-1380.

Endo, K. 1973. Hormonal regulation of mating in the butterfly, Polygonia C-auereum L. Develop. Growth and Differentiation. 15: 1-18.

Engleman, F. and R.H. Barth. 1968. Endocrine control of female receptivity in Leueophaea maderae (Blatteria). Ann. Entomol. Soc. Amer. 61: 503-505.

Feng, K.C. and W.L. Roelofs. 1977. Sex pheromone gland development in Redbanded Leafroller Moth, Argyrotaenia velutinana, pupae and adults. Ann. Entomol. Soc. Amer. 70 (5): 721-732.

Graf, J.F. 1975. Ecologie et ethologie d'Ixodes ricinus L. en Suisse (Ixodoidea:Ixodidae). Cinquieme note. Mise en evidence d'une pheromone sexuelle chez Ixodes ricinus. Acarologia 17 (3): 436-441.

Hagedorn, H.H., J.D. O'Connor, M.S. Fuchs, B. Sage, D.A. Schlaeger and M.K. Bohm. 1975. The ovary as a source of α-ecdysone in an adult mosquito. Proc. Nat. Acad. Sci. 72: 3255-3259.

Haggart, D.A. and E.E. Davis. 1981. Neurons sensitive to 2,6-dichlorophenol on the tarsi of the tick, Amblyomma americanum (Acari:Ixodidae). J. Med. Entomol. 18 (3): 187-193.

Hetru, C. and D.H.S. Horn. 1980. Phytoecdysteroids and zooecdysteroids. In: Hoffman, J.A. (ed.) Progress in ecdysone research. Elsevier/North Holland Biomedical Press, New York, NY. pp. 13-28.

Hoogstraal, H. 1956. African Ixodoidea. 1. Ticks of the Sudan (with special reference to Equatoria Province and with preliminary review of the genera Boophilus, Margaropus, and Hyalomma). Dep. Navy, Bur. Med. Surgery, Washington, D.C. 1101 pp.

Hoogstraal, H. 1967. Ticks in relation to human diseases caused by Rickettsia species. Annu. Rev. Entomol. 12: 377-420.

Hoogstraal, H. 1973. Viruses and ticks. Chap. 18. In: Viruses and invertebrates. A.J. Gibbs (ed.). Elsevier/North Holland Pub. Co., Inc., New York, NY. pp. 349-390.

Humason, G.L. 1972. Animal tissue techniques. (3rd. ed.). W.H. Freeman and Co., San Francisco. 641 pp.

Jefferson, R.N., H.H. Shorey, and L.K. Gaston. 1966. Sex pheromones of noctuid moths. X. The morphology and histology of the female sex pheromone gland of Trichoplusia ni (lepidoptera:Noctuidae). Ann. Entomol. Soc. Amer. 59 (6): 1166-1169.

Kellum, D. and R.S. Berger. 1977. Relationship of the occurrence and function of 2,6-dichlorophenol in two species of Amblyomma. (Acari: Ixodidae). J. Med. Entomol. 13 (6): 701-705.

Khalil, G.M., S.A. Nada, and D.E. Sonenshine. 1981. Sex pheromone regulation of mating behavior in the camel tick Hyalomma dromedarii (Ixodoidea:Ixodidae). J. Parasitol. 67 (1): 70-76.

Khalil, G.M., D.E. Sonenshine, O.M. Sallam, and P.J. Homsher. Mating regulation and reproductive isolation in the ticks, Hyalomma dromedarii and H. anatolicum excavatum (Ixodoidea:Ixodidae). Unpublished manuscript.

Kittredge, J.S. and F.T. Takahashi. 1972. The evolution of sex pheromone communication in the Arthropoda. J. Theor. Biol. 35: 467-471.

Leahy, M.G., G. Karuhize, C. Mango, and R. Galun. 1975. An assembly pheromone and its perception in the tick, Ornithodoros moubata (Murray) (Acari:Argasidae). J. Med. Entomol. 12: 284-287.

Leahy, M.G., and K.S. Booth. 1978. Perception of a sex pheromone, 2,6-dichlorophenol, in hard ticks. Proc. Tick Borne Diseases and their vectors. Univ. of Edinburgh. 1977. In press.

Mango, C.K.A., T.R. Odhiambo, and R. Galun. 1976. Ecdysone and the super tick. Nature (Lond.) 260: 318-319.

Mango, C.K.A. 1978. Effects of beta-ecdysone and ponasterone A on nymphs of the soft tick Ornithodoros moubata (O. p. porcinus, Walton 1962). In: Wilde, J. (ed.). Tick-borne Diseases and Their Vectors. pp. 35-37.

Oliver, J.H. Jr., P.R. Wilkinson, and G.M. Kohls. 1972. Observations on hybridization of three species of North American Dermacentor ticks. J. Parasitol. 58 (2): 380-384.

Petney, T.N. and C.M. Bull. 1981. A non-specific aggregation pheromone in two Australian reptile ticks. Anim. Behav. 29: 181-185.

Rechav, Y., S. Terry, M.M. Knight, and R.H.M. Cross. 1977. Chemoreceptor organs used in detection of pheromone(s) of the tick, Amblyomma hebraeum (Acarina:Ixodidae). J. Med. Entomol. 14 (1): 71-78.

Reum, L. and J. Koolman. 1979. Analysis of ecdysteroid by radioimmunoassay: Comparison of three different antisera. Insect Biochem. 9 :135-142.

Riddiford, L.M. and J.W. Truman. 1978. Biochemistry of insect hormones and insect growth regulators. In: M. Rockstein (ed.). pp. 307-357. Academic Press, New York, NY.

Roelofs, W.L. 1978. In: Rockstein, M. (ed.). Biochemistry of Insects. Academic Press, Inc., New York, NY. 649 pp.

Ryerse, J.S. 1980. The control of malpighian tubule developmental physiology by 20-hydroxyecdysone and juvenile hormone. J. Insect Physiol. 26: 449-457.

Sannasi, A. and T. Subramoniam. 1972. Hormonal rupture of larval diapause in the tick Rhipiciphalus sanguineus (Lat.). Experientia 28: 666-667.

Smith, R.D., and M. Ristic. 1977. Ehrlichia. In: Parasitic Protozoa. J.P. Kreier (ed.). Academic Press, New York, NY. 4: 295-328.

Sokal, R.R. and F.J. Rohlf. 1969. Biometry. The principles and practice of statistics in biological research. W.H. Freeman and Co., San Francisco, CA. 776 pp.

Sonenshine, D.E., R.M. Silverstein, E.C. Plummer, J.R. West and T. McCullough. 1976. 2,6-dichlorophenol, the sex pheromone of the Rocky Mountain wood tick, Dermacentor andersoni Stiles and the American dog tick, Dermacentor variabilis (Say). J. Chem. Ecol. 2: 201-209.

Sonenshine, D.E., R.M. Silverstein, L.A. Collins, M. Saunders, C. Flynt, and P.J. Homsher. 1977. Foveal glands, source of sex pheromone production in the ixodid tick Dermacentor andersoni Stiles. J. Chem. Ecol. 3: 695-706.

Sonenshine, D.E., P.J. Homsher, J.S. VandeBerg, and D. Dawson. 1981. Fine structure of the foveal glands and foveae dorsales of the American dog tick, Dermacentor variabilis (Say). J. Parasitol. 67 (5): 627-646.

Sonenshine, D.E., D.M. Ganisburg, and P.J. Homsher. Quinone inhibition of sex pheromone activity in the ticks Dermacentor andersoni Stiles and Dermacentor variabilis (Say). J. Parasitol (in press/a).

Sonenshine, D.E., R.M. Silverstein, and Y. Rechav. Tick pheromone mechanisms. In: The physiology of ticks. F.D. Obenchain and R. Galun (eds.). Pergammon Press (in press/b).

Sonenshine, D.E., G.M. Khalil, P.J. Homsher, and S.N. Mason. Evidence for the existence of genital sex pheromones in Dermacentor variabilis and D. andersoni. Exp. Parasitol. (in press/c).

Treverrow, N.L., B.F. Stone, and M. Cowie. 1977. Aggregation pheromones in two Australian hard ticks, Ixodes holocyclus and Aponomma concolor. Experientia, 33 (5): 680-683.

Uebel, E.C., M. Schwarz, R.W. Miller, and R.E. Menzer. 1978. Mating stimulant pheromone and cuticular lipid constituents of Fannia femoralis (Stein) (Diptera:Muscidae). J. Chem. Ecol. 4: 83-91.

Uilenberg, G. 1976. Tick-borne livestock diseases and their vectors. 2. Epizootiology of tick-borne diseases. World Animal Rev. 17: 8-15.

Vernick, S.H., S. Thompsen, D.E. Sonenshine, L.A. Collins, M. Saunders, and P.J. Homsher. 1978. Ultrastructure of the foveal glands of the ticks, Dermacentor variabilis (Say) and Dermacentor andersoni Stiles (Acari: Ixodidae). J. Parasitol. 64: 515-523.

Weseloh, R. M. 1980. Sex pheromone gland of the Gypsy Moth Parasitoid, Apanteles melanoscelus. Revaluation and ultrastructural Survey. Ann. Entomol. Soc. Amer. 73 (5): 576-580.

Wood, W.F., M.G. Leahy, R. Galun, G.D. Prestwich, J. Meinwald, R.E. Purnell, and R.C. Payne. 1975. Phenols as pheromones of ixodid ticks: A general phenomenon. J. Chem. Ecol. 1: 501-509.

Wright, J. 1969. Hormonal termination of larval diapause in Dermacentor albipictus. Science 163: 390-391.

Ziv, M., D.E. Sonenshine, R.M. Silverstein, J.R. West, and K.H. Gingher. Use of the sex pheromone, 2,6-dichlorophenol, to disrupt mating by the American dog tick, Dermacentor variabilis (Say). J. Chem. Ecol. (in press).

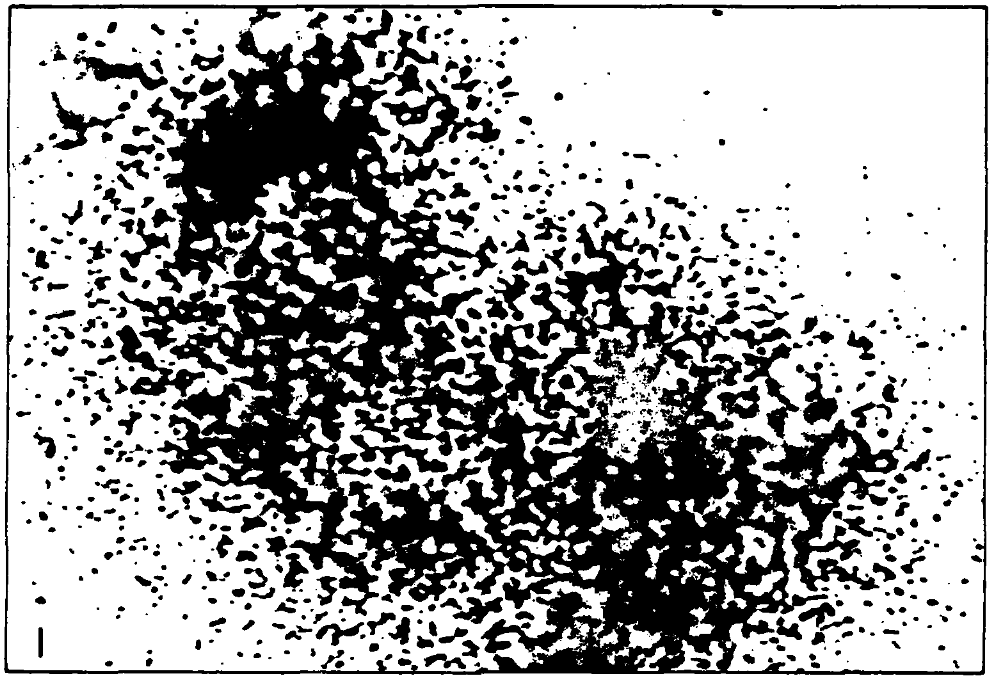


Figure 1. Autoradiograph of a frozen section of the foveal glands from unfed female _H. dromedarii_ that emerged from nymphs inoculated with ^{3}H alpha ecdysone. The intense accumulation of silver grains virtually obscures the large secretory cells of the gland lobes (1000 ×).

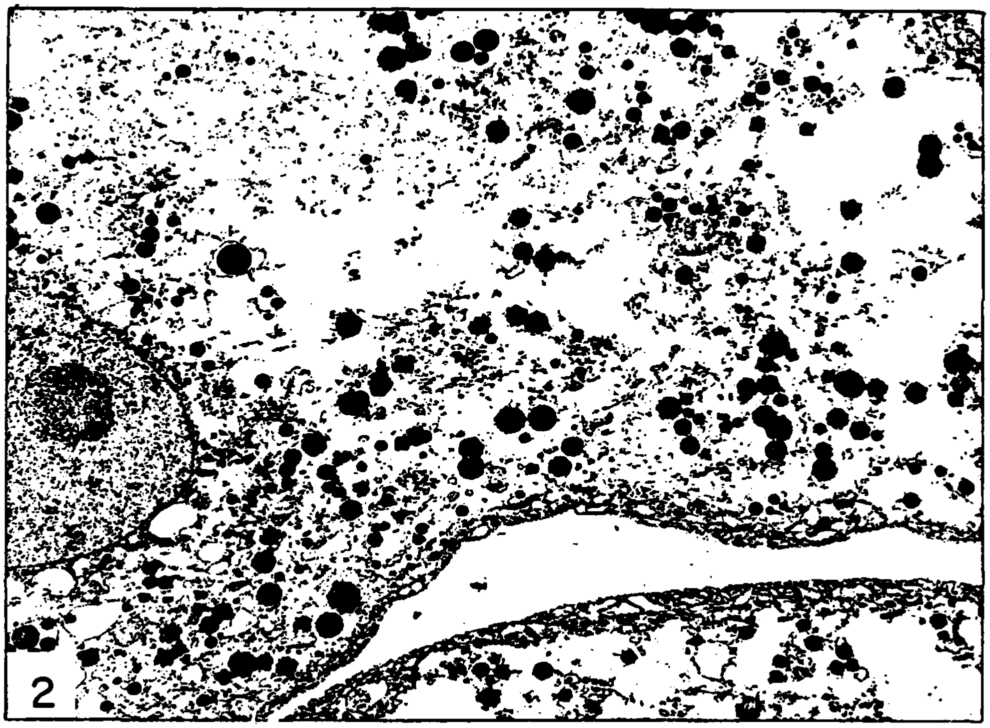


Figure 2. Transmission electron micrograph of a section of the secretory lobe of the sex pheromone gland of unfed female H. dromedarii from a beta-ecdysone inoculated nymph illustrating extensive vesicular disruption and release of neutral lipid secretory droplets (nld) free in the cytoplasm (n = nucleus, 5,640 ×).

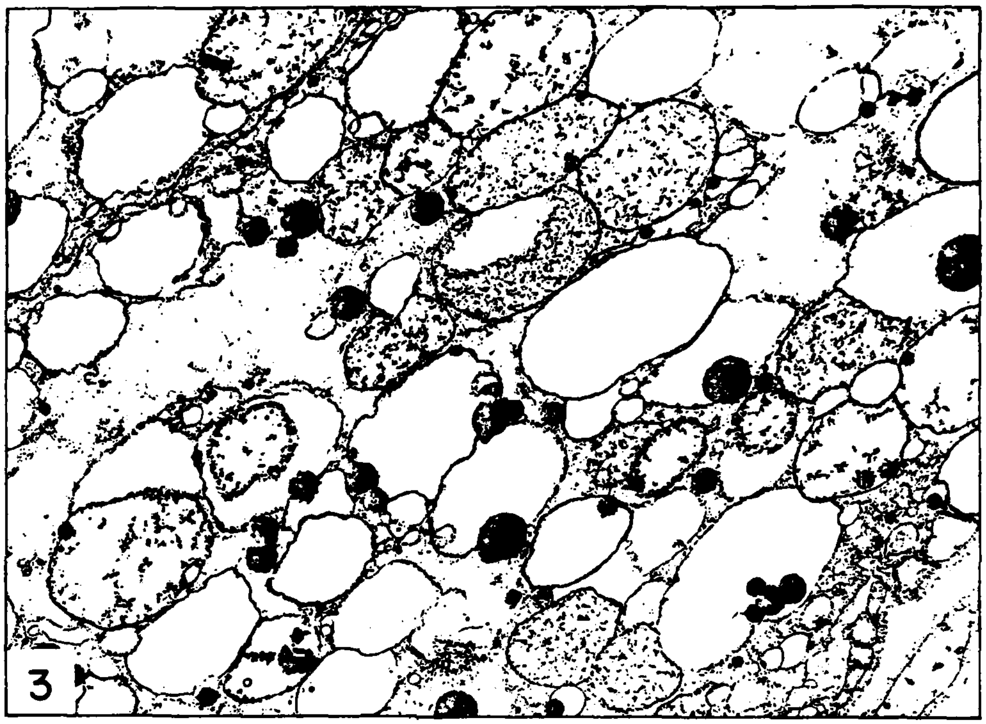


Figure 3. Transmission electron micrograph of a section of the secretory lobe of the sex pheromone gland of unfed female H. dromedarii from a 1% saline inoculated nymph illustrating the vesicle enclosed neutral lipid secretory droplets (nld). Most vesicles appear intact (vm = vesicle membrane; 13,000 ×).

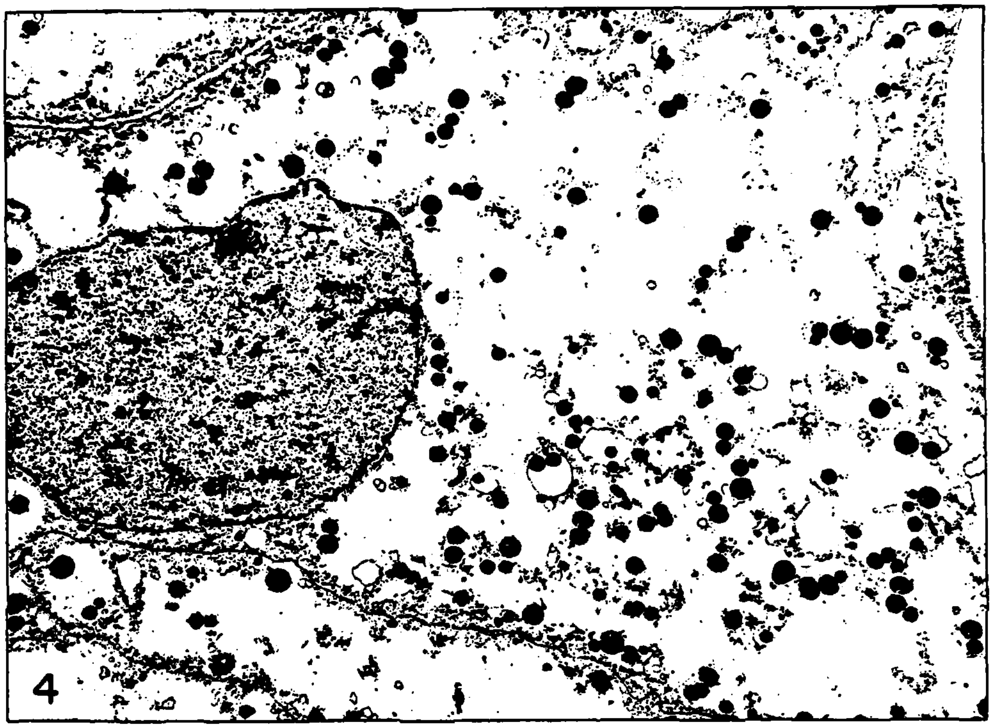


Figure 4. Transmission electron micrograph of a section of the secretory lobe of the sex pheromone gland of unfed female D. variabilis from a beta-ecdysone inoculated nymph illustrating vesicular disruption and neutral lipid secretory droplets (nld) free in the cytoplasm (n = nucleus; 8,100 ×).

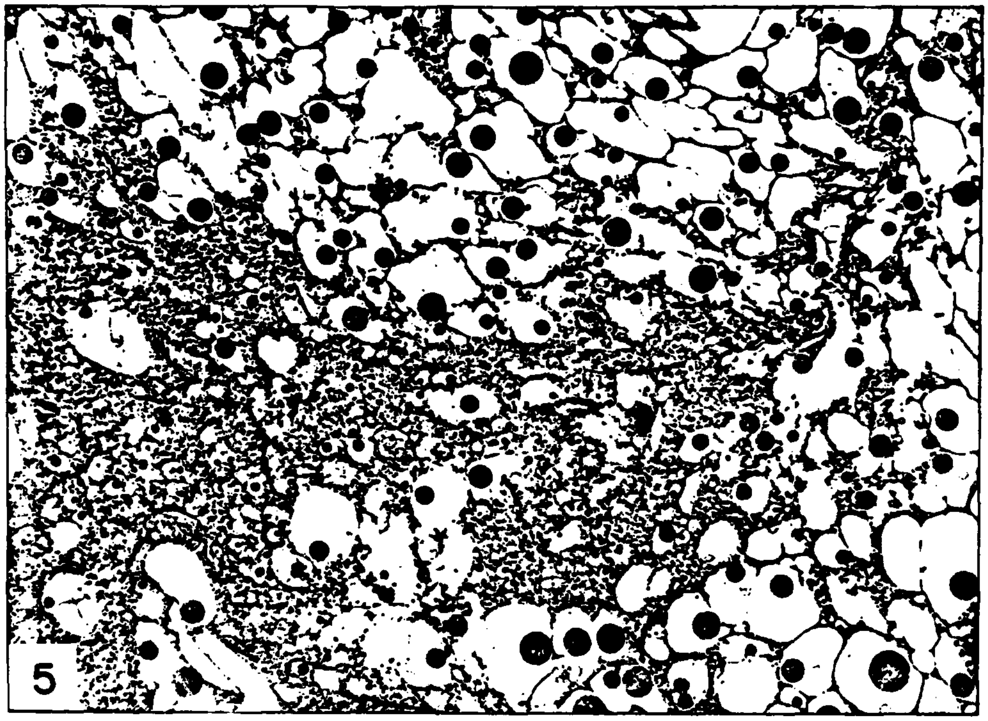


Figure 5. Transmission electron micrograph of a section of the secretory lobe of the sex pheromone gland of unfed female D. variabilis from a 1% saline inoculated nymph illustrating the extensive vesiculation of the cytoplasm of the lobe cell and the enclosure of the neutral lipid secretory droplets (nld), each within its own vesicle.

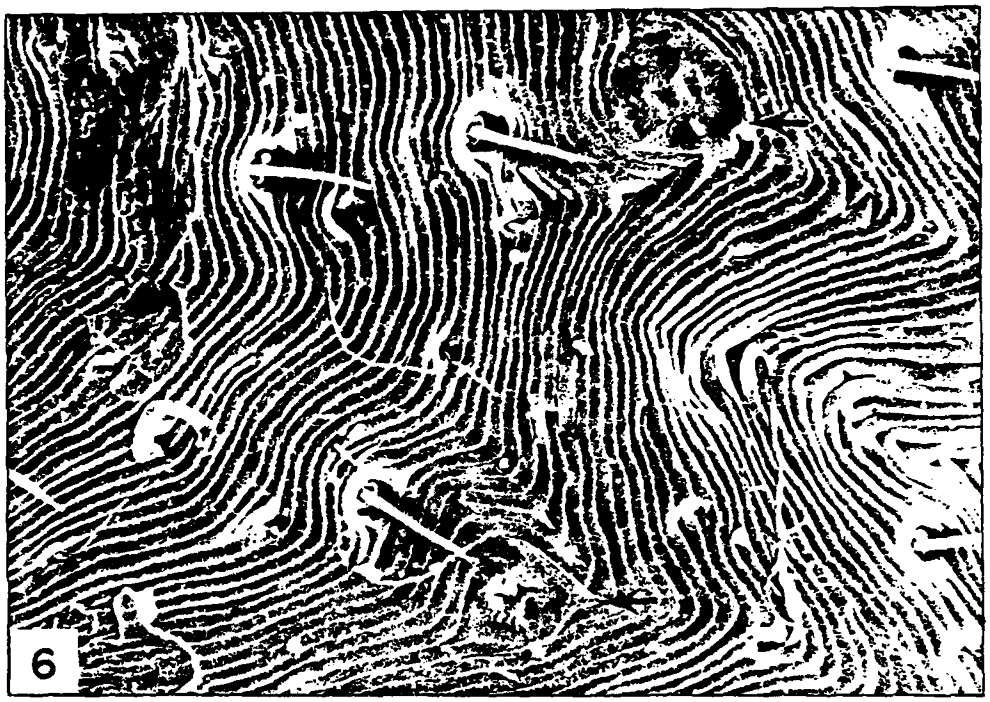


Figure 6. Scanning electron micrograph illustrating the primordia of the foveal dorsal s (arrow) on the dorsal alloscutal surface of an unfed H. dromedarii nymph. Three pores are visible in each primordium (1,050 ×).

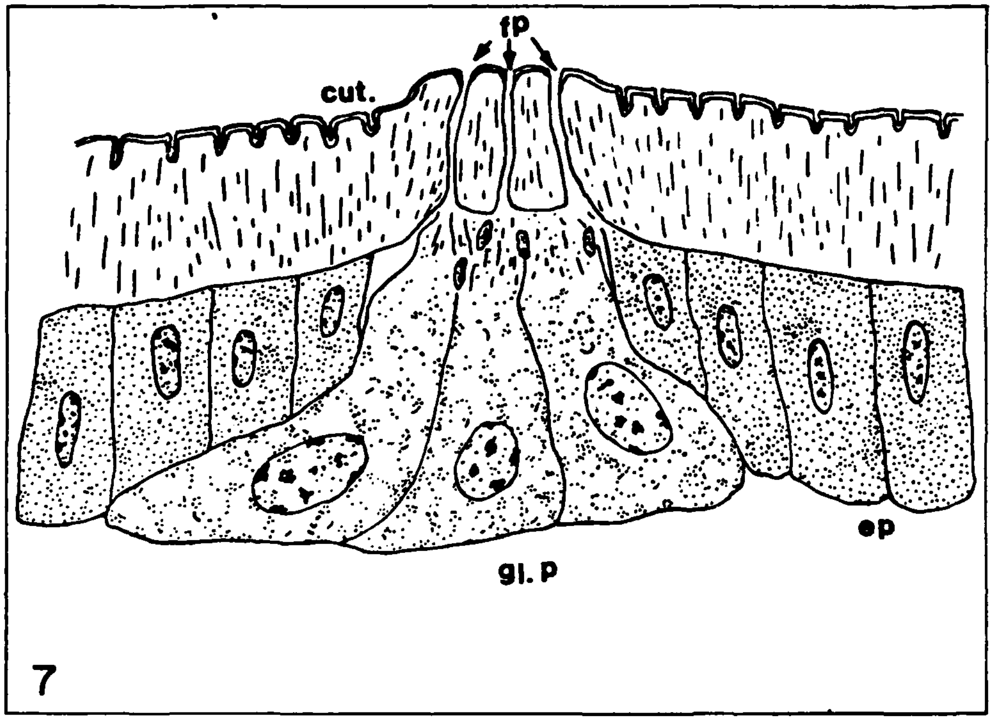


Figure 7. Diagrammatic representation from photomicrographs of a cross-section through the cuticle and underlying tissues of an engorged H. dromedarii nymph five days post-engorgement. The gland primordium (gl. p.), containing at least three cells, is associated with minute channels and pores (f. p.) in the cuticle (cut) (650 ×).

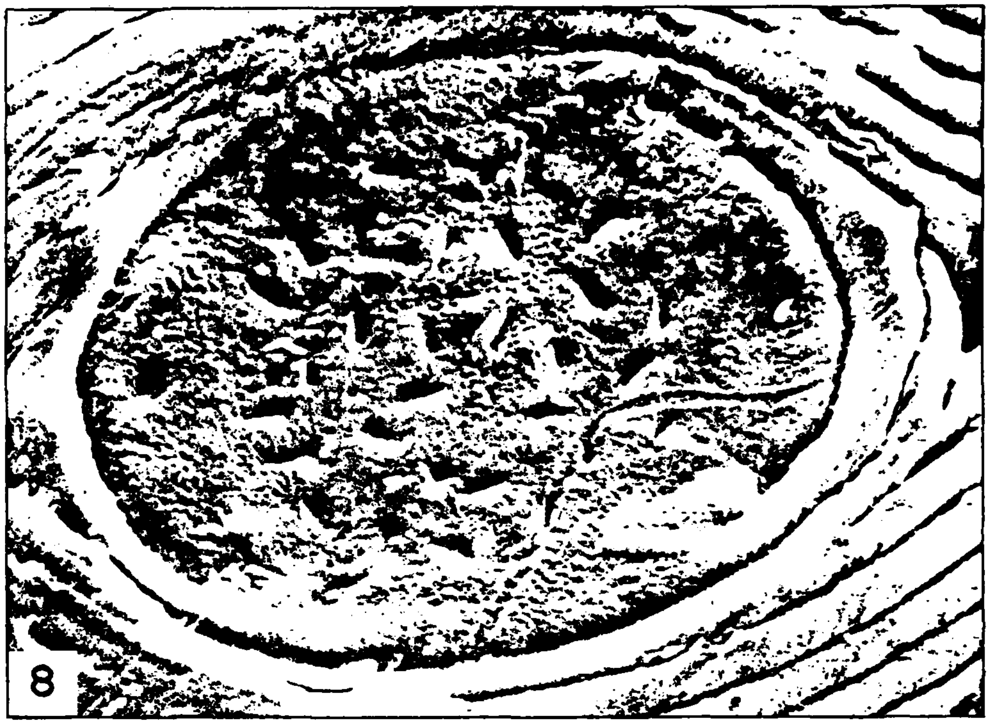


Figure 8. Scanning electron micrograph of the surface of the fovea dorsalis of adult H. dromedarii, unfed female with approximately 28 pores, illustrating the numerous slit-like pores in each fovea (1,440 ×).

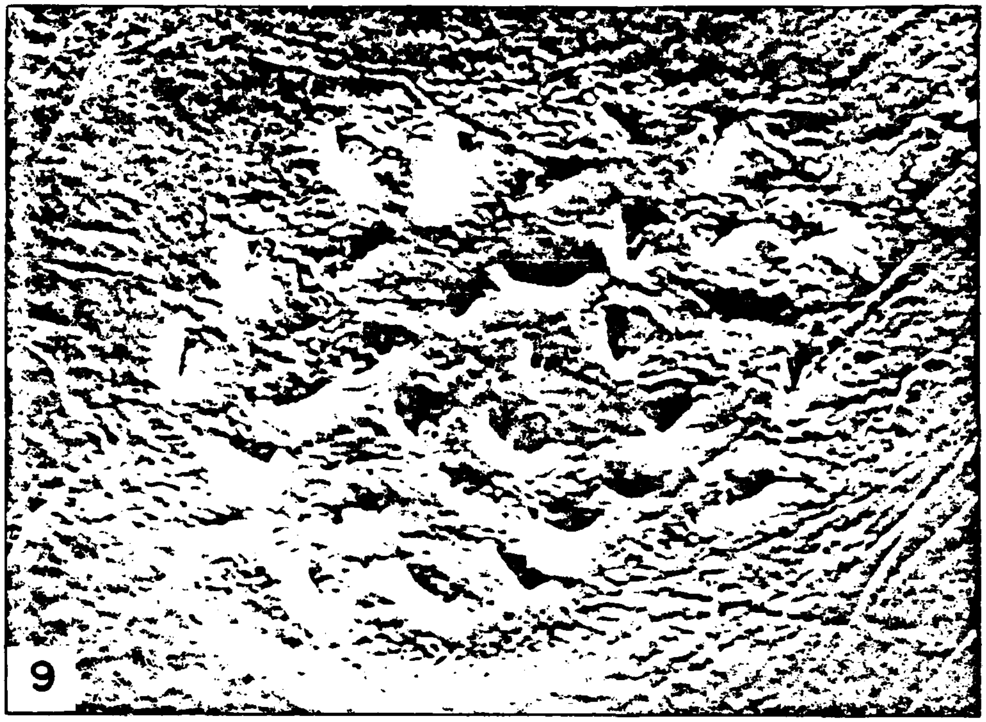


Figure 9. Scanning electron micrograph of the surface of the fovea dorsalis of adult H. dromedarii, unfed male with approximately 18 pores, illustrating the numerous slit-like pores in each fovea (2,060 ×).

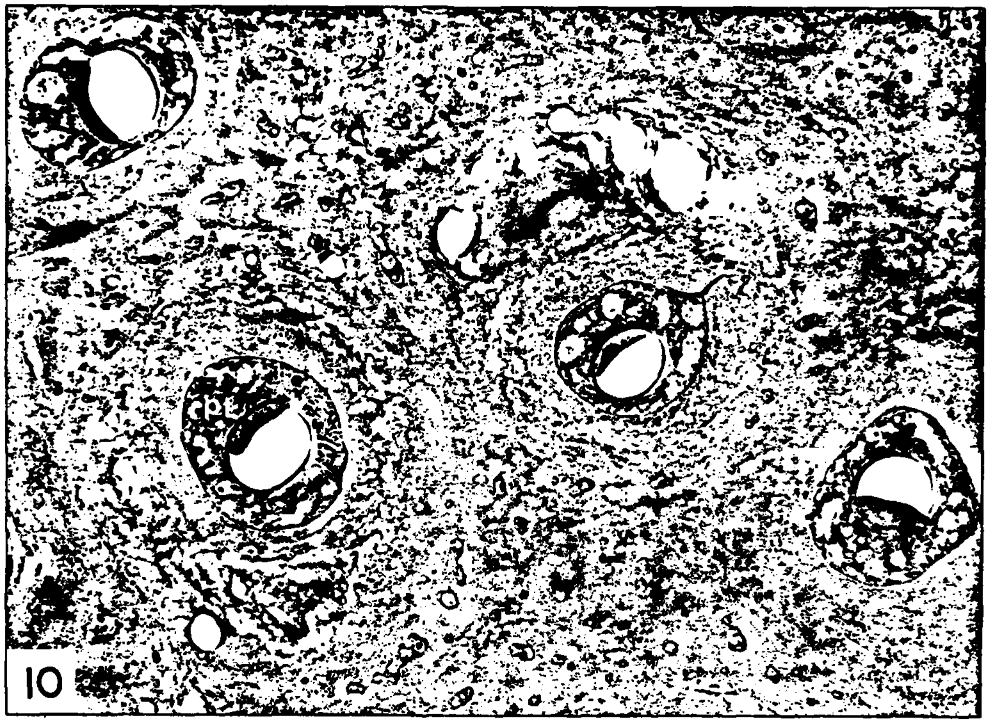


Figure 10. Transmission electron micrograph of the foveal pores of a mature unfed female H. dromedarii, pores and pore tubes (pt). The pore tubes are similar to one another, each with a patent lumen and an amorphous, kidney-bean-shaped object within. The tube lining is clearly differentiated from the cytoplasm of the pore tube cells (6,250 ×).

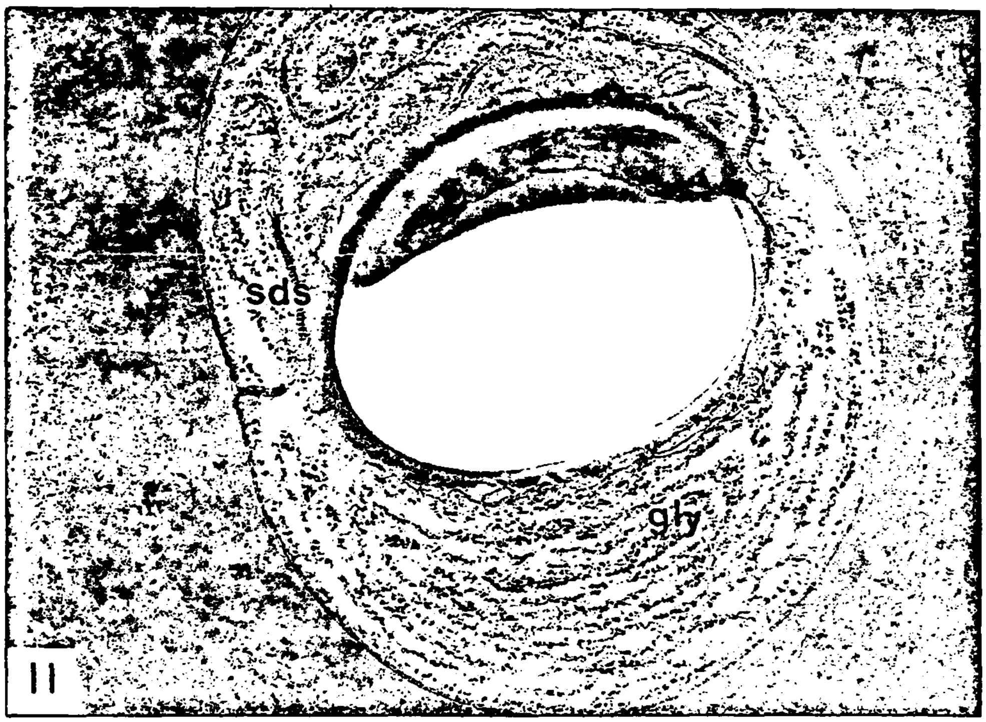


Figure 11. Transmission electron micrograph of the foveal pores of a mature unfed female H. dromedarii, enlargement of a pore tube. The intra-luminal object (noted in the figure) has an intensely electron-dense core surrounded by a less dense, amorphous zone. The relatively thick tube wall resembles cuticle in appearance. The cells surrounding the tube contain numerous minute, electron-dense granules in a somewhat vacuolated cytoplasm. The cells are joined by a septate desmosome (sds) (30,400 ×).

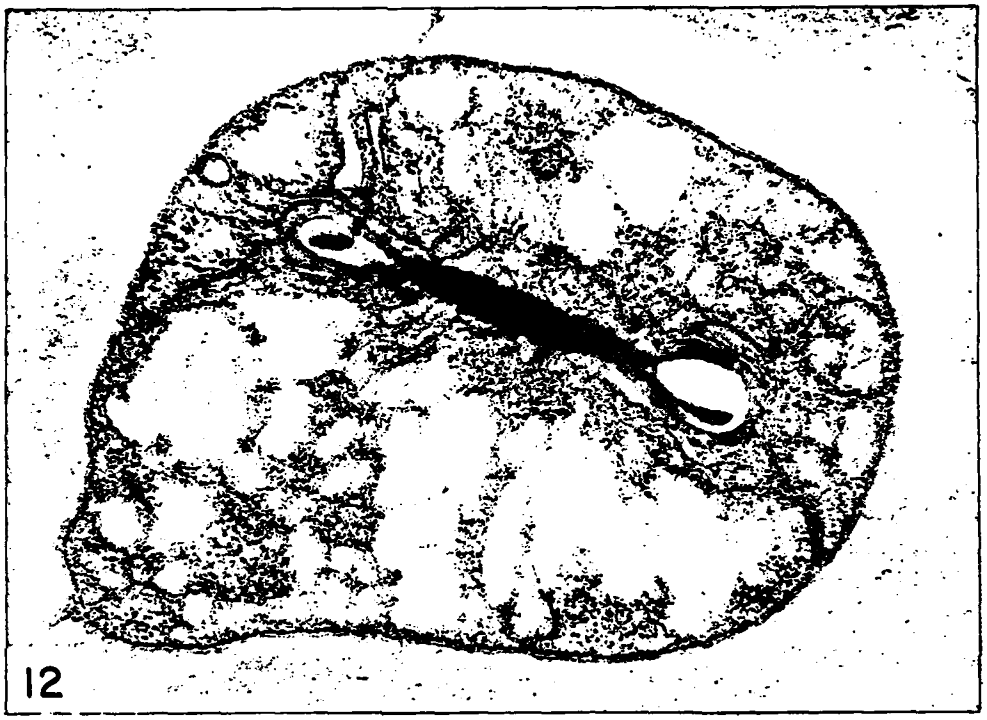


Figure 12. Transmission electron micrograph of the foveal pores of a mature unfed female H. dromedarii, pore tube with a closed lumen. The cells are much more high vacuolated than in the open pore tubes (39,117 ×).

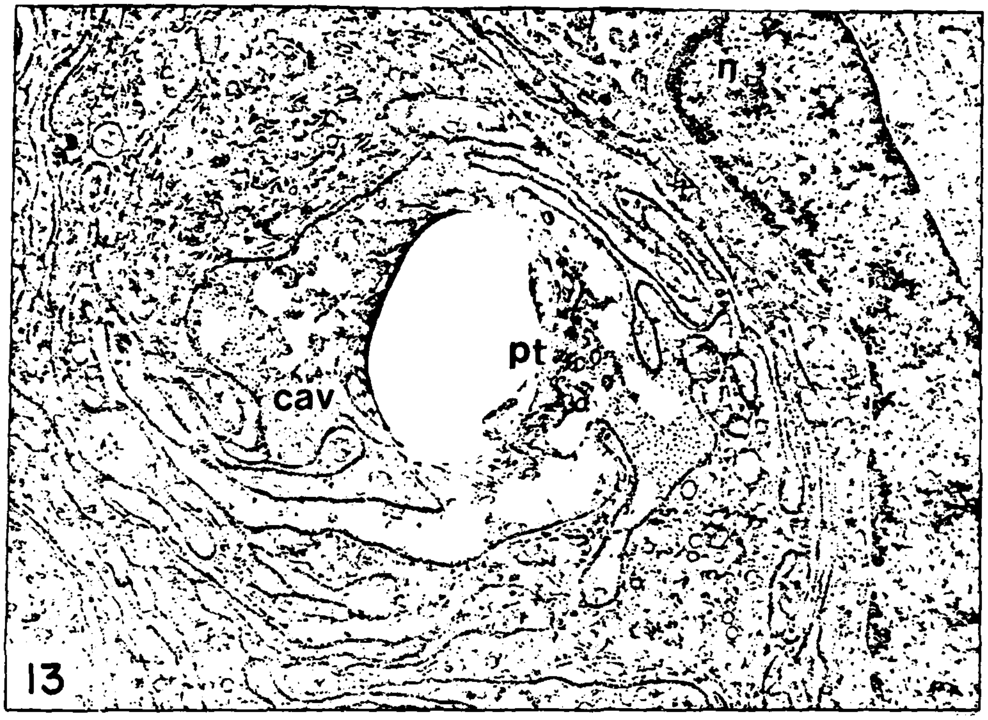
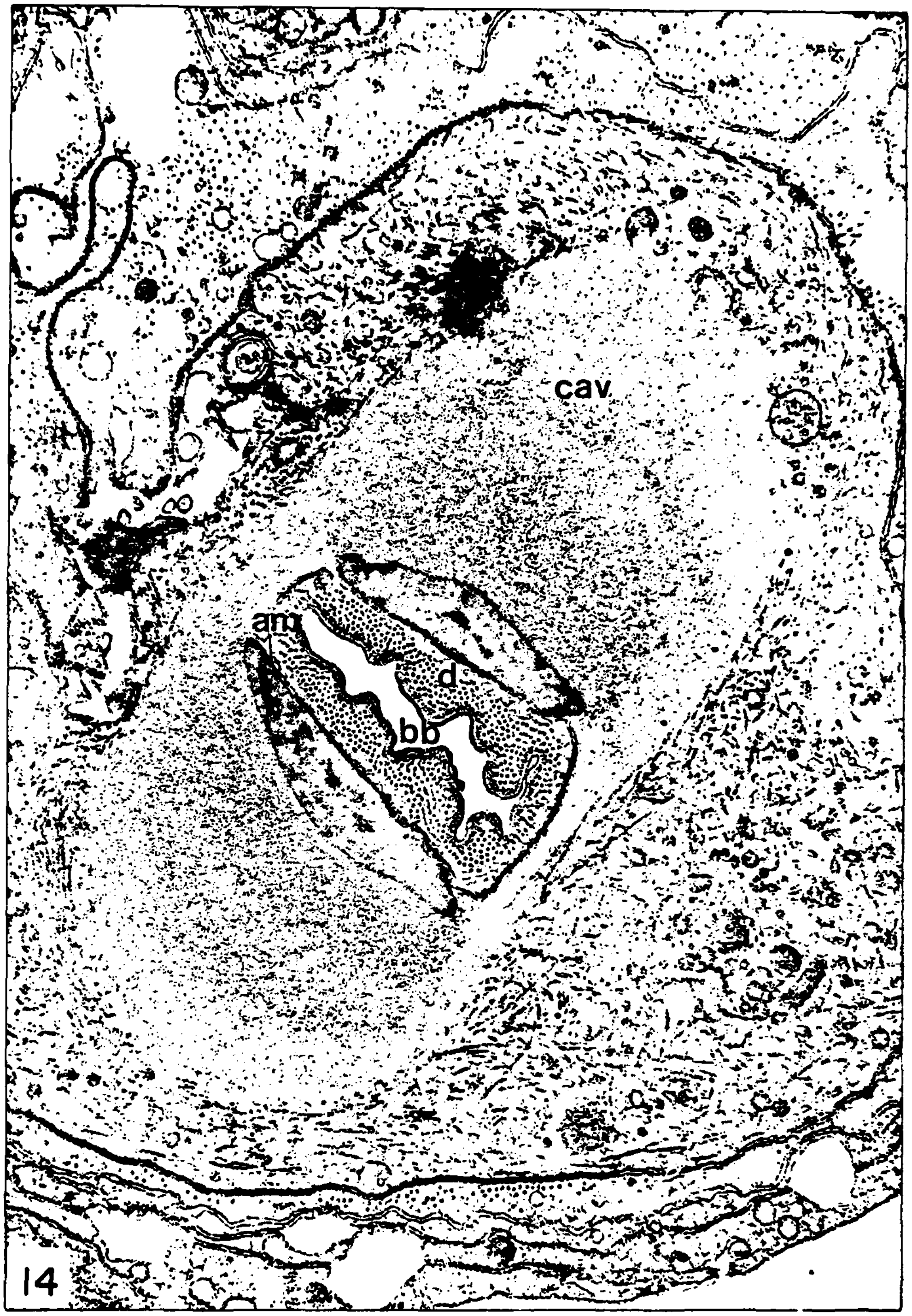


Figure 13. Transmission electron micrograph of the foveal pores of a mature unfed female H. dromedarii, pore tube (pt) extended into the zone of the ducts. Tube cells are absent. The pore tube is surrounded by numerous interdigitating, membranous extensions of the support cells typical of this zone (cav = cavity; n = nucleus of support cell; 10,886 ×).



cav
am
d
bb
14

Figure 14. Transmission electron micrograph of the duct zone of the foveal gland of mature unfed female H. dromedarii, ampulla (am), duct (d), cavity (cav), and surrounding support cells. The innermost structure is the terminus of a duct supported internally by structural microtubules. The inner lining bears a brush border (bb). Surrounding the duct is the ampulla, an extension of the lining wall of the pore tube. Surrounding the ampulla and duct is a small cavity filled with an intensely granular and presumably gelatinous substance near the interior, but with scattered fibrils and particles near the periphery. Folded membranes with numerous structural microtubules enclose the cavity (26,162 ×).

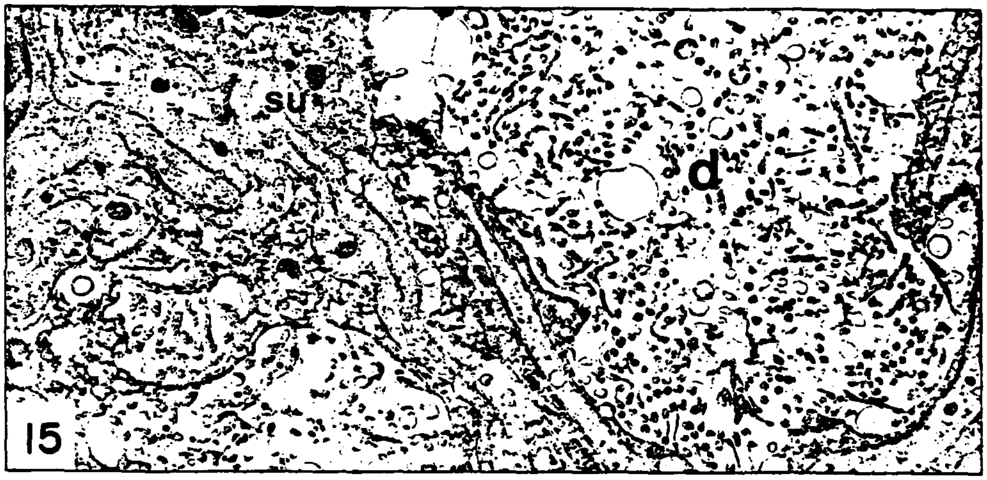


Figure 15. Transmission electron micrograph of the duct zone of the foveal gland of partially fed female H. dromedarii, cross-section through a duct (d). Note the numerous microvilli of varying length lining the duct lumen: occasional vesicles occur also. Highly folded membranes of a support cell (su) and another duct occur nearby (14,200 ×).

Figure 16. Transmission electron micrograph of the duct zone of the foveal gland of a partially fed female H. dromedarii, cross-section through a duct. Note the intensely electron-dense droplets, one in the duct, the other at its edge (a-row) (14,200 ×).

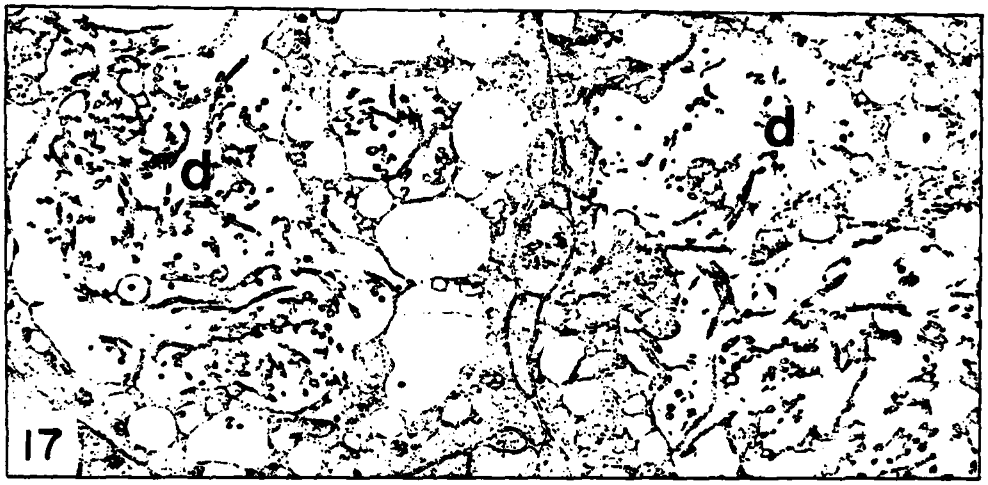


Figure 17. Transmission electron micrograph of the duct zone of the foveal gland of an unfed female H. dromedarii, cross-section through two ducts showing vesicles in and around the duct, but no electron-dense droplets (11,400 ×).

Figure 18. Transmission electron micrograph illustrating a section through a fiber cluster of the foveal nerve of an unfed female H. dromedarii. A fasicle (f) surrounds the nerve (fn). The neurilemma (nl) which forms a layer under the fasicle, surrounds the fiber cluster and also gives rise to extensions which ramify among the fiber to surround and enclose individual axons and dendrites. Glial cells (gl) with large nuclei are visible between the fibers (5,500 ×).

Figure 19. Transmission electron micrograph of the foveal nerve of an unfed female _H. dromedarii_, illustrating details of individual fibers. Note the cluster of neurosecretory vesicles (nsv) in one of the fibers (23,660 ×).

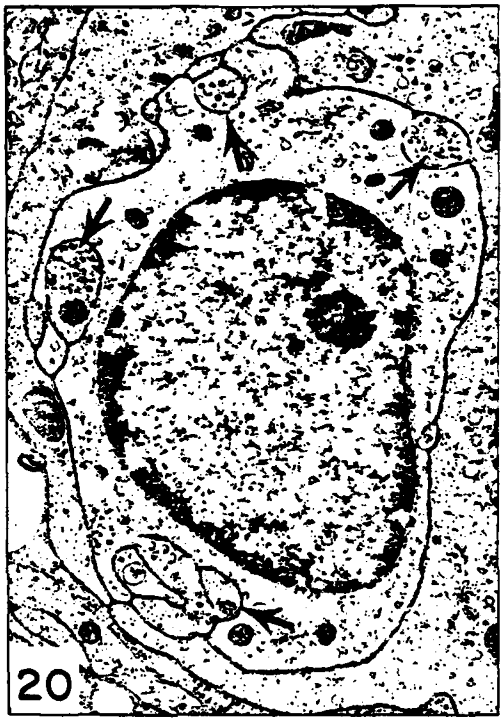


Figure 20. Transmission electron micrograph of a section illustrating branches of the foveal nerve of an unfed female H. dromedarii in the duct region of a day zero unfed female. Individual fibers and small fiber clusters (arrows) occur adjacent to a support cell, indicating ramification of the nerve fibers among the duct tissues. Electron-lucid vesicles are abundant in some of the nerve fibers (15,500 ×).

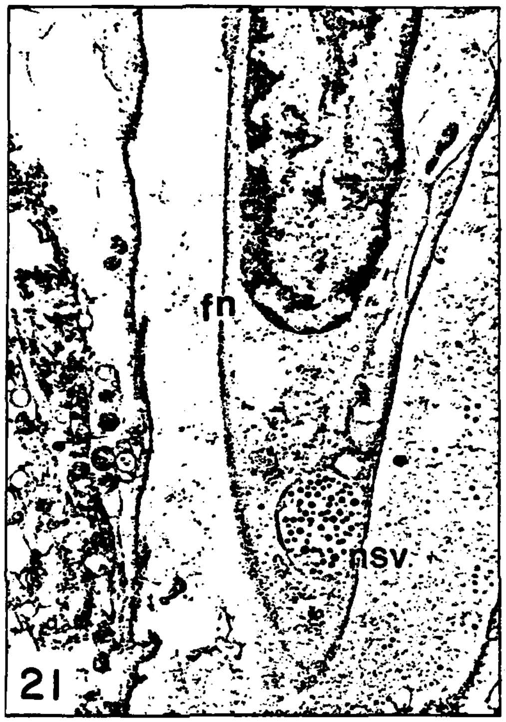


Figure 21. Transmission electron micrograph of a section of the foveal nerve (fn) of day zero unfed female H. dromedarii illustrating an accumulation of electron-dense neurosecretory vesicle (nsv) in one of the fibers (15,300 ×).

Figure 22. Scanning electron micrograph of the foveal gland of a mature, unfed female H. dromedarii. Lobe clusters of the paired foveal glands (fg) adjacent to the heart (h) (319 ×).

Figure 23. Scanning electron micrograph of the foveal gland of a mature, unfed female H. dromedarii. Enlargement illustrates surface detail of several lobes (1269 ×).

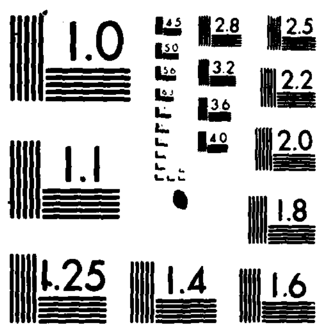
AD-A114 890 OLD DOMINION UNIV NORFOLK VA DEPT OF BIOLOGICAL SCIENCES F/G 6/1
HORMONAL INTERFERENCE WITH PHEROMONE SYSTEMS IN PARASITIC ACARI--ETC(U)
MAY 82 D E SONENSHINE, P J HOMSHER N00014-80-C-0546

UNCLASSIFIED NL

2 of 2

END
DATE
FILMED
6-82
DTIC

1489



MICROCOPY RESOLUTION TEST CHART
NATIONAL BUREAU OF STANDARDS-1963-A

Figure 24. Scanning electron micrograph of the foveal gland of a mature, unfed male H. dromedarii. Each lobe cluster contains only 8 or 9 small lobes, and the two clusters are so close together that they appear as one gland (468 ×).

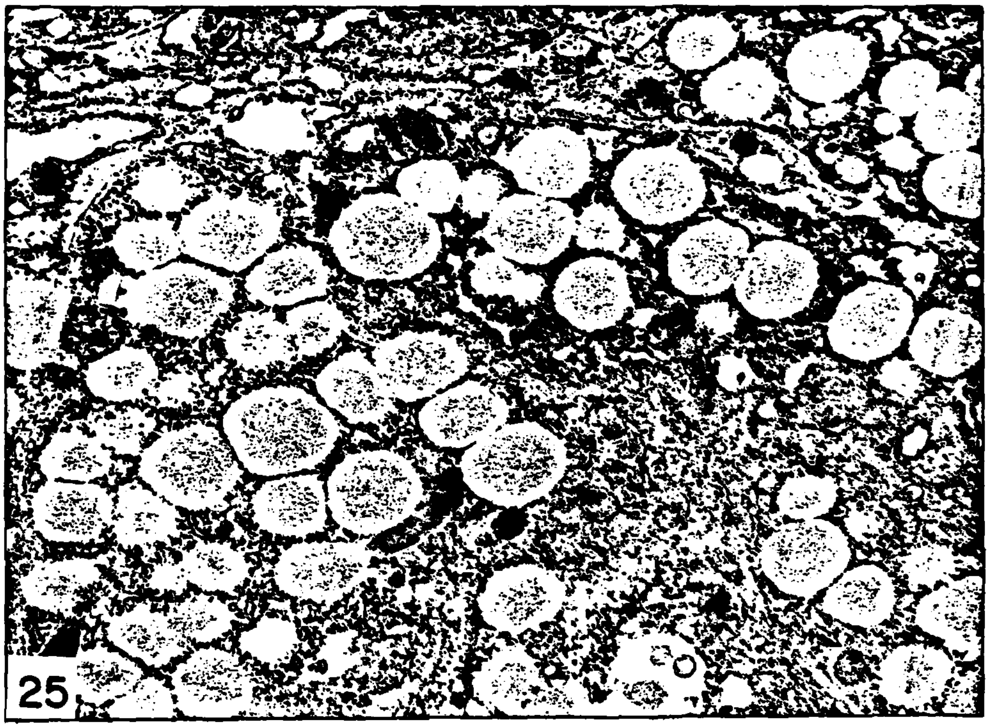


Figure 25. Section illustrates varying states of the secretory lobe contents in relation to age and feeding in H. dromedarii females. Intact vesicles with minute granular contents, but no electron-dense secretory droplets, in a day zero unfed female (12,713 ×).

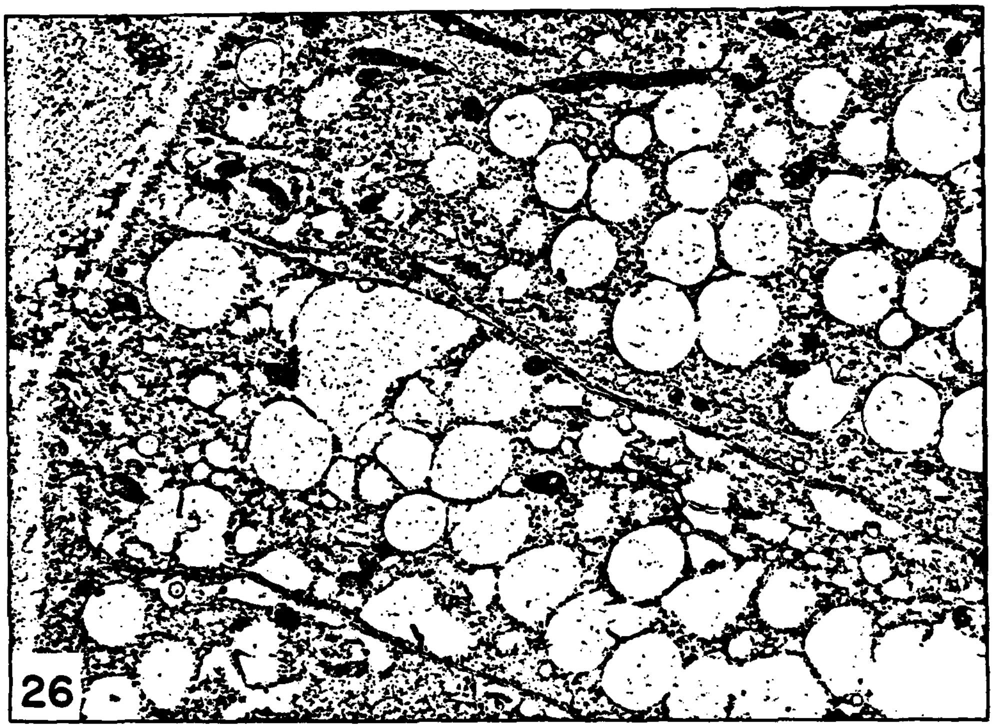
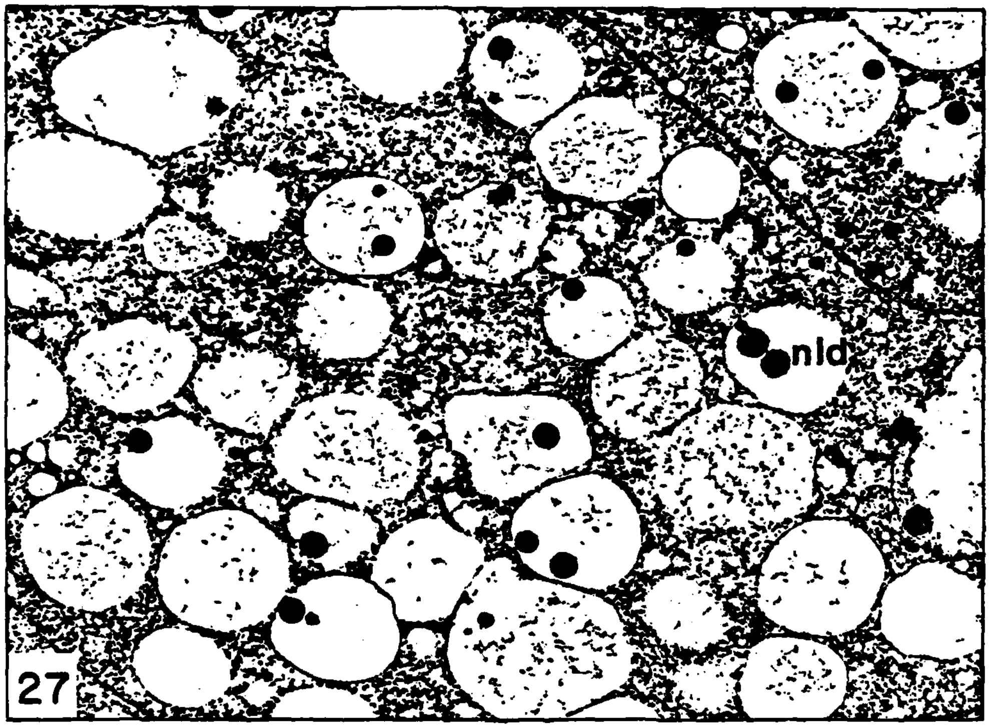


Figure 26. Section illustrates varying states of the secretory lobe contents in relation to age and feeding in _H. dromedarii_. Intact vesicles with amorphous contents, but no electron-dense secretory droplets, in a day zero unfed female (10,500 ×).



27

Figure 27. Section illustrates varying states of the secretory lobe contents in relation to age and feeding in H. dromedarii. Intact vesicles, many with 1, 2 or even more electron-dense neutral lipid secretory droplets (nld) in a secretory lobe of a mature, unfed female. The droplets are relatively small and uniform in size (9,000 ×).

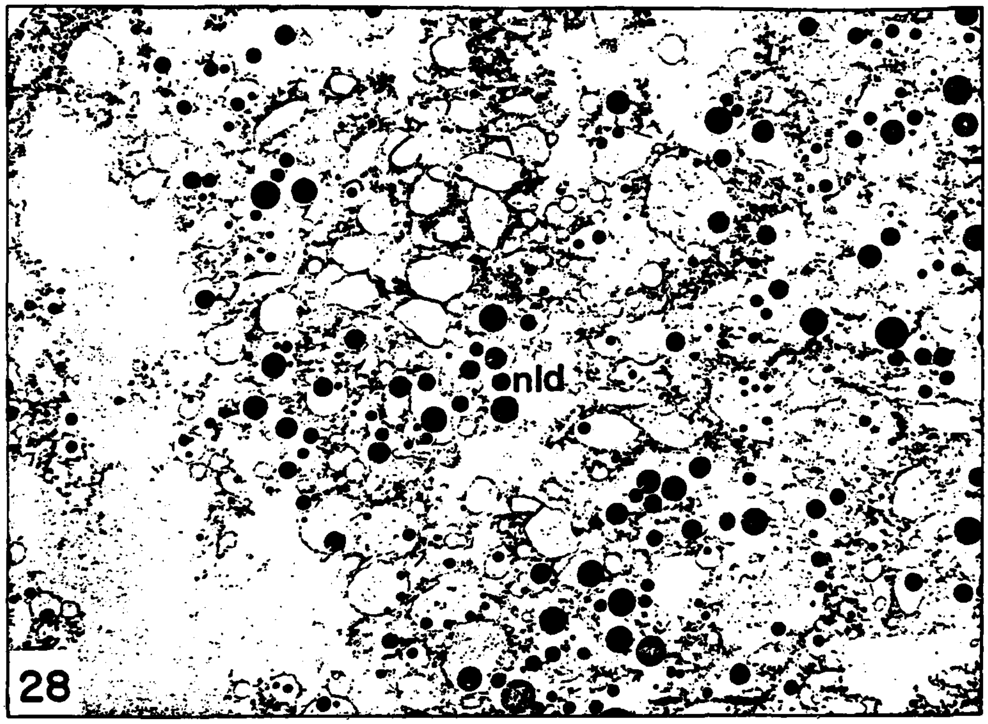


Figure 28. Part of a secretory lobe of a part-fed female with many electron-dense neutral lipid secretory droplets (nld) of varying size lying free in the cytoplasm. Many vesicles have disintegrated, leaving membrane fragments and particulate substance scattered throughout the lobe (6,250 ×).

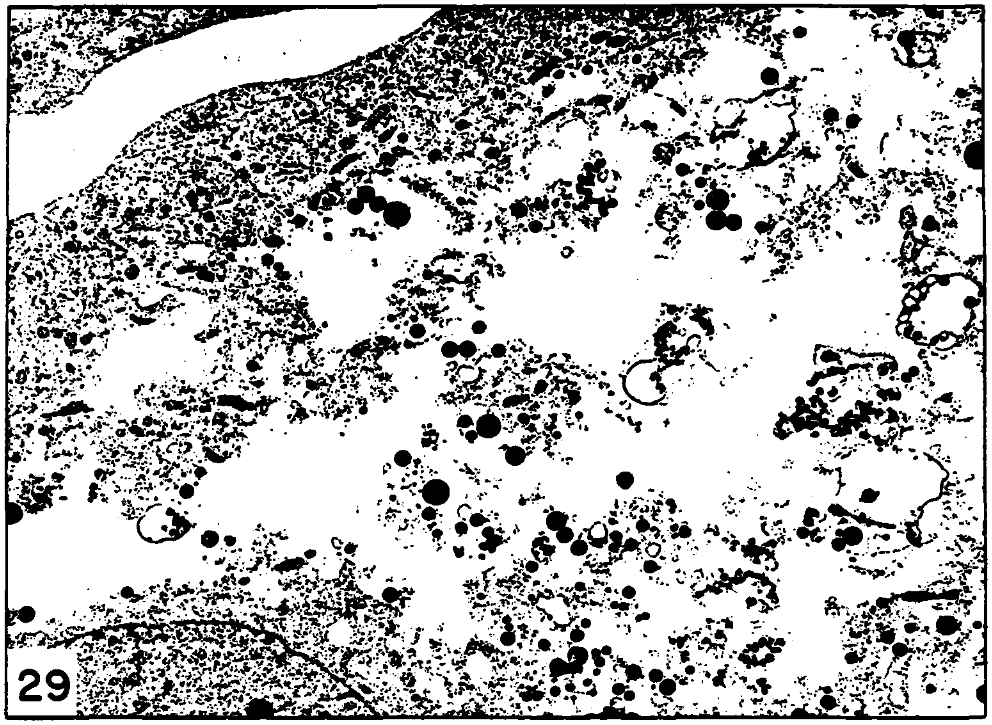


Figure 29. Section illustrates the depletion of the secretory droplets in the foveal gland of a mature, mated and repleting female H. dromedarii. The inner lobes are virtually devoid of droplets.

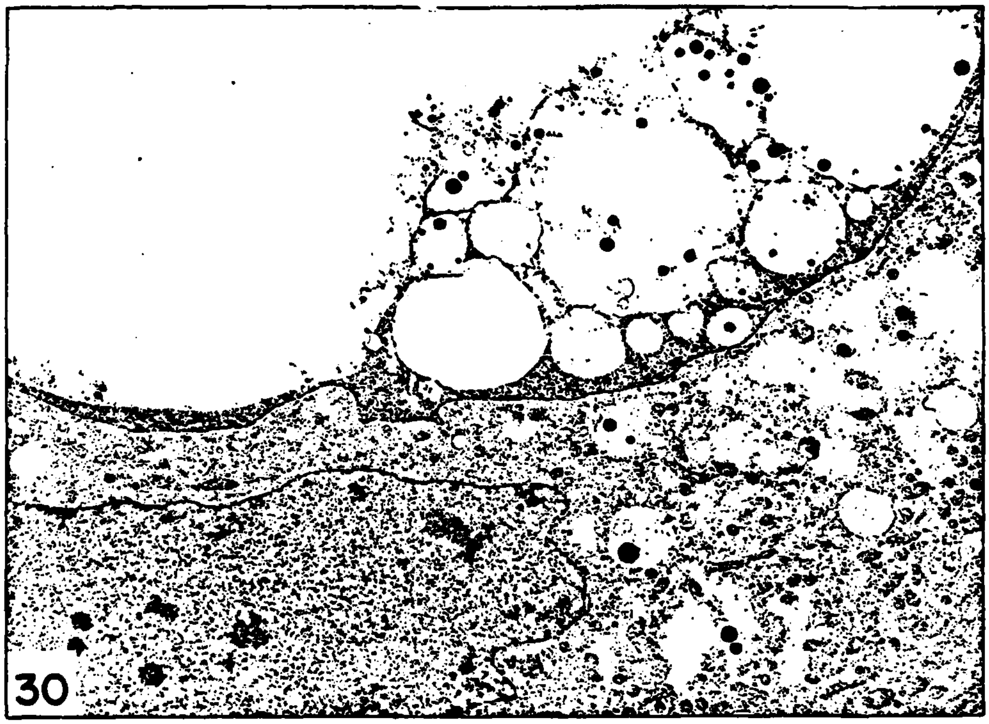


Figure 30. Section illustrates the depletion of the secretory droplets in the foveal gland of a mature, mated and repleting female H. dromedarii. The inner lobes are virtually devoid of droplets.

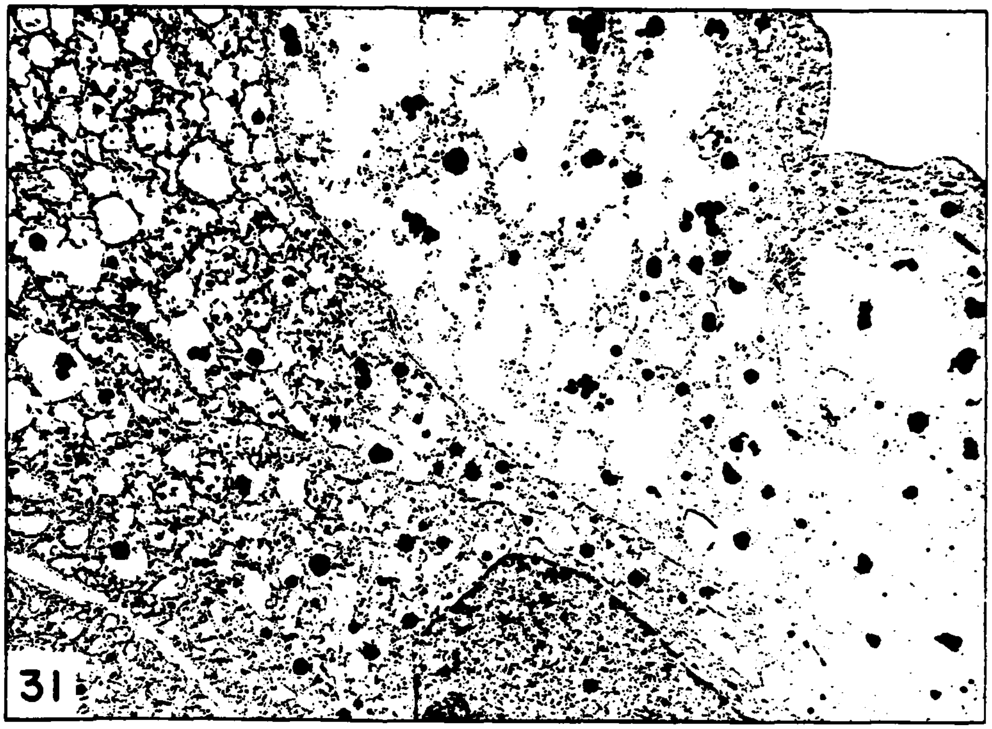


Figure 31. Section illustrates the ultrastructure of the foveal glands of a mature, unfed male H. dromedarii and the vesiculated cells with relatively few, scattered electron-dense fragments, extremely variable in size, appearing as small particles or aggregates. In the uppermost cell, relatively few vesicles occur, many membranes have been broken, and substantial areas of the cytoplasm appear empty (4,800 ×).

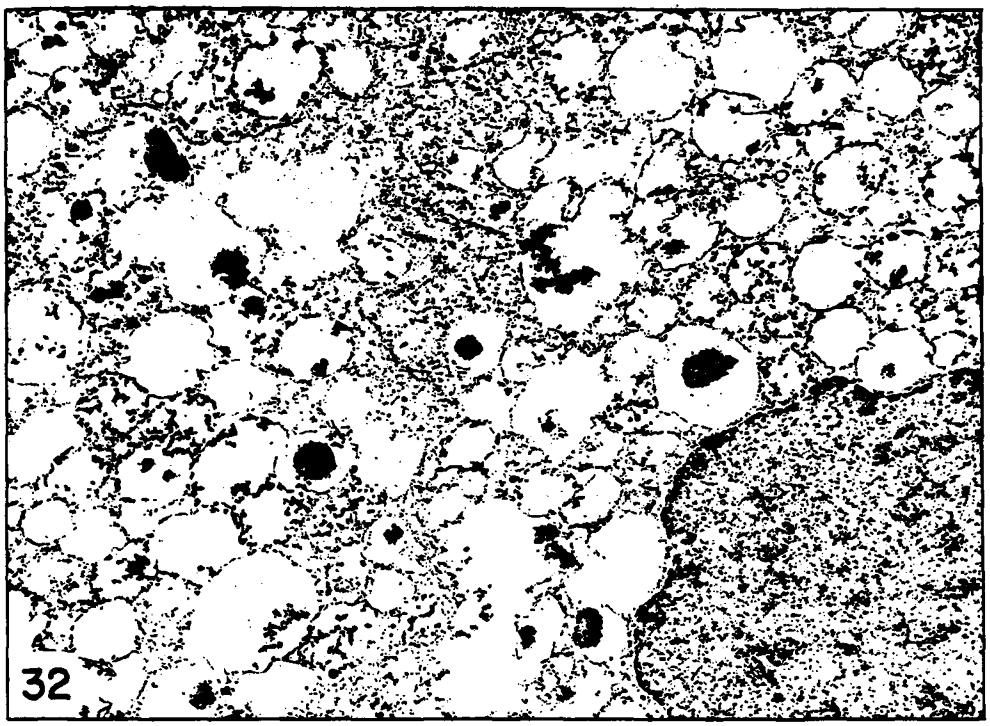


Figure 32. Section illustrates the ultrastructure of the foveal glands of a mature, unfed male H. dromedarii. Enlargement of a secretory lobe cell. The electron-dense material appears variable in shape and organization, and many are extremely irregular in shape (9,750 ×).

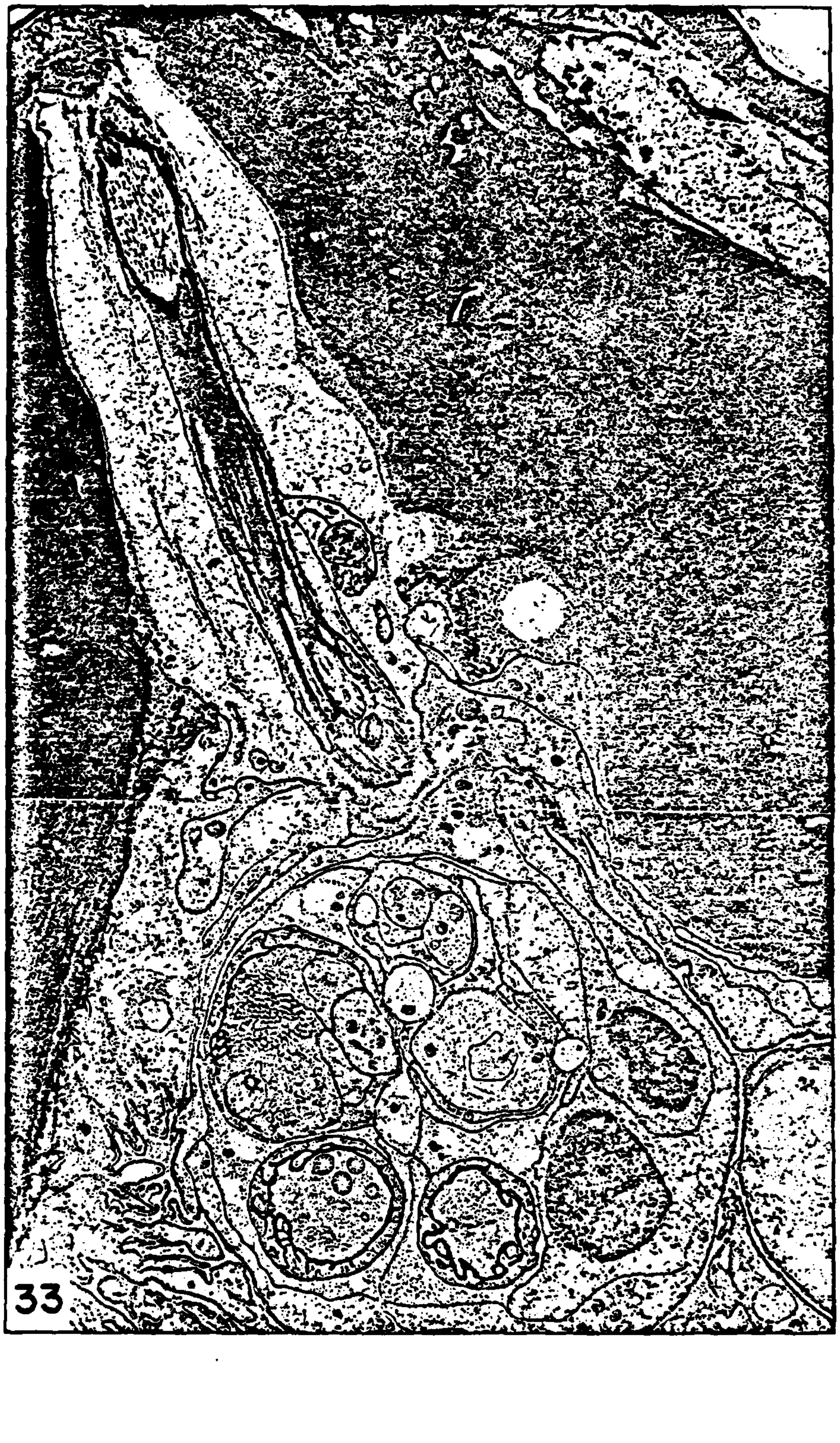

33

Figure 33. Transmission electron micrograph illustrating the nerve in the inner cheliceral digit of D. variabilis, with a branch leading to a pore near the surface.

OFFICE OF NAVAL RESEARCH
NAVAL BIOLOGY PROJECT
STANDARD DISTRIBUTION LIST

Number of Copies

(12)	Administrator Defense Technical Information Center Cameron Station Alexandria, VA 22314
(6)	Director Naval Research Laboratory Attn: Technical Information Division Code 2627 Washington, DC 20375
(3)	Office of Naval Research Naval Biology Project Code 443 800 N. Quincy Street Arlington, VA 22217

One copy to each of the following:

Office of Naval Research
Code 200
800 N. Quincy Street
Arlington, VA 22217

Office of Naval Research Eastern/
Central Regional Office
Building 114, Section D
666 Summer Street
Boston, MA 02210

Office of Naval Research Branch
Office
536 South Clark Street
Chicago, IL 60605

Office of Naval Research Western
Regional Office
1030 East Green Street
Pasadena, CA 91106

Technical Library
U.S. Army Natick Laboratories
Natick, MA 01760

Dr. A. L. Salfkosky
Scientific Advisor, Commandant of
Marine Corps (Code RD-1)
Washington, DC 20380

Assistant Commander for Research &
Development
Code 03
Naval Facilities Engineering Command
200 Stovall Street
Alexandria, VA 22332

Biological Sciences Staff
Code 112B
Naval Facilities Engineering Command
200 Stovall Street
Alexandria, VA 22332

Scientific Library
Naval Biosciences Laboratory
Naval Supply Center
Oakland, CA 94625

Enclosure (3)

Commander
Army Research Office
Research Triangle Park, NC 27709

National Environmental Research Center
Edison Water Quality Research Division
Edison, NJ 08817

Technical Advisory Division
National Marine Fisheries Service
Department of Commerce
Washington, DC 20235

Head, Disease Vector Control Section
BUMED (MED-31412)
Department of the Navy
Washington, DC 20372

Matthew Stevenson
National Academy of Sciences
Room JH 538
2101 Constitution Avenue
Washington, DC 20418

Director
National Library of Medicine
8600 Wisconsin Avenue
Bethesda, MD 20014

Commanding Officer
Navy Environmental Health Center
Naval Station
Norfolk, VA 23511

Chief, Entomology Research Branch
Prevention Medicine Division
U.S. Army Medical Research & Development Command
Fort Detrick
Frederick, MD 21701

David W. Taylor Naval Ship Research & Development Center
Code 2831
Annapolis, MD 21402

Health Effects Research
Environmental Protection Agency
ORD 683401
401 M Street, SW
Washington, DC 20468

Commanding Officer
Naval Medical Research & Development Command
National Naval Medical Center
Bethesda, MD 20014

Commandant, DAT
U.S. Coast Guard
400 Seventh Street, SW
Washington, DC 20511

Commandant, DAS
U.S. Coast Guard Research & Development Center
Avery Point
Groton, CT 06340

Commander, Naval Oceanography Command
NSTL Station
Bay St. Louis, MS 39529

Officer in Charge
Navy Disease Vector Ecology & Control Center
Naval Air Station
Alameda, CA 94501

Officer in Charge
Naval Disease Vector Ecology & Control Center
Naval Air Station
Jacksonville, FL 32212

David W. Taylor Naval Ship Research & Development Center
Code 286
Annapolis, MD 21402

Dr. John D.O'Connor
Department of Biological Sciences
University of California
Los Angeles, CA 90024

Mr. Pat Cave
Research Programing
Naval Facilities Engineering Command
Code 032F
200 Stovall Street
Alexandria, VA 22332

Mr. S. M. Hurley
Environmental Programs
Naval Facilities Engineering Command
Code 032P
200 Stovall Street
Alexandria, VA 22332

Dr. Martin Alexander
Department of Agronomy
Cornell University
Ithaca, NY 14850

Ms. C. Irene Belmore
Battelle Columbus Laboratories
William F. Clapp Laboratories, Inc.
Washington Street
Duxbury, MA 02332

Dr. G. Mallory Boush
University of Wisconsin
College of Agriculture & Life Sciences
Department of Entomology
Madison, WI 53706

Dr. Rita R. Colwell
Department of Microbiology
University of Maryland
College Park, MD 20742

Dr. D. A. Crossley
University of Georgia
Department of Entomology
Athens, GA 30602

Mr. Eugene Findl
BioResearch, Inc.
315 Smith Street
Farmingdale, NY 11735

Dr. Donald W. Hall
Associate Professor
Department of Entomology & Nematology
University of Florida
Gainesville, FL 32611

*Dr. Galila M. Khalil
Naval Medical Research Unit #3
Medical Zoology Department
FPO NY 09527
*Mark env. AIRMAIL

Dr. John R. Linley
Florida Medical Entomology Laboratory
P.O. Box 520
Vero Beach, FL 32960

Dr. Ralph Mitchell
Harvard University
Division of Engineering & Applied Physics
Cambridge, MA 02138

Dr. Claude H. Nash, III
SK&F Laboratories
P.O. Box 7929
Philadelphia, PA 19101

Dr. V. Romanovsky
Centre de Recherches et d'Etudes Oceanographiques
73-77, rue de Sevres
92100 Boulogne, France

Dr. Mary H. Ross
Department of Entomology
College of Agriculture & Life Sciences
Virginia Polytechnic Institute & State University
Blacksburg, VA 24061

Dr. Harry Hoogstraal, Head
Medical Zoology Department
Naval Medical Research Unit No. 3
Fleet Post Office
New York, N.Y. 09527 AIRMAIL

Dr. Neylan A. Vedros
Scientific Director
Naval Biosciences Laboratory
Naval Supply Center
Oakland, CA 94625

*Address outer envelope as follows: Commanding Officer, NAMRU-3, FPO, NY 09527. Address inner envelope as shown in above listing.

Dr. D. E. Weidhaas
Insects Affecting Man & Animals
Research Laboratory
USDA-SEA-AR, Southern Region
1600 S.W. 23rd Drive
P.O. Box 14565
Gainesville, FL 32604

Dr. James J. Whitesell
Biology Department
Valdosta State College
Valdosta, GA 31601

James H. Oliver
Department of Biology
Georgia Southern College
Statesboro, GA 30458

Paul J. Homsher
Department of Biological Sciences
Old Dominion University
Norfolk, VA 23508

Robert M. Silverstein
College of Environmental Science & Forestry
State University of New York
Syracuse, NY 13210