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Evidence for the Role of Ecdysteroids in the Genital Sex Pheromone of Two species of Hard Ticks, *Dermacentor variabilis* (Say) and *Dermacentor andersoni* Stiles

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EVIDENCE FOR THE ROLE OF ECDYSTEROIDS IN THE GENITAL SEX PHEROMONE OF TWO SPECIES OF HARD TICKS, DERMACENTOR VARIABILIS (SAY) AND DERMACENTOR ANDERSONI STILES

by

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Approved by:

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Daniel E. Sonenshine (Director)

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ABSTRACT

EVIDENCE FOR THE ROLE OF ECDYSTEROIDS IN THE GENITAL SEX PHEROMONE OF TWO SPECIES OF HARD TICKS, DERMACENTOR VARIABILIS (SAY) AND DERMACENTOR ANDERSONI STILES

DeMar Taylor
Old Dominion University, 1989
Director: Dr. Daniel E. Sonenshine

Neutering of part-fed females virtually eliminated copulatory behavior in Dermacentor variabilis and D. andersoni males. Extracts from the anterior reproductive tracts (ART) of part-fed (7 days) females restored the male copulatory behavior in conspecific neutered females, suggesting the presence of a genital sex pheromone (GSP). Similar extracts from unfed females did not restore the behavior, suggesting that the pheromone was produced during feeding. Perception of the GSP by sensillae on the male cheliceral digits was confirmed by electrophysiological techniques.

Ecdysteroids, specifically ecdysone and 20-OH-ecdysone were shown to be present in the anterior reproductive tracts in excess of amounts that could be explained by mere hemolymph contamination. Ecdysteroids were also found in washings of the vaginal lumen of these two species. D. andersoni females contained larger amounts of ecdysteroids than D. variabilis females. Males of D. variabilis and D.

andersoni responded positively to authentic ecdysone, and 20-OH-ecdysone in neutered female bioassays and electrophysiological assays. The strongest responses were to 20-OH-ecdysone in both species. No response was found with sterols. 20-OH-ecdysone and possibly ecdysone appear to be components of the genital sex pheromone (GSP) of D. variabilis and D. andersoni. Species recognition is facilitated by these components, but the complete mechanism is not yet fully understood. The importance of ecdysteroids in the evolutionary development of chemical communication systems in Arthropoda is also discussed.

ACKNOWLEDGEMENTS

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I would like to thank the many individuals who provided assistance and technical support. Particularly James S. Phillips for his help with assays and his continual encouragement and friendship.

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Finally, I thank God for His guidance and help in achieving my goals.

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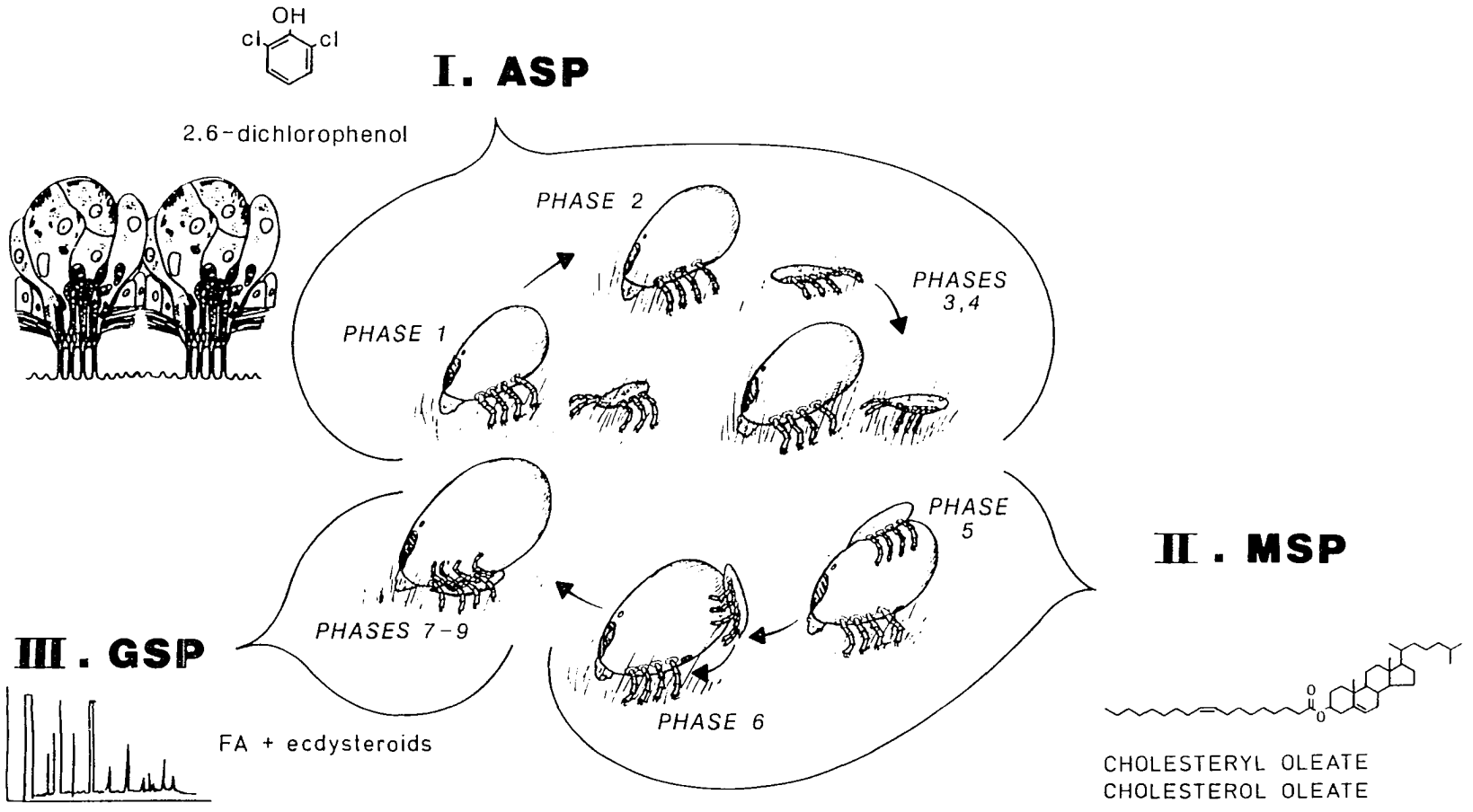
INTRODUCTION

Successful matings in most arthropods result from a series of specific behaviors, often involving a hierarchy of steps regulated by chemical or physical stimuli. Overall, these behaviors ensure conspecific matings but do not totally prevent congeneric matings (Matthews and Matthews, 1978).

Courtship in ixodid ticks, which appears to be regulated largely by pheromones, follows this arthropod pattern. Most ticks must attach to a host for several days and obtain a blood meal before they are sexually active. Ticks of different species frequently attach to the same host, suggesting that sex attractant pheromones have species specific qualities. We now know that females of many species release the same sex attractant pheromone, 2,6-dichlorophenol (2,6-DCP) (Khalil et al., 1981; Sonenshine et al., 1976). Despite this seemingly generic sex attractant, interspecific matings are not common in ixodid ticks (Oliver, 1974). For example, a male of Dermacentor variabilis or D. andersoni is attracted to a female by 2,6-DCP, mounts it and proceeds through as many as nine steps of courtship (Sonenshine, 1985). The stages of courtship are outlined in Fig. 1. The first 4 phases of courtship behavior are controlled by this volatile

Figure 1. Representation of the behavioral stages that occur during courtship in Dermacentor variabilis and D. andersoni. Phases 1-4: feeding female releases volatile attractant sex pheromone (ASP), 2,6-dichlorophenol, this excites males to detach (Phase 1), begin searching for the female (Phase 2), male finds the emitting female, mounts it and orients to the foveal gland, (source of ASP). Phases 5-6: These stages are controlled by the mounting sex pheromone (MSP), cholesteryl oleate. Males probe the dorsal surface of the female, turn posteriorly, crawl over the female's body and proceed to the ventral surface. The female lifts her body to facilitate these movements. Phases 7-9: These stages are regulated by the genital sex pheromone (GSP). The male positions itself at the genital aperture, placing its legs between the females, and flexes its capitulum to probe. The chelicerae are inserted into the genital aperture and copulation takes place.



attractant sex pheromone (ASP). When detected, feeding males are stimulated to detach and search for a potential mate. Males that encounter pheromone emitting females, mount and proceed to the subsequent stages of courtship behavior. This pheromone has been reported in 14 species of ticks in 5 genera (Sonenshine, 1985). It does not convey species recognition except in two species of Hyalomma (Khalil et al., 1983). During the next two phases of courtship behavior (Phases 5 & 6) the male probes the dorsal surface of the female with its mouthparts, turns and crawls around to the ventral surface. These behaviors are regulated by the mounting sex pheromone (MSP) (Hamilton & Sonenshine, 1988). Recently, the MSP of D. variabilis has been identified to be cholesteryl oleate (Hamilton et al., in preparation). Males also respond to the MSP in heterospecific as well as conspecific pairing in Dermacentor species. Therefore the MSP does not provide the information for species recognition, either. The remaining phases of the courtship process are concerned with locating and probing the genital aperture and, if stimulated, releasing the copulatory response (phases 7-9). In the genus Dermacentor conspecific mating is completed when males probing the gonopore and vulva with their chelicerae percieve the genital sex pheromone (GSP) found in these organs (Sonenshine et al., 1982, 1985b). At this point, unless the male has mounted a conspecific female, copulation (insertion of a spermatophore into the gonopore)

will not be completed. The presence of this third pheromone (GSP) in D. variablis and D. andersoni has been established by Sonenshine et al. (1982), and shown to be perceived by sensory structures on the cheliceral digits (Sonenshine et al., 1984). The vestibular vagina and oviducts of the anterior reproductive tract are postulated as the sites of production of the genital sex pheromone. The composition of the pheromone is likely a mixture of at least two components (Sonenshine et al., 1985b). Allan et al. (1988) have shown that a narrow range of saturated free fatty acids in D. variabilis and D. andersoni serve as a component of the GSP. They also noted that the same range of fatty acids was present in the vaginal lumen of both species. Further studies by Allan et al. (in press) showed that D. variabilis responded to much lower concentrations of fatty acids than D. andersoni. Males responded positively to extracts of the anterior reproductive tract organs (ART) as well as to artificial fatty acid mixtures. Earlier work has also implicated ecdysteroids as components of the genital sex pheromone (Sonenshine et al., 1985b; Brossut, personal communication), but the findings were inconclusive. Therefore, it is my hypothesis that ecdysteroids are also a component of the genital sex pheromone of D. variabilis and D. andersoni and may be necessary for the recognition of a conspecific mate.

Studies on ecdysteroids in insects and crustaceans are extensive and much is known of their origin and function in

these arthropods. Reviews of this work are published in books edited by Gilbert et al. (1980), Hoffmann (1980), Downer & Laufer (1983), Hoffmann & Porchet (1984), Kerkut & Gilbert (1985ab) and others. Ecdysteroids have also been found to function as pheromones in some crustaceans. Kittredge et al. (1971) showed that crustecdysone (20-hydroxyecdysone) functions both as a moulting hormone and a sex pheromone in the lined shore crab, Pachygrapsus crassipes. Reviews of work on ecdysteroids in acari have been done by Binnington and Obenchain (1982), Solomon et al., (1982) and most recently Diehl et al. (1986). Sonenshine (1987) has reviewed the hormonal regulation of sex pheromone function in ticks. Ecdysteroids have been shown to have a variety of functions in most arthropods.

The first evidence of ecdysteroids in ticks was reported by Delbecque et al. (1978). Exogenous ecdysteroids have been observed to induce supermoulting (Campbell & Oliver, 1984; Connat et al., 1983; Kitaoka, 1972), accelerate moulting (Khalil et al., 1984), influence oogenesis (Connat et al., 1983; Kitaoka, 1972), induce salivary gland degeneration (Harris & Kaufman, 1981) and affect fovea dorsalis development and sex pheromone activity (Dees et al., 1984ab). These experiments demonstrated that ecdysteroids are probably found in ticks endogenously. The evolution of sophisticated analytical methods, discussed in detail by Hoffman & Hetru (1983), Lafont & Koolman (1984) and Lafont et al. (1980), such as

radioimmunoassay (RIA), high-performance liquid chromatography (HPLC), gas chromatography (GC), mass spectrometry or fragmentography and NMR spectroscopy have increased our knowledge of arthropod ecdysteroid chemistry, biosynthesis, metabolism and mode of action. They have also helped to establish the presence of ecdysteroids in ticks. Ecdysteroids have been found in larval (Diehl et al., 1986; Dees et al., 1984b) and nymphal ticks (Germond et al., 1982; Solomon et al., 1982; Diehl et al., 1982; Ellis & Obenchain, 1984; Bouvier et al., 1982). These workers have shown the presence of 20-hydroxyecdysone and ecdysone in immature stages of argasid and ixodid ticks. They have also concluded, that like with other arthropods, 20-hydroxyecdysone is the active moult-controlling hormone. Little work has been done on ecdysteroids in males. However, Dees et al. (1984b) have shown the presence of ecdysteroids in D. variabilis and H. dromedarii males. Dumser & Oliver (1981) have hypothesized that ecdysteroids might be important in the control of spermatogenesis in males. Ecdysteroids in adult female ticks have been studied in more detail (Connat et al., 1985; Wigglesworth et al., 1985; Dees et al., 1984b) and have been shown to be important in moulting and salivary gland degeneration. They also appear to control hormonal regulation of other processes such as diapause, oogenesis, and embryogenesis (Diehl et al., 1986). Ecdysteroids have also been shown to

occur in eggs of several species of ticks (Whitehead et al., 1986; Dees et al., 1984b).

The purpose of this study was to determine if and what ecdysteroids are present in the anterior reproductive tract and the vaginal lumen and whether they can be detected by males and induce mating thus functioning as a component of the genital sex pheromone in two ixodid species, D. variabilis and D. andersoni.

MATERIALS AND METHODS

Ticks

The ticks used in these experiments were reared in the laboratory as described by Sonenshine et al. (1976). Dermacentor variabilis was colonized from wild specimens collected near Suffolk, Virginia; D. andersoni was colonized from specimens obtained from the U.S. Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana. Except when feeding, all ticks were kept in an Aminco-Aire Climate Laboratory incubator (American Instrument Co., Sliver Spring, MD) at $27^{o}\pm1^{o}$ C and $92\pm2\%$ relative humidity. Immature D. variabilis were fed on albino rats, adults on laboratory rabbits. D. andersoni larvae were fed on hamsters, nymphs and adults on rabbits.

Chemicals

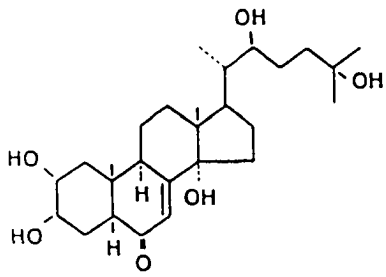
Solvents used for high performance liquid chromatography (HPLC) were HPLC-grade (Burdick & Jackson, Muskeegan, MI). Methanol and hexane used for extraction and in the preparation of standards for bioassays were HPLC-grade solvents further purified by glass distillation (2x). Solvents for gas chromatography (GC) were nanograde toluene (Mallinckrodt, Inc., St. Louis, MO) and ethyl ether (Burdick & Jackson, Muskeegan, MI) further purified by

glass distillation (2x). Ecdysone (E), 20-hydroxyecdysone (20E), 2-deoxyecdysone (DAE), 2-deoxy-20-hydroxyecdysone (DBE), makisterone A (MAK), cholesta-3,5-diene (C35), 5α-cholestan-3β-ol-6-one (6KC), 5-cholesten-3β-ol-7-one (7KC), cholesterol (CHO), 5β-cholestan-3α-ol (5BC), and 5α-cholestan-3β-ol (5AC) were all obtained from Sigma Chemical Co. (St. Louis, MO) (Fig. 2 & 3). Inokosterone (INO) and Tri-(TMS)-5β-cholest-7-en-3,5,14-triol-6-one were donated by Dr. M.J. Thompson of the Insect Physiology Laboratory, USDA, Beltsville, MD. Tritiated ecdysone (α-(23,24-^{3}H(N))-(45 Ci/mmol) was obtained from New England Nuclear Co. (NEN), Boston, MA. This compound was assayed for impurities by thin layer chromatography (TLC) on Bakerflex IB-2 silica gel plates (J.T. Baker Chemical Co., Phillipsburg, NJ) in chloroform:methanol 4:1; no significant impurities were detected. Tritiated cholesterol (1,2,6,7-^{3}H(N))- (97.1 Ci/mmol) obtained from the same source was also found to have no significant impurities (Silica Gel IB-2; benzene:ethyl acetate (9:1)). Borate buffer was prepared as a 1 liter solution containing 6.18 g boric acid, 9.54 g $Na_2B_4O_7 \cdot 10H_2O$, and 4.38 g NaCl adjusted to a pH of 8.4.

Extracts

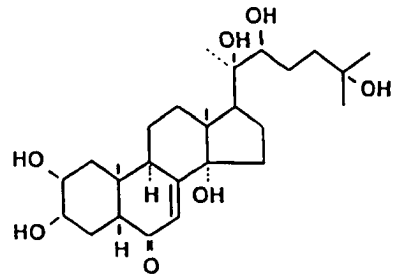
Three different methods were used in preparation of extracts for bioassay and chemical analysis. These methods are detailed below; 1) flush of the vaginal lumen, genital pore and anal pore with methanol, hereafter referred to as

Figure 2. Chemical structures of ecdysteroids used in this study.



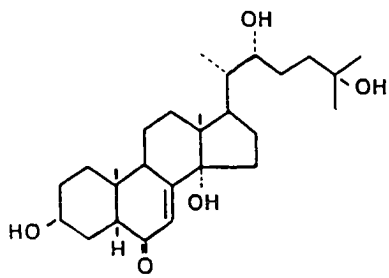
ECDYSONE

(2β, 3β, 14α, 22, 25-pentahydroxy-5β-cholest-7-en-6-one)

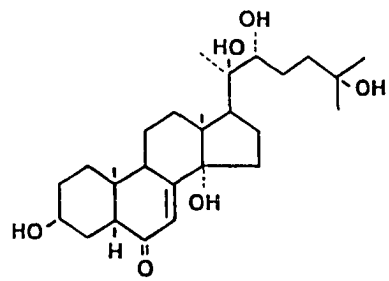


20-HYDROXYECDYSONE

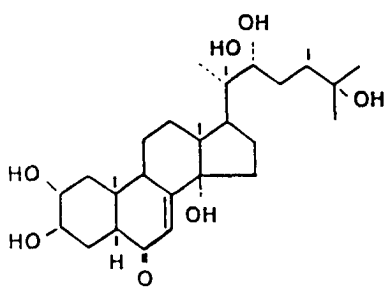
(2β, 3β, 14α, 20, 22, 25-hexahydroxy-5β-cholest-7-en-6-one)



2-DEOXYECDYSONE

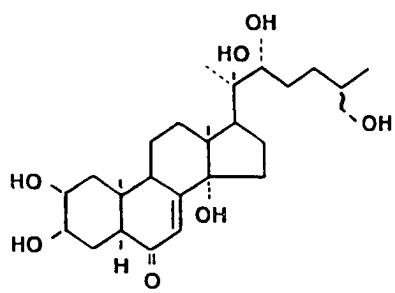


2-DEOXY-20-HYDROXYECDYSONE



MAKISTERONE A

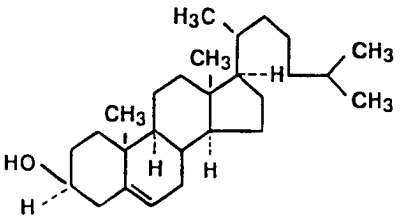
(20-hydroxy-24-methylecdysone)



INOKOSTERONE

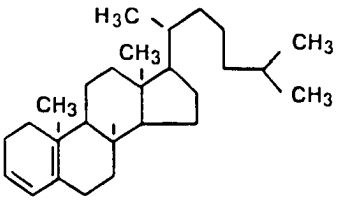
(25-deoxy-20,26-dihydroecdysone)

Figure 3. Chemical structures of sterols used in this study.

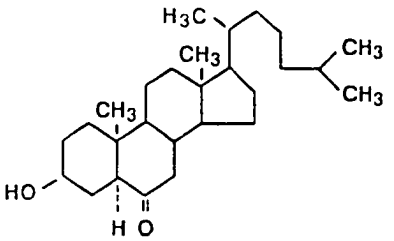


CHOLESTEROL

(Cholest-5-en-3β-ol)

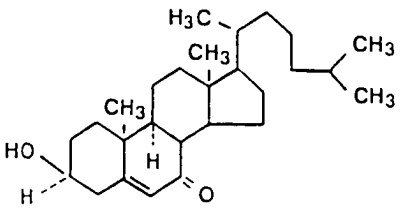


Cholesta-3,5-diene



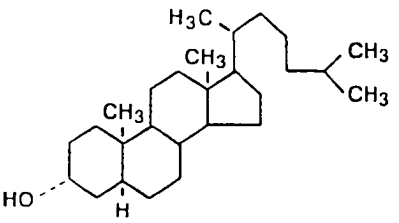
5α-Cholestan-3β-ol-6-one

(6-ketocholestanol)

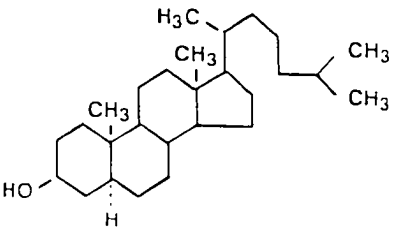


5-Cholesten-3β-ol-7-one

(7-ketocholesterol)



5β-Cholestan-3α-ol

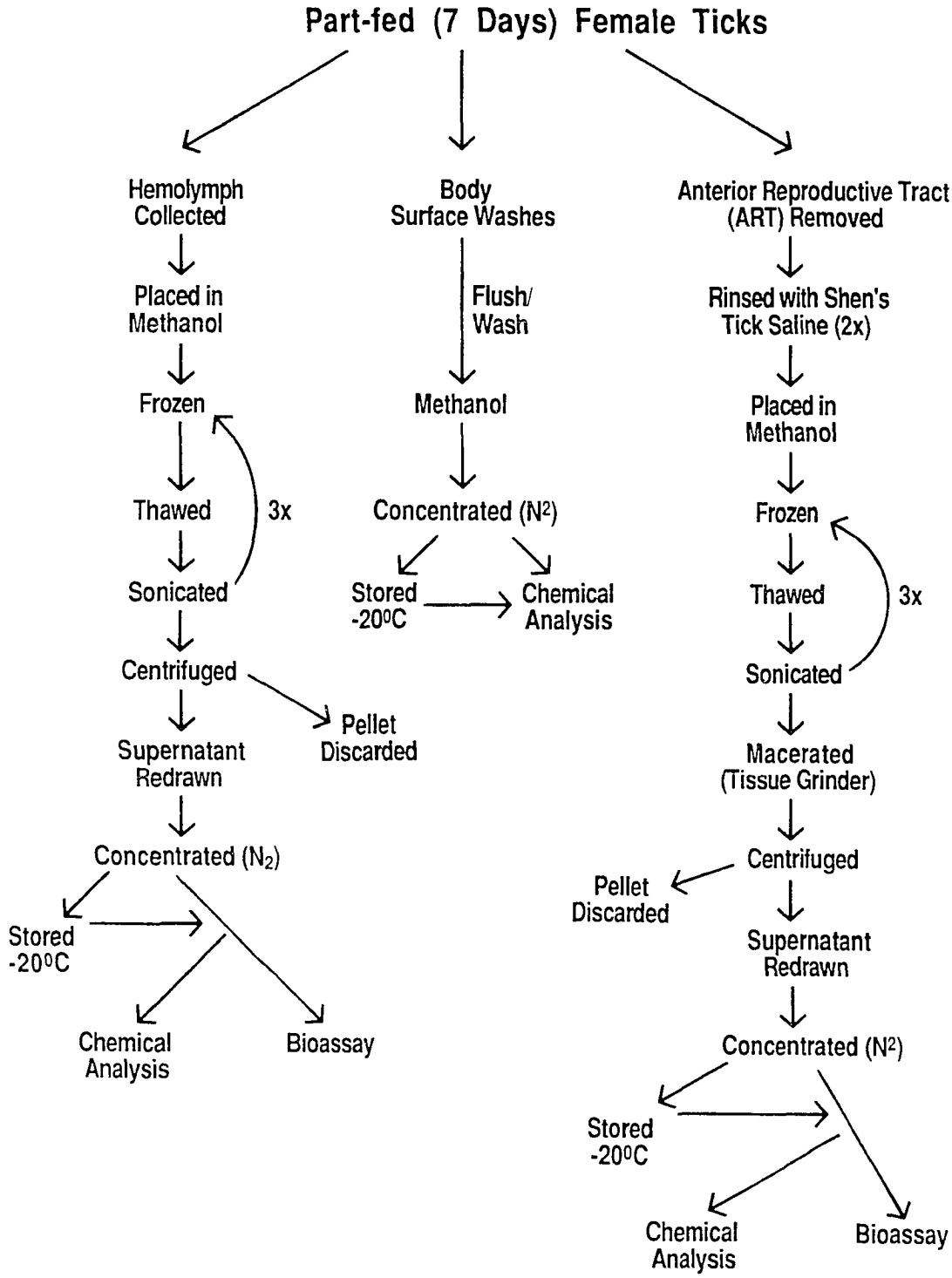


5α-Cholestan-3β-ol

(Cholestanol)

vulval wash (VW) extract, genital wash (GW) extract and anal wash (AW) extract, 2) excision and collection of the anterior reproductive tract in methanol hereafter called the ART extract, and 3) collection of hemolymph in methanol, hereafter referred to as hemolymph (HL) extract (Fig. 4). The VW extract was prepared by using a glass micropipette that was drawn to a tip diameter of 10-20 u with an microelectrode puller (Industrial Science, Flusing NY). The micropipette was held on a Drummond Dialamatic Microdispenser (Drummond Scientific Co., Broomhall, PA) and mounted on a Leitz micromanipulator (Bunton Instrument Co., Baltimore, MD). The micropipet was manipulated into the gonopore of a part-fed (7 days) virgin female tick viewed under a Wild stereomicroscope (Wild Heerbrugg Ltd., Heerbrugg, Switzerland). Approximately 1 ul of glass distilled HPLC methanol was forced into the gonopore and then withdrawn immediately. If the methanol became contaminated with hemolymph, by puncturing of the reproductive tract, the sample was discarded. Each female was washed once, the VW extract collected in a microvial, the wash of many individuals combined (approximately 40/extract) and stored at -20^{o} C for chemical analysis. Genital washings of normal part-fed females (GW) and neutered part-fed females (GWN) and anal washings of normal part-fed females (AW) were prepared as described above, except that the outer surface around the pores was washed instead of the lumen. Each extract contained washings of

Figure 4. Flow diagram of the techniques used in the preparation of the extracts for chemical analysis and bioassay.

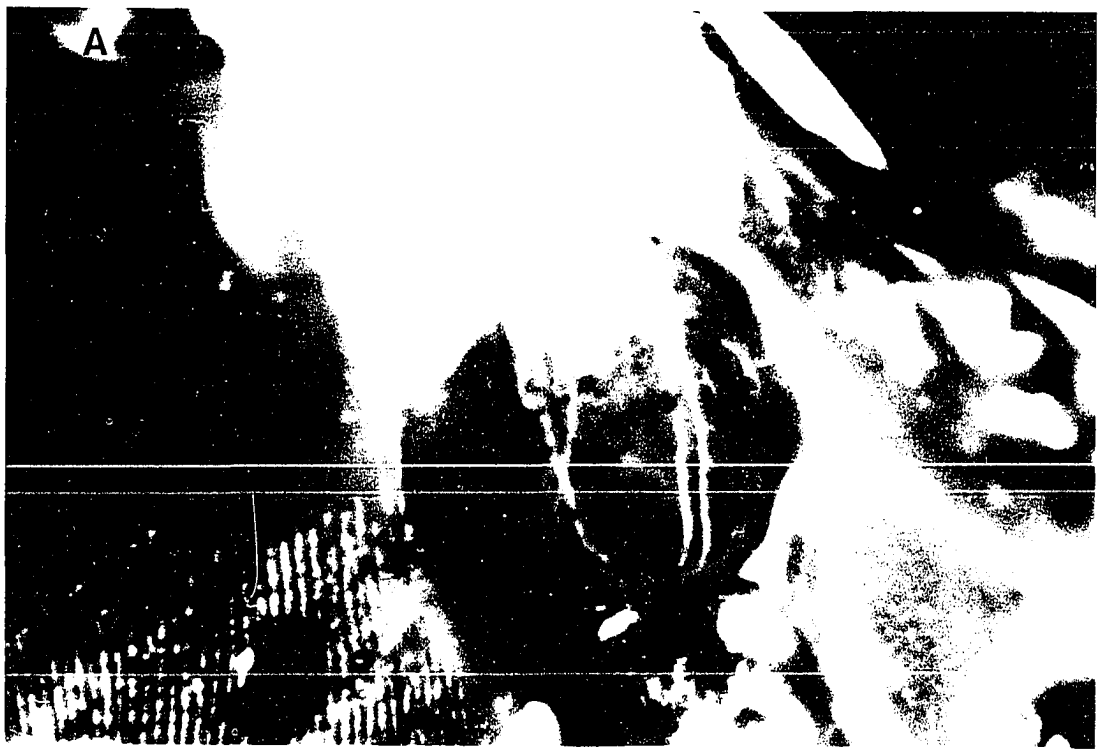


approximately 30 females, adjusted to a concentration of 0.5 female equivalents (FE)/ul.

ART extracts were prepared by excising the gonopore, vestibular vagina, lobular and tubular accessory glands, receptaculum seminis, uterus, oviducts and a small (0.5 mm^2) area of cuticle surrounding the gonopore (Fig. 5 & 6) and placing all of these structures in Shen's tick saline (Oliver, 1972). Hemolymph and midgut contaminants were removed by rinsing the ART twice in tick saline and the tissues were placed in 100% glass distilled methanol. When sufficient tissues were collected (approximately 100 ARTs/extract) they were frozen (liquid N_2), thawed, and sonicated three times. Tissues were then macerated using a Wheaton Ten Broeck glass tissue grinder (American Scientific Products, Columbia, MD) and centrifuged at 3200 rpm for 5 min. (American Scientific Products Co., MCGaw Park, IL). The supernatant was withdrawn, the pellet was reextracted in methanol, centrifuged and the supernatant added to the previous sample. The extract was then concentrated under N_2 to a concentration of 0.5 FE/ul and stored at -20^{o} C for bioassay and chemical analysis.

Hemolymph was collected from part-fed virgin females for chemical analysis. The hemolymph was collected from an incision in the foreleg, drawn into drummond micro-capillary pipettes, extracted with methanol, centrifuged, concentrated (N_2) to a concentration of approximately 0.5

Figure 5. Photographs of the anterior reproductive tract organs (ART) of a D. variabilis part-fed female tick. (A) exposed by cutting around the organs and (B) removed and pictured as the parts that were used in the preparation of the extracts.



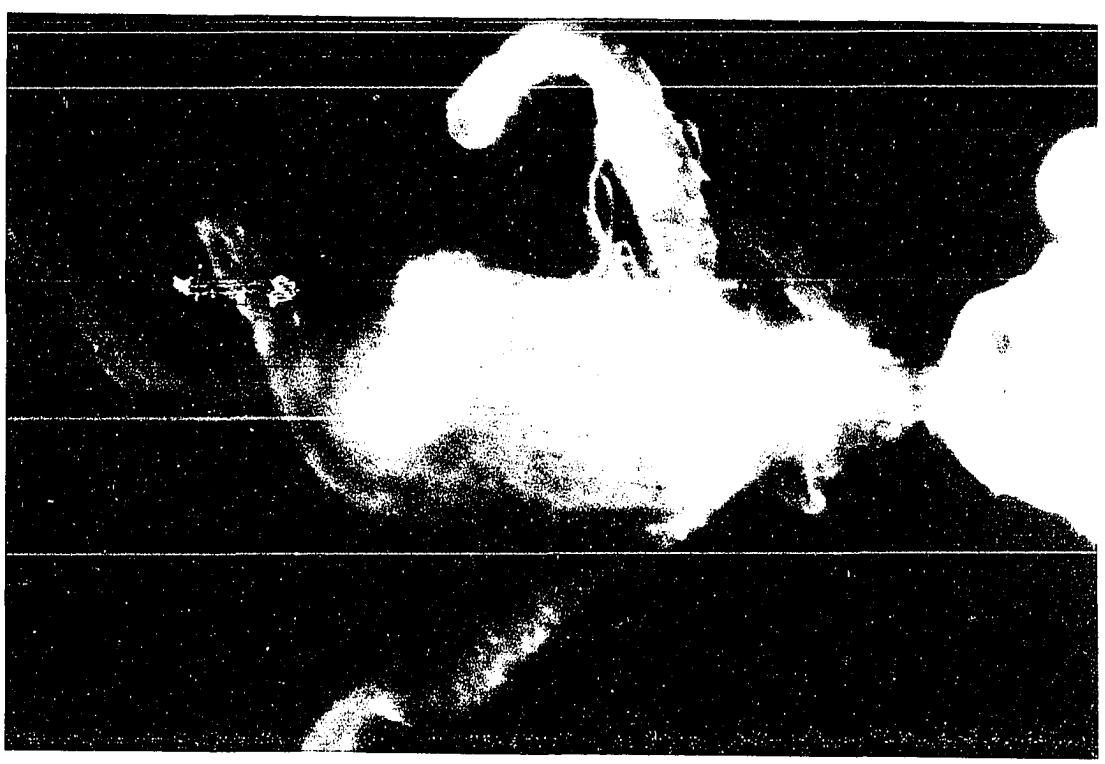


Figure 6. Stereodiagrammatic representation of the anterior reproductive tract (dorsolateral view) of a feeding, virgin female, Dermacentor variabilis. G = genital aperture; VS = vestibular sinus; LAG = lobular accesory gland; VV = vestibular vagina; TAG = tubular accessory gland (only one of a pair of glands is shown); CV = cervical vagina; RS = receptaculum seminis; M = muscle; CT = connecting tube; U = uterus; OV = oviduct.

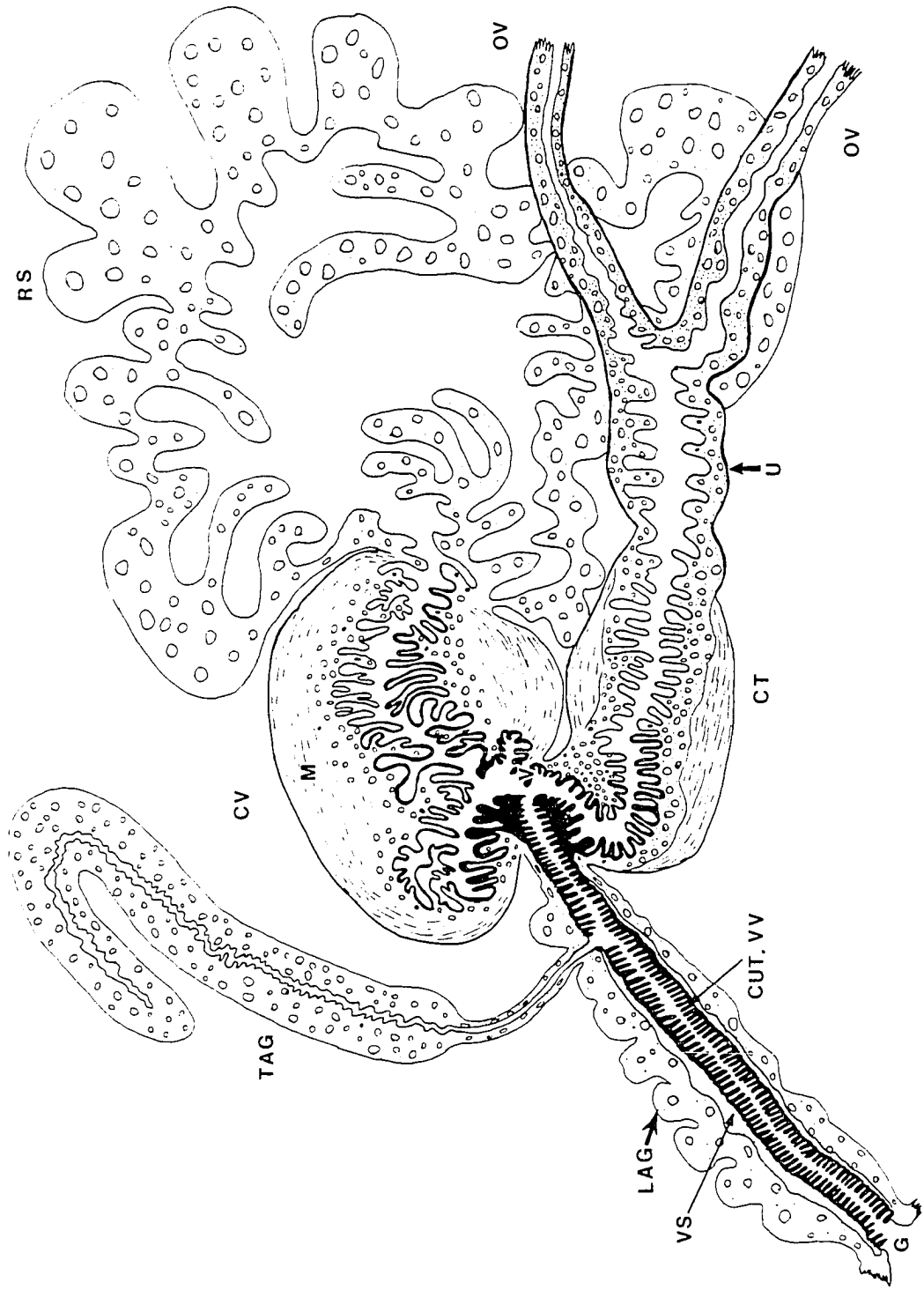


Figure 7. Flow diagram of the "neutered" female bioassay.

ul/ul and stored at -20° C. If any midgut or other tissues contaminated the sample, it was discarded.

Bioassays

A behavioral assay by the "neutered" female technique (Fig. 7), described by Sonenshine et al. (1985b), was used to measure the responses of sexually-active males (fed 7 days) to the extracts. Fed neutered females were used for the assay of standards, extracts, and controls. Each neutered female had the same physical appearance externally as untreated females, except for a small scar below the gonopore (Fig. 8).

Neutering was done in the following manner. An incision was made in the ventral body cuticle immediately below the gonopore and microforceps were inserted to remove the vagina and uterus. Surviving females were allowed to feed on a rabbit for 7 days. Each partially fed "neutered" female was then removed from the rabbit, the area around the gonopore was washed with a Q-tip soaked in hexane, acetone, and then methanol and the cuticle around the gonopore scraped to remove any residual pheromone. Sexually-active males then were used to "screen" and eliminate incompletely neutered females. This was done by allowing 2 different males three trials to proceed through the steps of courtship without allowing the males to place a spermatophore in the gonopore of the female. This can be accomplished because in normal matings probing will last for 10-20 min before a spermatophore is formed and placed

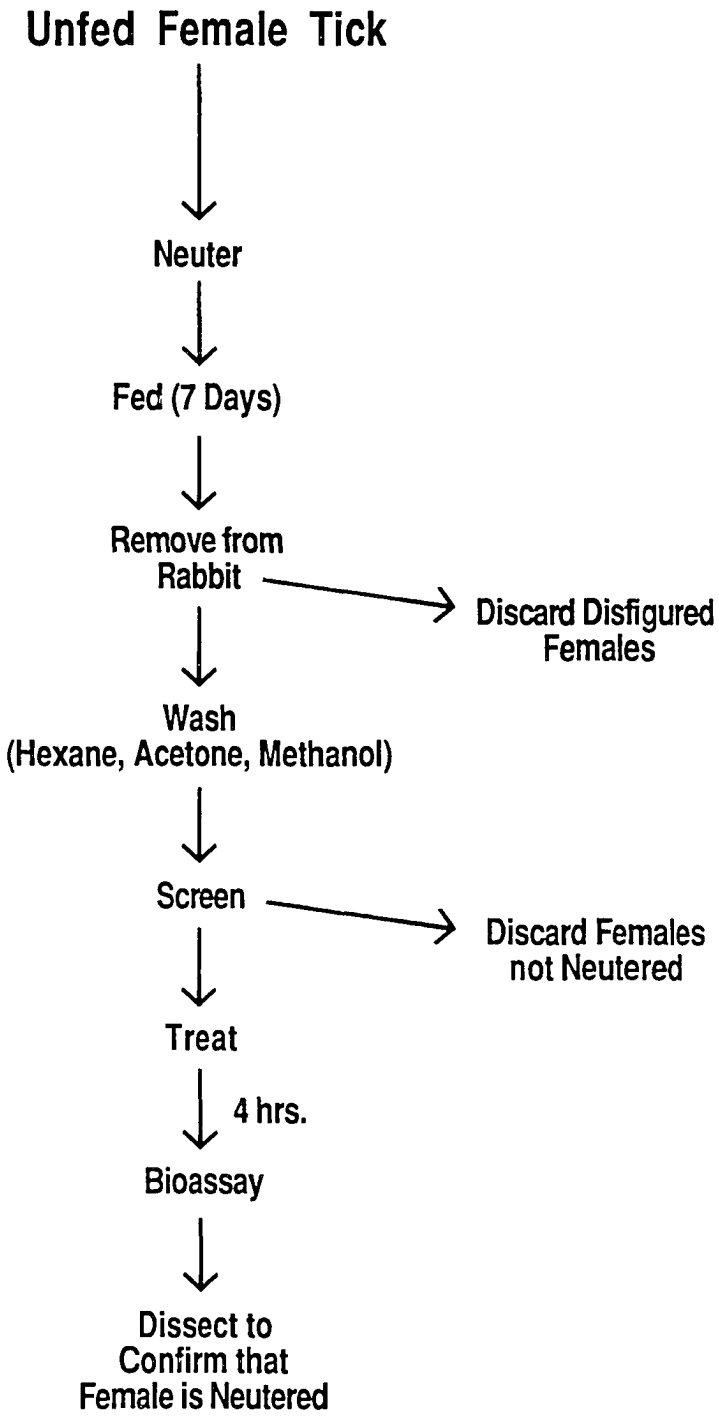
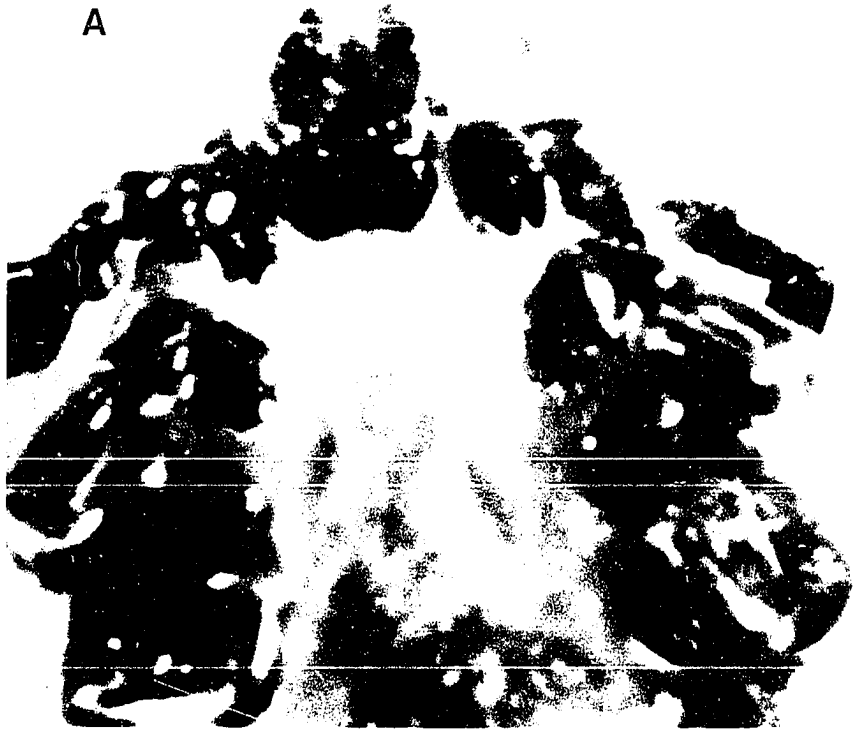


Figure 8. Photographs of the ventral surface of A) normal (not neutered) female and B) neutered female after feeding for 7 days. Represents the condition of females used in the bioassay.

A



B

in the gonopore. In this screening procedure males were allowed only to position themselves for probing and then removed. Distorted, incompletely neutered or moribund females were discarded. Males were also "screened" for appropriate sexual behavior by allowing them to exhibit the same stages of courtship with normal females. Extracts were introduced into the female tick's gonopores with micropipets, mounted on a Leitz micromanipulator while viewing the specimen through a stereoscopic microscope. The minute volume of the female vaginal tract precluded delivery of all but minute volumes at a time. The extract was repeatedly introduced into the gonopore in small amounts and allowed to dry until the desired amount was applied to the female. Four hours were allowed for solvent evaporation before the treated females were used for bioassay. All treatments were numbered randomly and applied so that the chemical being assayed was unknown.

Males were used to test pheromone activity of the treatments. Each male was released on the dorsal surface of a female and allowed to proceed through the steps of courtship and mating (Sonenshine, 1985; Sonenshine et al., 1985b). Positive responses were considered to consist of either deposition of a spermatophore or extended probing. Each male was allowed three trials, and up to 5 males were tested for each female. Bioassay responses to standards and controls were compared to ART extracts and unneutered females.

To evaluate the bioassay response, males were monitored and dorsal orientation (D), ventral orientation (V), probing behavior (P) and copulation (deposition of a spermatophore) (C) were recorded and the percent of trials in which males completed each step of the courtship determined. The length of time the male spent probing was measured and recorded. In addition a scoring system was developed, hereafter referred to as the "neutered" female assay. Although recorded and evaluated, dorsal and ventral orientation were not treated as part of this response, because these behaviors are controlled by other pheromones. This scoring system gave the highest value to copulation by the first males, less value to copulation by one of the subsequent males, and the least value to precopulatory probing behavior to determine an overall value for the bioassay response (Allan et al., 1988). The scores were assigned as follows: 100% if the first male copulated, 70% for the second male, 65% for the third and 60% for the fourth or fifth males. Dermacentor andersoni males are less likely to copulate (Allan et al., 1988) so they were given extra points for extended probing as outlined below; 2% for each trial in which probing lasted 6-10 min, 4% for 11-20 min, 6% for 20-100 min and 10% if they probed for more than 100 min.

After the trials, each female was dissected and examined with the stereoscopic microscope to ensure that the vagina had been severed and that the wound scars did

not occlude the gonopore. Females that did not meet these criteria were discarded, and additional tests were conducted.

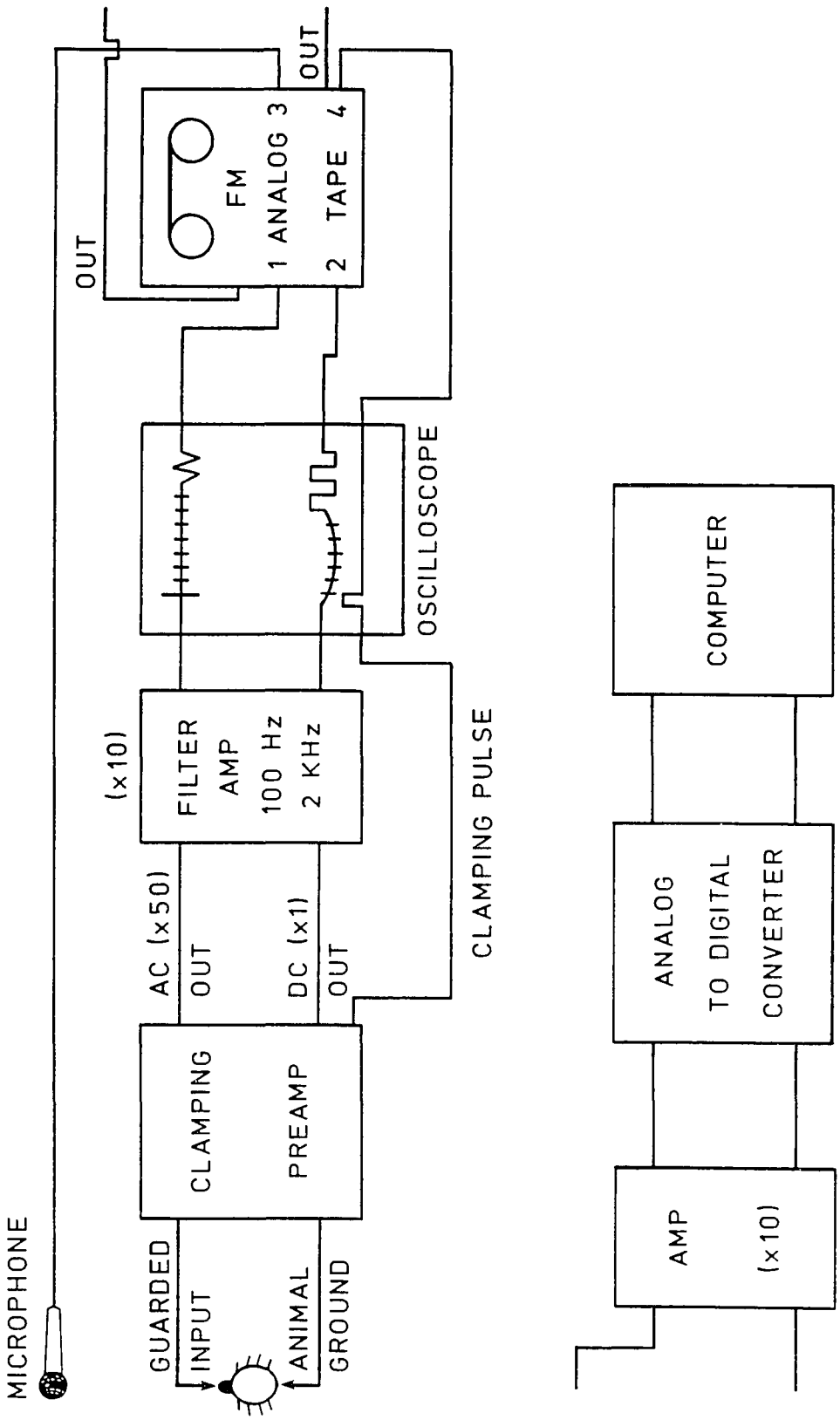
Controls included (1) sham-controls, i.e., "neutered" females treated with solvent (methanol) only, and (2) females that had not been neutered.

Statistics were done on the bioassay data using a SAS statistical package on an IBM 8083 computer. Analysis was by one-way ANOVA's using orthogonal contrasts to compare the extracts and the authentic standards against the methanol controls. Courtship and neutered bioassay data were subjected to an arcsin transformation and Duncan's multiple range tests used at the 5% level (Sokal and Rohlf, 1981). Untransformed means and standard errors are presented in the tables and figures.

Electrophysiogical Assays

Electrophysiology was used to assay male cheliceral digit responses to extracts and standards. The Dermacentor variabilis and D. andersoni males used in electrophysiological bioassays were removed from the rabbit after feeding for 7 days. Electrophysiological bioassays were performed at the laboratory of Dr. Frank Hanson at the University of Maryland-Baltimore County in Catonsville, MD. The electrophysiological and recording equipment were as described by Frazier & Hanson (1986). A general schematic of the electronic setup is shown in Fig 9. Standard electrophysiological techniques for recording from contact

Figure 9. Schematic of the set-up used for the electrophysiological assays.



chemosensilla were used (Hodgson et al., 1955).

Male ticks were restrained by wrapping them in a thin layer of Tackiwax^{R} (Boekel Ind., Philadelphia, PA). The palps were removed and pressure was applied to the body to cause the males to extend their cheliceral digits. Tackiwax^{R} was also placed around the mouthparts without covering the cheliceral digits. This procedure was necessary to keep the mouthparts extended and the sensory areas exposed for electrode placement. Glass capillary electrodes were drawn on a microelectrode puller to 10-20 um tip diameters. The indifferent electrode was filled with 0.1 M NaCl and the tip of the electrode was inserted into the posterior end of the animal, thereby also serving as a mount for the tick. The recording electrode, 5-10 um in diameter, filled with the test solution, was placed over the sensory area of a cheliceral digit. The following chemicals were tested: (1) NaCl at 3 concentrations (0.1, 0.01 and 0.001M); (2) crude extracts at 1 FE, (3) 20-hydroxyecdysone and (4) ecdysone at 10 ug/ul (2.08×10^{-2} and 2.2×10^{-2} M, respectively), 0.1 ug/ul (2.08×10^{-4} and 2.2×10^{-4} M) and 0.001 ug/ul (2.08×10^{-6} and 2.2×10^{-6} M). All chemicals were dissolved in 0.1 M NaCl solution. The recording electrode was frequently placed over nonsensory areas to avoid misinterpetations due to electrode contact noise. All tests were analyzed as described by Hanson et al. (1986).

High Performance Liquid Chromatography (HPLC)

High performance liquid chromatography was done on ART, HL and VW extracts to determine what ecdysteroids were present and to prepare extracts for RIA or liquid scintillation counting. This was done with a Waters System comprised of a pair of Model 510 pumps, Model 721 System Controller, Model 730 Data Module, Model U6K septum-less injector, a Z-module for column support and a Model 441 fixed wave length detector equipped with a 254 nm filter (Waters & Associates, Milford, MA). A 5-um Novapak C-18 cartidge, 8 mm I.D. by 15 cm long was used. The solvents used were HPLC-grade methanol and 20 mM Tris/$HClO_4$ buffer or MilliQ-water with 1% acetic acid.

Separation was accomplished by using the following gradient at a flow of 1ml/min: (1) 35% to 45% methanol, from 0-5 min; (2) constant conditions from 5-10 min; (3) 45% to 65% methanol, from 10-20 min; (4) constant conditions from 20-25 min; (5) 65% to 100% methanol, from 25-35 min; (6) constant conditions from 35-40 min; (7) 100% to 35% methanol, from 40-60 min. Authentic ecdysteroids were analyzed with the same gradient and their retention times compared with the samples. VW extracts were injected directly onto the HPLC for separation. HL and ART extracts were purified by using Waters C-18 Sep-paks cartridges before injection on the HPLC. Procedures were similar to those described by Lafont et al. (1982) with modifications. Extracts were reconstituted in 10% methanol, injected on

the cartidge, rinsed with 30% methanol and the ecdysteroids were eluted from the cartridge with 70-75% methanol. Any remaining free or conjugated ecdysteroids were then removed with 100% methanol. Samples were filtered with 0.2 um Acrodisc filters (Gelman Sciences Inc., Ann Arbor, MI), concentrated (N_2), injected on the HPLC and collected at 1.0 min intervals with a Pharmacia fraction collector FRAC-100 (Pharmacia Fine Chemicals, Piscataway, NJ).

Radioimmunoassay (RIA)

Samples separated by HPLC were assayed by the RIA procedure of Hagedorn (personal communication) and Borst and O'Connor (1972,1974) as described in Dees et al. (1984a) to determine ecdysteroid composition. Antiserum was a gift from Dr. J. Koolman. The lyophilized antiserum was reconstituted in 500 ul distilled water, divided into five 100 ul aliquots and stored at -80° C until used. A 100 ul aliquot was removed, thawed and 10 ul diluted to a 1:10 dilution. An antiserum check was done to determine the best dilution for 50% binding. The remaining stock was then diluted and used in the RIA of crude extracts.

Standards were prepared with authentic ecdysone using known quantities ranging from 25 pg/100 ul to 1000 pg/100 ul. All samples were done in duplicate and standards and controls in triplicate. Samples from HPLC separation were dried (N_2), reconstituted in borate buffer (pH 8.4) and pipetted into 6 x 50 mm culture tubes. Standards and samples were all added to the tubes in 100 ul quantities.

Borate buffer (100 ul) was put in similar tubes for positive antiserum controls and negative (no antiserum) controls. ^{3}H ecdysone (45 Ci/mmol) was adjusted to 4000 cpm/50 ul in borate buffer and 50 ul added to each tube, including controls and standards. Then 50 ul of rabbit serum was added to the negative control tubes and 50 ul of diluted antiserum added to all of the other tubes including the positive controls. All samples were immediately mixed (vortex mixer) and incubated for 24 hrs at room temperature. After incubation, samples were cooled (4^{o} C), 200 ul saturated ammonium sulfate (SAS) added and mixed immediately. Samples were kept at 4^{o} C overnight to allow proteins (γ-globulins) to precipitate, centrifuged at 2500 rpm for 15 min at 4^{o} C and the supernatant was aspirated and discarded. The precipitate was resuspended and washed in 400 ul borate buffer and SAS (1:1) and centrifuged. The supernatant was again removed, discarded and the precipitate resuspended in 25 ul ethanol. The samples were then prepared for counting by adding 600 ul of RIA Fluor^{R} (New England Nuclear Corp., Boston, MA), mixed with a vortex mixer and centrifuged. Samples were counted using a Beckman Model LS-250 or a Beckman Model LS-1701 liquid scintillation counter (Beckman Instruments Inc., Silver Spring, MD). Efficiency of the separations and RIA was determined by eluting known amounts of authentic E and 20E with Waters C-18 sep-paks, followed by separation and

identification by HPLC and RIA. The recovery was 63.7% for E and 21.5% for 20E using this method.

The quantities (pg) of ecdysteroids in the samples were calculated from radioassay (CPM) data by computer analysis using "Analysis of Radioimmunoassay" (V.B. Faden and D. Rodbard, NIH, 1975 edition). The number of pg/10 FE are presented in the figures. In the HL samples the pg/ul were converted to pg/10 FE by multiplying by 15.9 ul/FE for D. varibilis (Sonenshine et al., 1985a) and 23.0 ul/FE for D. andersoni (Binnington & Obenchain, 1982).

^{3}H Ecdysone Injections

Tritiated ecdysone was injected into ticks to determine the fate of ecdysone in the hemolymph and anterior reproductive tract of these animals. Labelled ecdysone ^{3}H was dried (N_2), reconstituted in Shen's solution and injected into feeding adult virgin female ticks attached to rabbits. The rabbits were sedated with Acepromazine (AVECO Co., Fort Dodge, IA) and the ticks were inoculated on day 3 of feeding with approximately 0.5 uCi/tick using a 50 ul Hamilton syringe and 30 gauge needle. The ticks were allowed to feed an additional 4 days after treatment. Following feeding, they were removed, hemolymph and ART's collected and extracts prepared as described previously. These extracts were separated by HPLC and all peaks collected at 1.0 min intervals. They were then dissolved in scintillation cocktail (Dimilume, Packard Inst. Co., Oak Grove, IL) and

assayed for radioactivity with a Beckman Model LS-250 or a Beckman Model LS-1701 liquid scintillation counter LS-250. Counting efficiences after quench correction (external standard) were 36.0% and 39.2%, respectively.

^{3}H Cholesterol Injections

To determine if cholesterol was utilized in the biosynthesis of ecdysteroids in the ART and HL extracts, ^{3}H cholesterol was injected into these ticks. Labelled ^{3}H cholesterol was dried (N_2) and dissolved in a mixture of ethyl oleate and olive oil, 1:1 (v/v). Aliquots containing about 0.5 uCi were injected through the body wall (posterior end) into feeding female ticks (3 days) on a tranquilized rabbit with a 50 ul Hamilton syringe and 30 gauge needle. The ticks were allowed to feed (4 additional days), removed and hemolymph and ART's collected and extracts prepared as described above. These extracts were analyzed by HPLC and 1.0 min peaks collected, cocktail added and the samples assayed for radioactivity with a Beckman Model LS-250 or a Beckman Model LS-1701 liquid scintillation counter. Counting efficiences after quench correction (external standard) were 30.2% and 31.7%, respectively.

Esterase Hydrolysis

Aliquots of ART and HL extracts from ^{3}H cholesterol injected ticks were subjected to enzyme hydrolysis (Connat et al., 1986) to determine if undetected ecdysteroids were

present as conjugated ecdysteroids. The RIA does not detect conjugates of the ecdysteroids. Samples were dried (N_2) and reconstituted in 600 ul borate buffer (pH 8.4). Next, 50 IU of porcine liver esterase (E.C. 3.1.1.1, 3.2 M $NH_4(2)SO_4$) (Sigma Chemical Co., St. Louis, MO) was added to the sample and incubated overnight at 37° C. After 24 hrs, 300 ul of methanol was added and the reaction stopped. The sample was mixed and separated by C-18 cartridges as described above. The 75% and 100% fractions were combined, dried (N_2), separated by HPLC and collected at 1.0 minute intervals. Cocktail was then added and radioactivity counted with a Beckman Model LS-250 liquid scintillation counter.

Gas Chromatography

Ecdysteroid content of the ART and HL extracts was further examined by gas chromatography (GC) using a Packard Model 439 gas chromatograph equipped with an electron capture detector and a Shimadzu C-R3A Chromatopac recording integrator. Because ecdysteroids are not volatile and are thermally unstable they were derivatized to their trimethylsilyl (TMS) ethers in order to be detected by GC.

Derivatization of standards and extracts were done as described by Bielby et al. (1986). Approximately 25 ug of standards, 20 F.E. of ART extracts and 20 ul of HL extracts were evaporated to dryness under N_2 in Reacti-Vials (Pierce Chemical Co., Rockford, IL). The sample was then dissolved

in 65 ul pyridine and 35 ul trimethylsilylimidazole (TMSI) (Pierce Chemical Co., Rockford, IL) and incubated at 140° C for 60 hrs. A sample of 10 ul of derivatized standard was removed and diluted with toluene to give a concentration of 1 or 2 ng/ul. The resulting products were injected on a DB-1 capillary column, 0.324 mm x 15 m with a 0.25 um film thickness (J&W Scientific, Folsom, CA). Isothermal separation was used for the ART extracts with a column temperature of 275° C, the injector 285° C and the detector 275° C. The carrier gas was N_2 at 3-5 ml/min. A thermal gradient was used for the HL extracts. The temperature of the column was held at 250° C for 5 min and then raised 5°/min to a temperature of 275° C, and then held at this temperature for 10 min. The injector and detector temperatures remained the same as above.

Derivatized ART and HL extracts were concentrated and cleaned before injection by dissolving them in 1 ml toluene which was then loaded onto a precleaned silica Sep-pak cartridge (Waters & Associates, Milford, MA). The TMSI derivatives were eluted from the cartridge with 3 ml of double glass distilled ethyl ether and concentrated (N_2) before injection. Efficiency of derivatization recovery was estimated by comparison with known quantities of derivatized ^3H E, separated by TLC (methanol-washed Whatman Silica Gel HPTLC LHPK Plates; toluene:ethyl acetate, 7:3 v/v for 15 cm) and counted by liquid scintillation. The recovery was found to be approximately 72.0%.

RESULTS

Bioassays

The percentage of trials in which Dermacentor variabilis and D. andersoni males oriented to the dorsal surface (D), moved to the ventral surface (V), probed the gonopore (P) and placed a spermatophore in the gonopore (C) of a conspecific female during bioassays are presented in Tables 1 and 2. The mean behavioral score (R) as determined by the neutered female assay scoring system is also included. In both species the percent of trials in each step of the courtship (D,V,P and C) were significantly higher for the unneutered females as compared to the neutered females. D. variabilis (Table 1) males exposed to neutered females treated with ART extracts showed no significant increase in dorsal orientation (D) or ventral orientation (V) when compared to the methanol controls. However, there was a significant increase in the number of trials in which probing behavior (P) occurred at 0.1 FE (63.1%) and 0.5 FE (62.2%) when compared to the methanol controls (40.4%). Additionally, copulation (C) occurred at significantly higher rates, 0.05 FE (32.0%), 0.1 FE (33.8%) and 0.5 FE (41.0%), when compared to the methanol controls (4.8%). The mean behavioral scores (R) were also significantly different than the controls at these same

Table 1. Comparison of D. variabilis (DV) male responses to part-fed neutered DV females treated with DV anterior reproductive tract extracts from part-fed females (ART) and anterior reproductive tract extracts from DV unfed females (UART) and methanol controls. Also DV male responses to normal (not neutered) DV females (positive controls).

Treatment	#reps	Trials	Mean Positive Responses[1] D[2]	V	P	C	Mean Behavioral Scores R[3]
ART 10 FE	25	305	88.3±1.8^{cd4}	64.0±3.4^{cde}	28.4±3.8^{cde}	5.9±2.0^{e}	19.2±6.3^{def}
5 FE	25	314	92.0±1.7^{bcd}	70.6±3.4^{bcd}	45.3±7.2^{bc}	10.5±5.5^{de}	18.4±6.9^{def}
1 FE	25	309	92.9±1.2^{bc}	73.2±4.0^{bcd}	46.6±6.8^{bc}	18.1±6.3^{cde}	28.7±7.8^{cde}
0.5 FE	25	278	94.9±1.5^{ab}	82.1±3.9^{ab}	62.2±7.6^{b}	41.0±8.9^{b}	52.4±9.7^{b}
0.1 FE	25	246	95.2±1.5^{ab}	87.3±3.6^{ab}	63.1±7.5^{b}	33.8±8.3^{bc}	49.2±9.3^{bc}
0.05 FE	25	263	92.3±1.5^{bcd}	77.3±3.9^{bc}	45.6±7.9^{bc}	32.0±9.5^{bcd}	33.2±9.4^{bcd}
0.01 FE	25	333	88.3±1.5^{cd}	63.5±3.9^{cde}	31.2±6.1^{cde}	12.0±6.6^{cde}	13.3±6.6^{def}
UART 10 FE	15	225	89.4±1.6^{cd}	51.1±4.8^{e}	12.4±2.7^{e}	0.0±0.0^{e}	0.0±0.0^{e}
1 FE	15	198	88.0±1.9^{cd}	60.0±6.4^{cde}	24.9±8.4^{cde}	10.0±7.2^{de}	14.1±8.8^{def}
0.1 FE	15	201	88.9±2.0^{cd}	63.3±6.2^{cde}	26.7±8.4^{cde}	13.3±9.1^{cde}	14.1±9.0^{def}
0.01 FE	15	211	87.6±1.9^{d}	58.2±5.8^{de}	20.0±6.3^{de}	6.7±6.7^{e}	7.7±6.7^{def}
MeOH Control	30	433	92.1±1.5^{bcd}	69.6±3.2^{bcd}	40.4±4.9^{cd}	4.8±3.3^{e}	5.5±3.5^{ef}
Not Neutered	25	57	99.5±0.5^{a}	95.9±1.9^{a}	88.8±4.5^{a}	76.3±7.2^{a}	91.2±4.2^{a}

[1]Average percentage of trials in which a behavior occurred. Mean ± standard error.

[2]D = dorsal orientation, V = Ventral orientation, P = Probing of the gonopore, and C = Copulation (i.e., male formation and placement of a spermatophore in the gonopore of a female).

[3]R = Behavioral response score based on the neutered female scoring system. Copulation on 1st male = 100%, 2nd male = 70%, 3rd male = 65%, 4th or 5th males = 60%.

[4]Statistical analysis was done by Duncan multiple range tests on the Arcsin transformation of the data. Those with different letters are significantly different at the 0.05 level.

Table 2. Comparison of D. andersoni (DA) male responses to part-fed neutered DA females treated with DA anterior reproductive tract extracts from part-fed females (ART) and anterior reproductive tract extracts from DA unfed females (UART) and methanol controls. Also DA male responses to normal (not neutered) DA females (positive controls).

Treatment	#reps	Trials	Mean Positive Responses[1] D[2]	V	P	C	Mean Behavioral Scores R[3]
ART 10 FE	25	375	85.2±1.6^{bc4}	46.9±4.6^{b}	14.7±4.6^{b}	0.0±0.0^{c}	0.2±0.2^{c}
5 FE	25	375	87.2±1.2^{bc}	56.8±3.3^{b}	16.3±2.4^{b}	0.0±0.0^{c}	0.6±0.2^{c}
1 FE	25	304	95.1±1.3^{abc}	86.8±2.3^{a}	64.6±6.2^{a}	20.0±6.8^{ab}	34.6±7.2^{b}
0.5 FE	25	338	94.1±1.6^{abc}	83.7±3.3^{a}	62.5±6.9^{a}	9.3±4.7^{bc}	31.1±7.3^{b}
0.1 FE	25	299	92.8±1.7^{abc}	79.8±4.2^{a}	53.7±7.5^{a}	22.0±7.5^{ab}	36.2±8.4^{b}
0.05 FE	25	347	89.9±1.3^{bc}	51.7±4.9^{b}	20.5±5.6^{d}	8.0±5.5^{bc}	9.0±5.5^{bc}
0.01 FE	25	375	88.5±1.7^{bc}	49.3±4.6^{b}	16.8±4.0^{b}	0.0±0.0^{c}	1.3±0.5^{c}
UART 10 FE	15	225	85.3±2.2^{bc}	49.3±4.7^{b}	14.7±4.2^{b}	0.0±0.0^{c}	0.5±0.3^{c}
1 FE	15	225	87.1±1.9^{bc}	50.7±5.5^{b}	10.7±2.7^{b}	0.0±0.0^{c}	1.1±0.5^{c}
0.1 FE	15	225	85.3±2.0^{bc}	56.0±3.7^{b}	17.3±2.6^{b}	0.0±0.0^{c}	1.3±0.6^{c}
0.01 FE	15	225	89.3±1.6^{bc}	56.9±3.3^{b}	18.7±3.9^{b}	0.0±0.0^{c}	1.3±0.8^{c}
MeOH Control	30	436	81.3±2.8^{c}	51.3±4.7^{b}	17.8±4.0^{b}	3.3±3.3^{c}	4.0±3.3^{c}
Not Neutered	25	182	96.3±1.2^{a}	88.5±3.1^{a}	55.3±7.3^{a}	33.5±7.5^{a}	56.2±8.6^{a}

[1]Average percentage of trials in which a behavior occurred. Mean ± standard error.

[2]D = dorsal orientation, V = Ventral orientation, P = Probing of the gonopore, and C = Copulation (i.e., male formation and placement of a spermatophore in the gonopore of a female).

[3]R = Behavioral response score based on the neutered female scoring system. Copulation on 1st male = 100%, 2nd male = 70%, 3rd male = 65%, 4th or 5th males = 60%. Additional points for extended probing included 2% for 6-10 min, 4% for 11-20 min, 6% for 20-100 min and 10% if the male probed over 100 min.

[4]Statistical analysis was done by Duncan multiple range tests on the Arcsin transformation of the data. Those with different letters are significantly different at the 0.05 level.

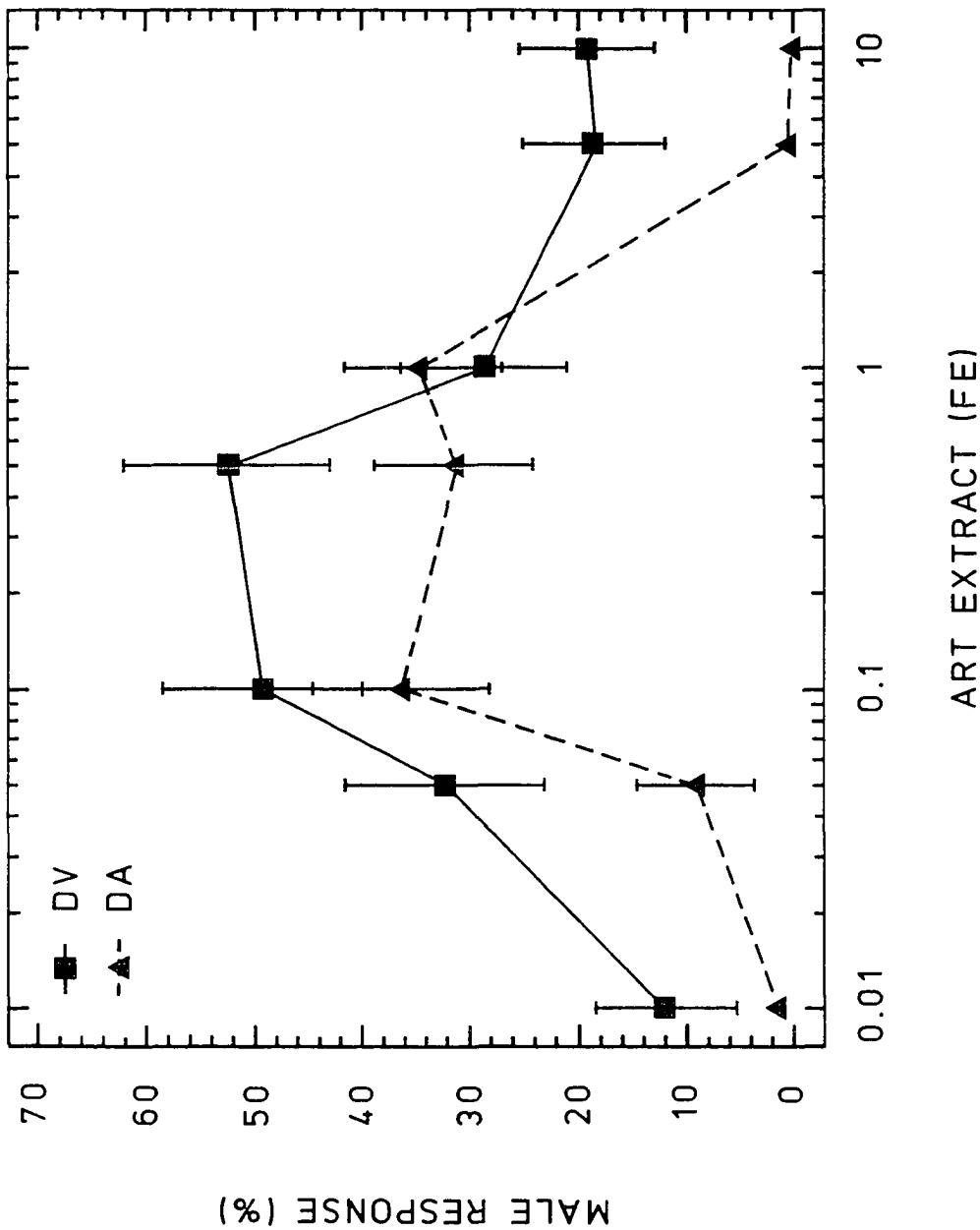
concentrations. Placing extracts made from the ART organs of unfed females did not excite male probing (P) or copulatory responses (C); there was no difference between these responses and the methanol controls. The behavioral scores were also not different than the controls. Clearly, treatment of the neutered females with ART extracts from part-fed females partially restored the male copulatory response eliminated by the neutering procedure.

Dermacentor andersoni male dorsal orientation (D) was not significantly different between the treatments and the methanol controls. In contrast to D. variabilis, ventral orientation (V) was significantly higher in D. andersoni than the methanol controls (51.3%) when the neutered females were treated with 0.1 FE (79.8%), 0.5 FE (83.7%) and 1 FE (86.8%) of ART extracts from part-fed females. These were also not significantly different than the unneutered control (88.5%). Similar to D. variabilis the percentage of trials in which males probed were significantly higher in the treatments with ART extracts at 0.1 (53.7%), 0.5 (62.5%) and 1.0 FE (64.6%) than the methanol controls (17.8%). These were not significantly different than the unneutered control (55.3%). Additionally, the number of trials in which copulation occurred in D. andersoni were significantly greater than the methanol controls (3.3%) in treatments of 0.1 and 1 FE ART extract from part-fed females and occurred in 22.0 and 20.0% of the trials, respectively. These were also not

significantly different than the unneutered control (33.5%). The percentage of trials in which copulation occurred in the methanol controls was only 3.3%. The mean behavorial score (R) also showed a significant increase at 0.1, 0.5 and 1.0 FE. As in D. variabilis, treatment with extracts of ART organs from unfed females did not excite male probing (P) or copulatory responses (C). In summary, the treatment of neutered females with ART extracts from part-fed females also restored the male copulatory response in this species. In contrast to D. variabilis these same extracts also resulted in increased ventral orientation in D. andersoni.

Concentration of the ART extract from part-fed females appeared to be important in the degree of male response to the treated females. Fig. 10 illustrates male responses to neutered females treated with different concentrations of ART extracts as determined by the scoring system. The strongest positive responses by D. variabilis males were to the ART extract at a concentration of 0.5 FE and by D. andersoni males to the ART extract at a concentration of 0.1 FE, 52.4% and 36.2%, respectively. Orthoganol contrasts showed D. variabilis positive responses to the ART extract were significantly different than the methanol controls at 0.05 FE ($p=0.0063$), 0.1 FE ($p=0.0001$), 0.5 FE ($p=0.0001$) and 1 FE ($p=0.0122$). However, D. andersoni responses were weaker and were significant at 0.05 FE ($p=0.0095$), 0.1 FE ($p=0.0001$), 0.5 FE ($p=0.0001$) and 1 FE

Figure 10. Effect of different concentrations (FE) of anterior reproductive tract (ART) extracts on the mean behavioral score of male D. variabilis and D. andersoni in the neutered female bioassay. Bars represent standard error of the mean. (N=25).



(p=0.0172). In both species, the responses appear to follow a dose-response curve.

Males of D. variabilis and D. andersoni responded positively to neutered females treated with various concentrations of 20E (Fig. 11) and E (Fig. 12). The male responses to 20E appear to follow a dose-response curve in both species. However, in D. variabilis the curve is sharper. The strongest positive responses in D. variabilis to 20E were higher than to E, 53.4% at 10 ng and 40.0% at 100 ng, respectively. The highest response to 20E occurred at one log concentration lower than for E (Fig. 11 & 12). Responses to 20E were significantly different from the controls at 1 ug (p=0.0075), 0.01 ug (p=0.0001), 0.001 ug (p=0.0105), and 0.000001 ug (p=0.0446) (orthongal contrasts). Responses to ecdysone were significantly different than the controls at 10 ug (p=0.0090), 1 ug (p=0.0124), 0.1 ug (p=0.0013) and 0.001 ug (p=0.0237). Male D. andersoni responses to 20E were also higher than to E, 32.4% at 0.0001 ug and 19.6 % at 1 ug, respectively. These responses to 20E were at a concentration 3 logs lower than for E. Responses to 20E at 1 ug (p=0.0111), 0.01 ug (p=0.0316), 0.001 ug (p=0.0020) and 0.0001 ug (p=0.0337) were significantly different than the controls. Responses to E are not significantly different than the controls at any concentration. In summary, D. andersoni also responded much more strongly to 20E than E.

Figure 11. Effect of different concentrations of authentic 20-hydroxyecdysone (20E) on the mean behavioral score of male D. variabilis and D. andersoni in the neutered female bioassay. Bars represent standard error of the mean. (N=25).

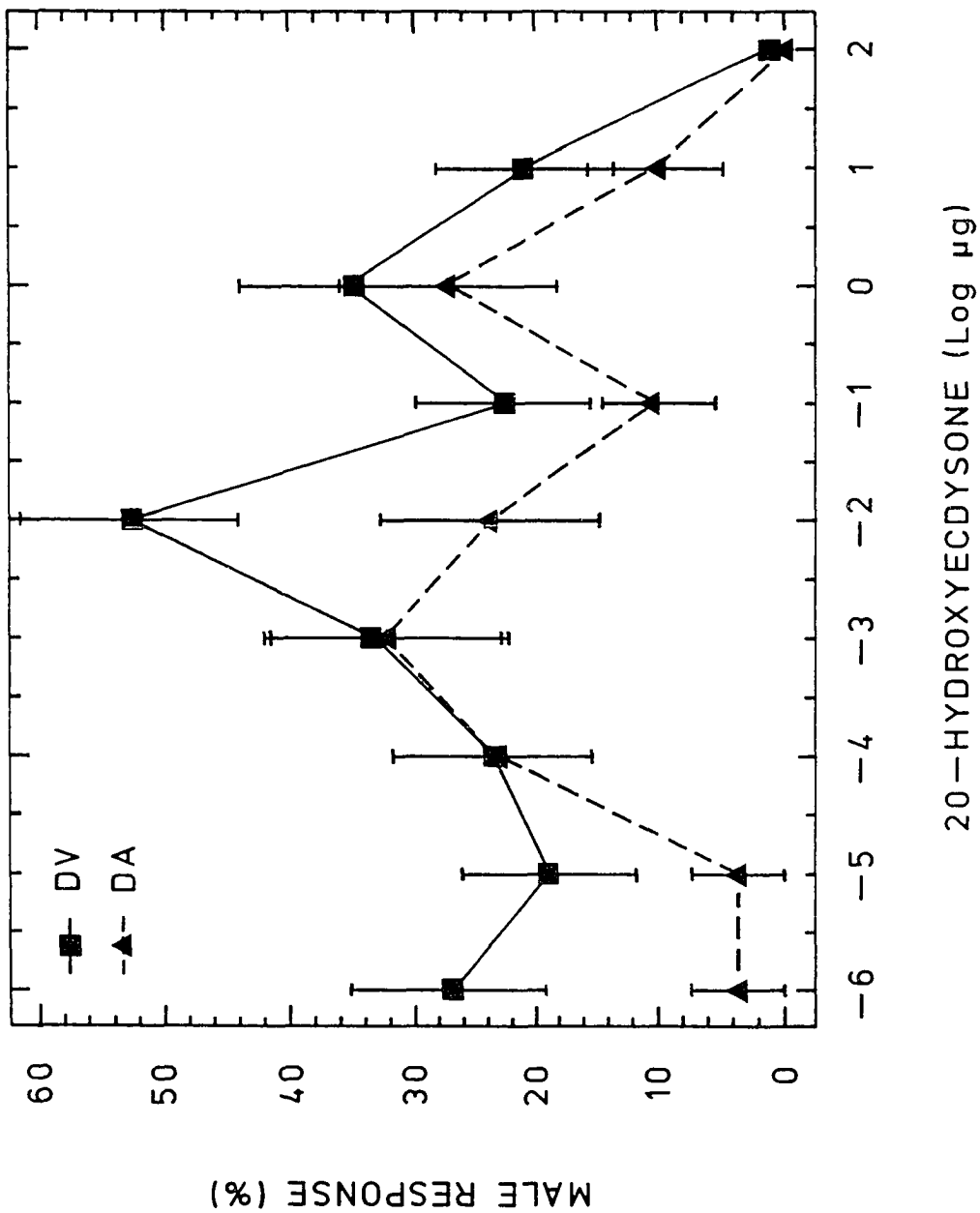
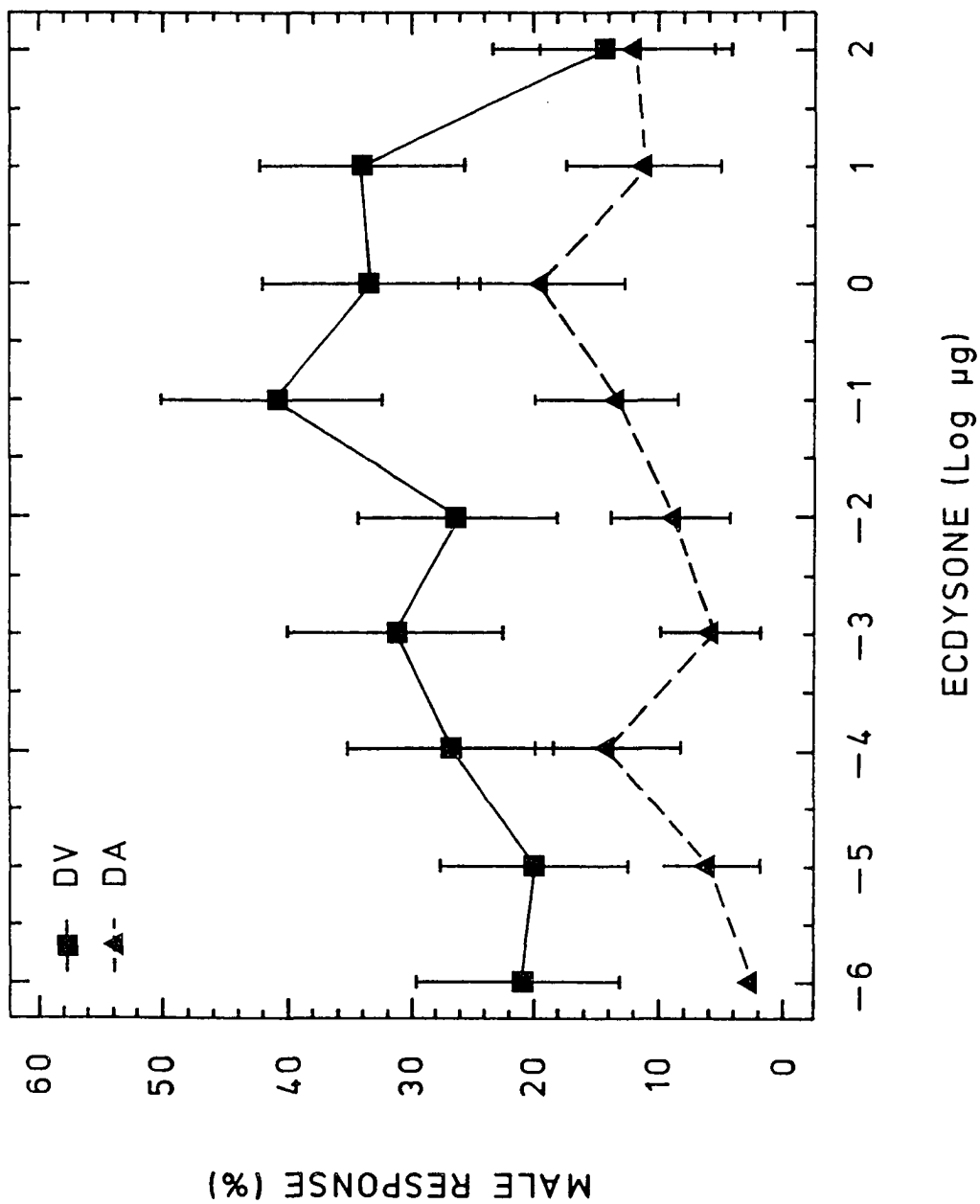


Figure 12. Effect of different concentrations of authentic ecdysone (E) on the mean behavioral score of male D. variabilis and D. andersoni in the neutered female bioassay. Bars represent standard error of the mean. (N=25).

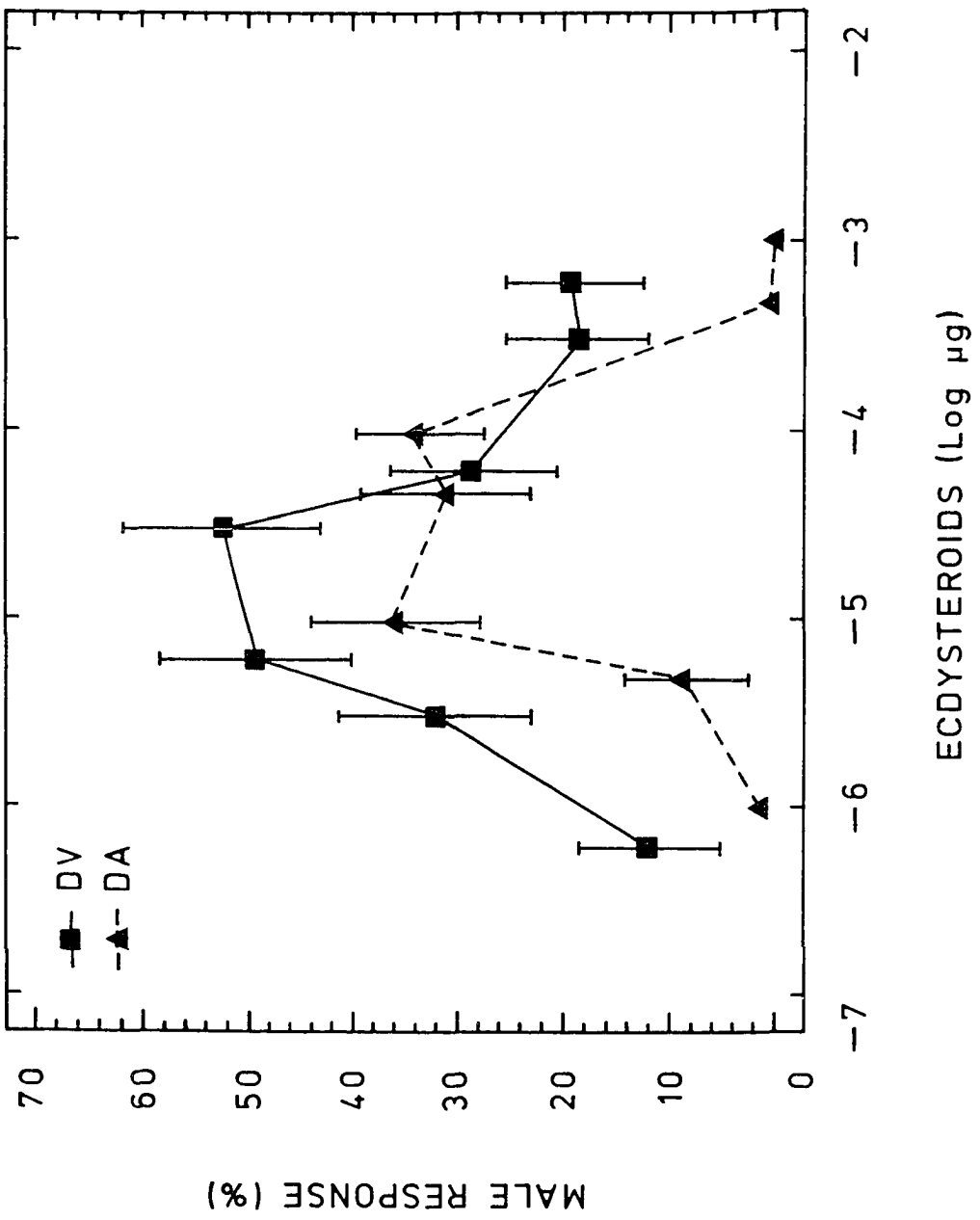


Comparisons were also made between 20E and E treatments and the ART extract by Duncan's multiple range tests. In D. variabilis responses to 20E at 1 ug, 0.01 ug and 0.001 ug were not significantly different than responses to ART extract (0.5 FE). Responses to E at 10 ug, 1 ug, 0.1 ug and 0.001 ug were the same as responses to the ART extract (0.5 FE). In D. andersoni the responses to 20E at 1ug, 0.01 ug, 0.001 ug and 0.0001 ug were not significantly lower than to the ART extracts (0.1 FE). Responses to all concentrations of E were significantly lower than the ART extracts (0.1 FE).

Bioassays were also done with heterospecific neutered females at the same concentrations of 20E and E (not shown in the figures). Responses were very low and not significanlty different than the controls. However, it is interesting to note that one copulation occurred at 0.001 ug 20E in each of the two species.

The results of the radioimmunoassay demonstrated concentrations of ecdysteroids in the ART extract of 58.8 pg/female in D. variabilis and 92.1 pg/female in D. andersoni (see Radioimmunoassay below). Using this data on observed total tick ecdysteroid content, the amount of ART extract administered may be expressed in terms of concentration of these compounds, enabling us to replot the bioassay responses. When re-evaluated on this basis we get curves (Fig. 13) that can be compared with the 20E bioassay results (Fig. 11). It is apparent that the strongest male

Figure 13. Effect of different concentrations of ART extracts expressed as ug of ecdysteroids as determined by RIA on the mean behavioral score of male D. variabilis and D. andersoni in the neutered female bioassay. Bars represent standard error of the mean. (N=25).



mating responses occurred at lower concentrations of natural ecdysteroids (Fig. 13) as compared with the 20E standards. The peak male responses to natural ecdysteroids were at 2.94×10^{-6} ug in D. variabilis and 9.21×10^{-6} ug in D. andersoni, whereas responses to authentic 20E were highest at 0.01 ug (2.08×10^{-5} M) and 0.001 ug (2.08×10^{-6} M), respectively.

The mean behavioral scores of male D. variabilis and D. andersoni to conspecific neutered females treated with 4 other ecdysteroids and 6 sterols were compared to male responses to 20E, E, ART extract and methanol controls (Fig. 14 & 15). Responses of D. variabilis (Fig. 14) to ART extract ($p=0.0014$), MAK ($p=0.0012$), DBE ($p=0.0001$), E ($p=0.0013$), 20E ($p=0.0001$) and C35 ($p=0.0069$) were significantly higher than the methanol controls. The highest response was to 20E (53.4%). In addition, only two other ecdysteroids gave responses similar to the ART extract (52.4%), E (40.0%) and DBE (40.8%).

The response of D. andersoni males to the same ecdysteroids and sterols are presented in Figure 15. D. andersoni males also showed a greater response to 20E ($p=0.0020$) than any other treatment except the ART extract ($p=0.001$). In contrast to the D. variabilis, D. andersoni also responded positively to treaments with cholesterol ($p=0.0318$). Responses to all other treatments were not significantly different than the methanol controls.

Figure 14. Mean behavioral scores of *D. variabilis* male responses to ecdysteroid and sterol standards, ART extract (0.5 FE) and methanol controls. Mean ± standard error of 25 - 30 replicates. Different letters represent statistical differences between the means as determined by Duncan's multiple range tests.

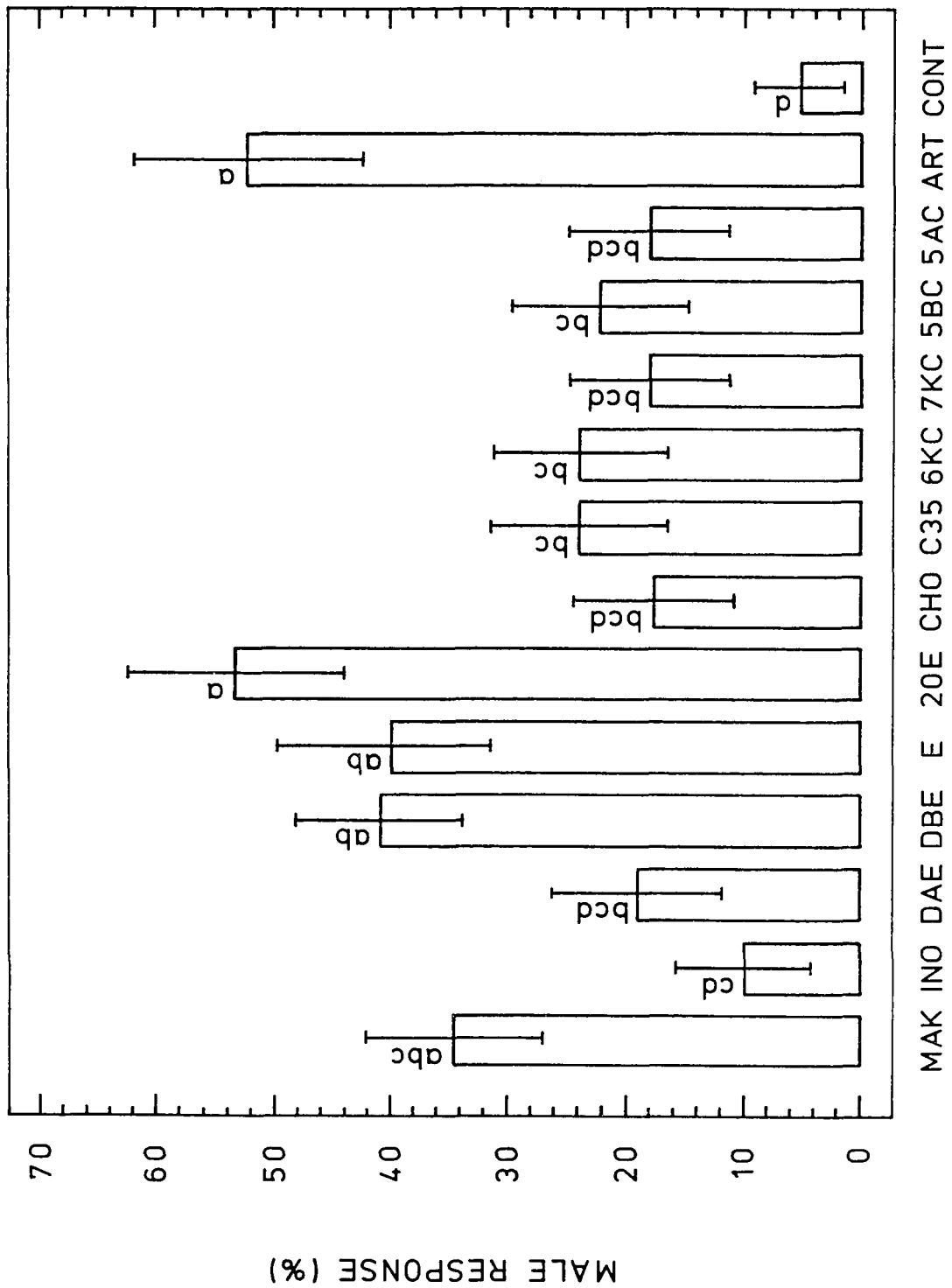
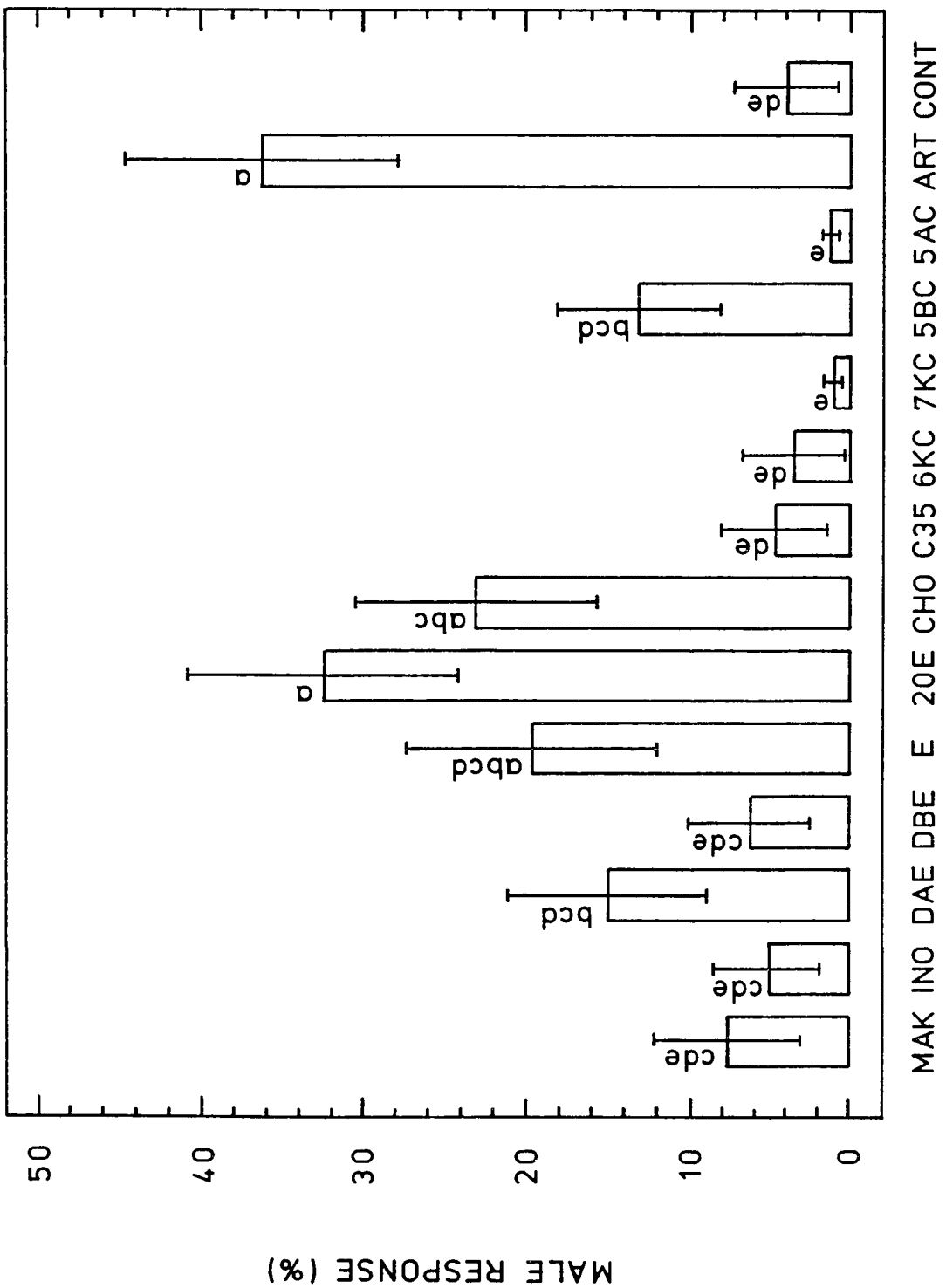


Figure 15. Mean behavioral scores of *D. andersoni* male responses to ecdysteroid and sterol standards, ART extract (0.1 FE) and methanol controls. Mean ± standard error of 25 - 30 replicates. Different letters represent statistical differences between the means as determined by Duncan's multiple range tests.



Electrophysiological Assays

Representative oscilloscope tracings illustrating the responses of the cheliceral sensilla of D. variabilis and D. andersoni to various chemical stimuli are presented in Figure 16. The number of spikes/msec were determined by counting the spikes in several different tracings using an enlarged view of the tracings as enhanced by a computer program. There was no difference between the 3 concentrations of NaCl tested. Therefore, all other chemicals were dissolved in a 0.1 M NaCl solution to insure good conductivity (Table 3). The lower concentrations of ART extract and 20E were not significantly different from the NaCl controls. D. andersoni spike frequency was higher than D. variabilis for all chemicals tested. In D. variabilis the highest responses were to the ART extract. Strong responses were also observed with 20E and E at concentrations of 1 ug/ul. The greatest responses were to 20E in D. andersoni. In addition D. andersoni responded strongly to the ART extract. Responses to E were not different from the controls in this species. Results of stimulation with lower concentrations of E were ambiguous.

Radioimmunoassay (RIA)

Crude extracts of the ART, HL, VW, GW, GWN, and AW were analyzed by radioimmunoassay for total ecdysteroids (Fig. 17). Larger amounts of ecdysteroids were found in the ART (54.7 pg/tick) and VW (38.8 pg/tick) extracts of D. andersoni than in D. variabilis, 16.0 pg/tick and 4.9

Figure 16. Oscilloscope tracings illustrating the responses of D. andersoni and D. variabilis cheliceral digit sensilla to chemical stimuli administered by microelectrodes. (A) Response of the inner cheliceral digit sensilla of D. andersoni to 0.1 M NaCl solution. (B) Response of the inner cheliceral digit sensilla of D. andersoni to 20-hydroxyecdysone (2.08×10^{-2} M). (C) Response of the inner cheliceral digit sensilla of D. andersoni to ART extract (1 FE). (D) Response of the inner cheliceral digit sensilla of D. variabilis to 0.1 M NaCl solution. (E) Response of the inner cheliceral digit sensilla of D. variabilis to 20-hydroxyecdysone (2.08×10^{-2} M). (F) Response of the inner cheliceral digit sensilla of D. variabilis to ART extract (1 FE).

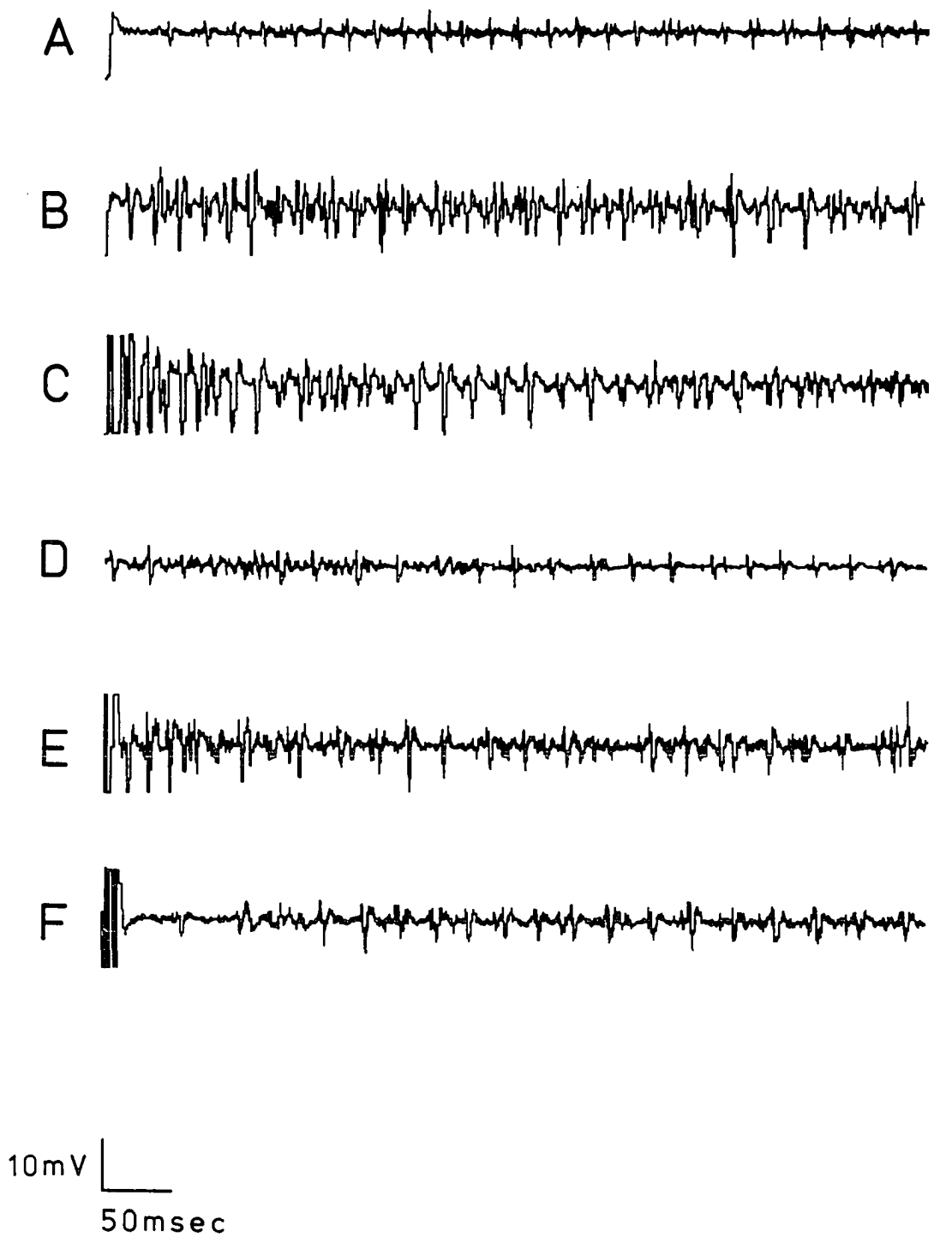
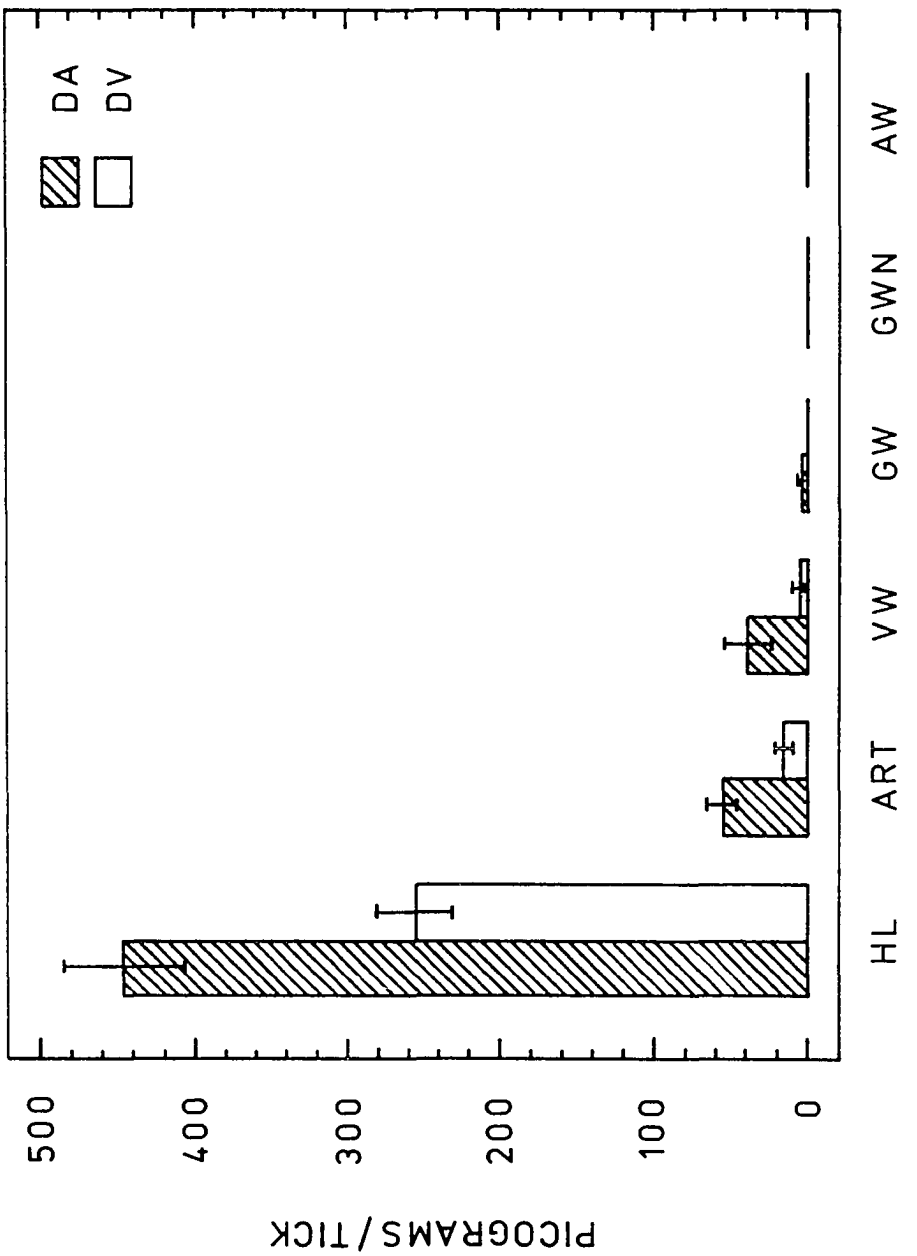


Table 3. Results of chemical stimulation of the inner cheliceral digits of D. variabilis and D. andersoni males with various chemicals. The values represent mean spikes/msec ± standard error. N = the number of preparations/chemical.

Treatment	D. variabilis spikes/msec	N	D. andersoni spikes/msec	N
NaCl 0.1 M	115.0±3.5	3	117.2±14.6	9
0.01 M	110.0±4.5	3	103.8±5.9	4
0.001 M	115.0±14.0	2	107.5±3.5	2
20E 10 ug	154.0±8.6	5	177.2±6.2	9
0.1 ug	107.5±24.7	2	118.3±8.7	6
0.001 ug	127.5±3.5	2	121.7±7.4	3
ART 1 FE	163.3±6.3	6	162.5±5.7	6
E 10 ug	155.0±7.1	7	123.3±3.7	6

Figure 17. The number of picograms of immunoreactive material in extracts of the hemolymph (HL), anterior reproductive tract (ART), vaginal lumen (VW), body surface around the genital pore of normal part-fed (7 days) females (GW), body surface around the genital pore of females neutered then fed for 7 days (GWN), and the body surface around the anal pore of normal part-fed females. Mean number of picograms $\pm$ the standard error presented.



pg/tick, respectively. Both D. variabilis and D. andersoni had large amounts of ecdysteroids in the hemolymph samples, 254.4 pg/tick and 446.2 pg/tick, respectively. Ecdysteroids were found in washes of the body surface immediately surrounding the genital pore of part-fed normal (not neutered) females in both species. These amounts were much larger in D. andersoni (4.07 pg/tick) extracts than in D. variabilis (0.37 pg/tick). Following neutering and feeding (7 days) ecdysteroids were not found in washes of the body surface around the genital area. Ecdysteroids were not found on the body surface around the anal pore of normal females.

Figures 18 thru 23 show the results of analysis of ecdysteroid content in the HL, ART and VW extracts by RIA after purification by HPLC. HL extract samples of D. variabilis (Fig. 18) and D. andersoni (Fig. 19) females had large amounts of total immunoreactive material. There were 1069.8 pg/10 FE in D. variabilis. The largest peak 858.6 pg coeluted with 20E. In addition, smaller amounts coeluted with other known compounds, specifically, E (111.4 pg), DBE (49.0 pg) and DAE (16.2 pg). In D. andersoni there were approximately 1609.7 pg/10 FE of RIA-positive material. The largest peaks coeluted with E (713.4 pg) and 20E (740.3 pg). In addition, there were 2 other peaks that coeluted with DBE (69.1 pg) and DAE (28.9 pg). A small unkown peak of 57.0 pg occurred between 22-23 min. These values are higher then what was found in the RIA of HL

Figure 18. Immunoreactive fractions of D. variabilis hemolymph (HL) extract. Extract was separated by Waters C-18 sep-pak cartidges then by HPLC and 1-min collections were assayed by RIA. Figure shows amounts detected, expressed in picograms (pg) per 10 ticks, superimposed on the HPLC chromatogram. Retention times for ecdysone (E), 20-hydroxyecdysone (20E), 2-deoxyecdysone (DAE) and 2-deoxy-20-hydroxyecdysone (DBE) are shown on the chromatogram ( ). HPLC conditions were a flow of 1 ml/min of methanol:Tris/$HClO_4$ buffer over a linear gradient; (1) 35 to 45% methanol from 0-5 min, (2) constant conditions from 5-10 min, (3) 45 to 65% methanol, from 10-20 min, (4) constant conditions from 20-25 min, (5) 65 to 100% methanol, form 25-35 min, (6) constant conditions from 35-40 min, (7) 100 to 35% methanol from 40-60 min.

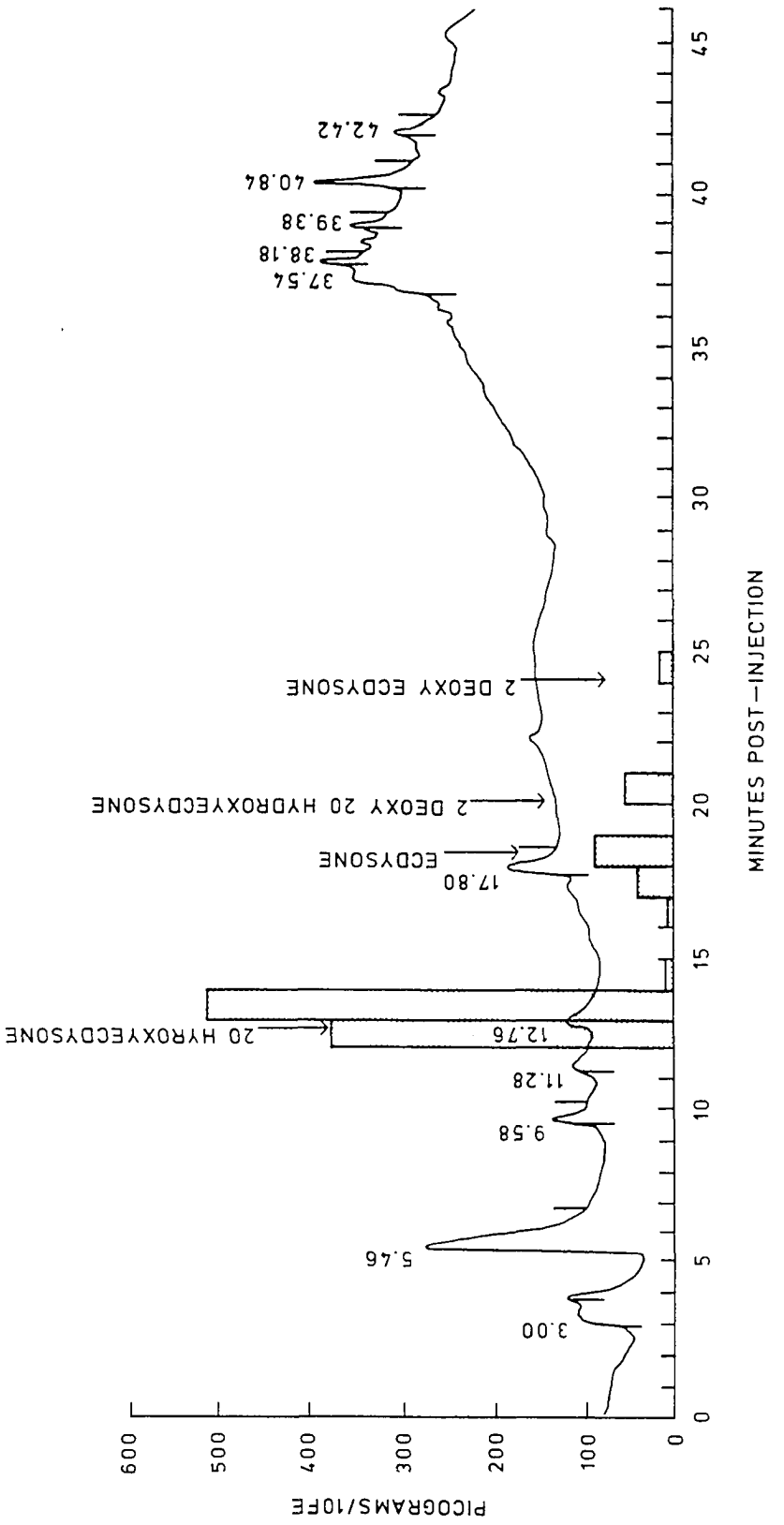
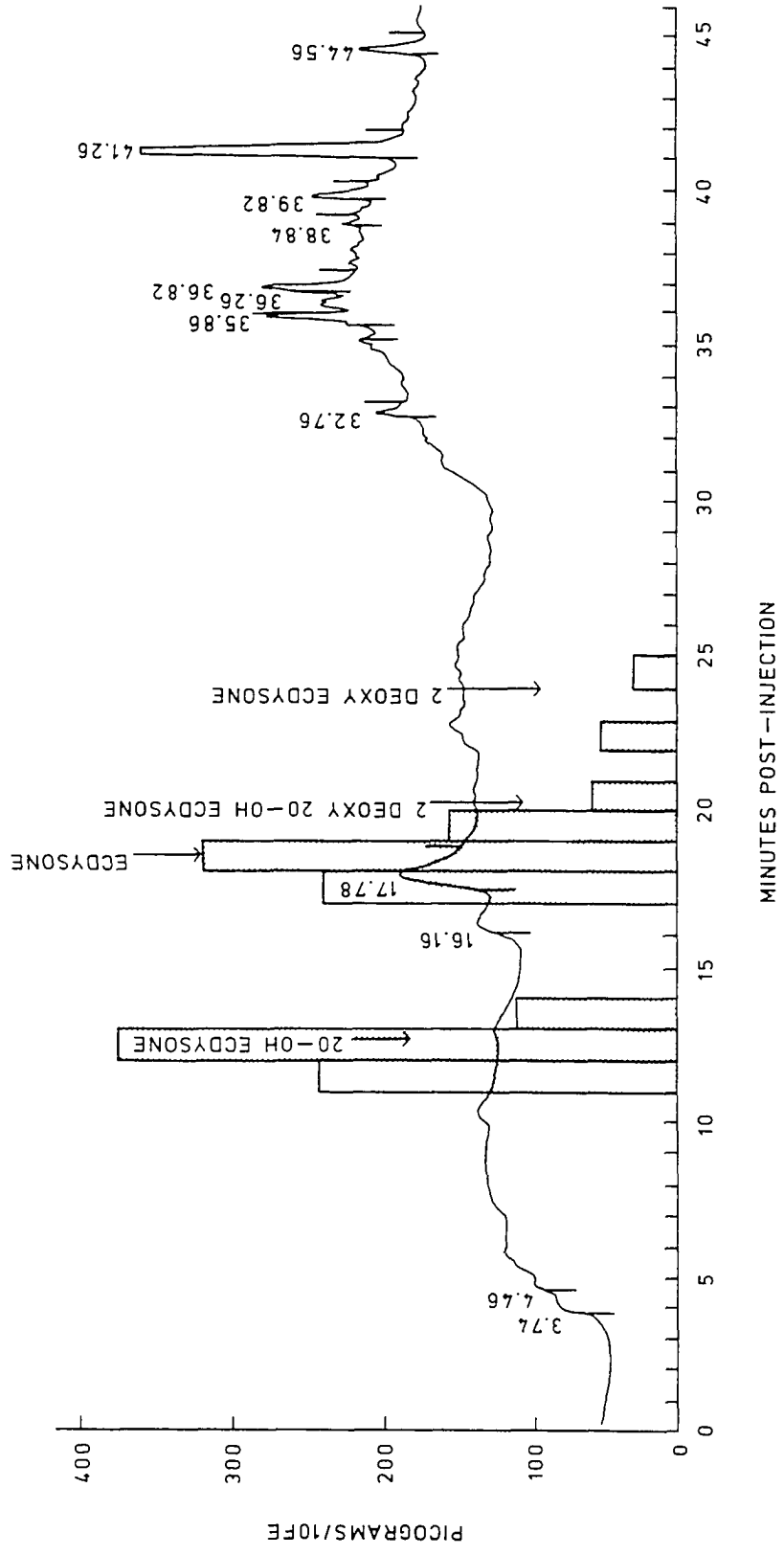


Figure 19. Immunoreactive fractions of D. andersoni hemolymph (HL) extract. Extract was separated by Waters C-18 sep-pak cartidges then by HPLC and 1-min collections were assayed by RIA. Figure shows amounts detected, expressed in picograms (pg) per 10 ticks, superimposed on the HPLC chromatogram. Retention times for ecdysone (E), 20-hydroxyecdysone (20E), 2-deoxyecdysone (DAE) and 2-deoxy-20-hydroxyecdysone (DBE) are shown on the chromatogram (↓). HPLC conditions were the same as described in Figure 18.



extract that had not been purified by HPLC. The hemolymph of D. variabilis appears to contain larger amounts of 20E, whereas D. andersoni has almost equal amounts of both 20E and E.

RIA-positive fractions of the ART extracts of D. variabilis and D. andersoni are presented in figures 20 and 21, respectively. There were a total of 587.7 pg/10 FE in D. variabilis ART extract. The largest peak coeluted with 20E (442.5 pg). In addition there were peaks that coeluted with E (105.6 pg) and DAE (11.3 pg). Small unknown peaks occurred between 8-9, 14-16 and 22-23 min. The total ecdysteroid content for the D. andersoni ART extract was 921.2 pg/10 FE. The largest peak coeluted with 20E (671.6 pg). Two other peaks coeluted with the ecdysteroids E (158.2 pg) and DAE (88.3 pg). Clearly, D. andersoni contained a substantially larger amount of both 20E and E than D. variabilis.

RIA-positive fractions from the VW extracts of D. variabilis and D. andersoni are presented in Fig. 22 and Fig 23, respectively. In D. variabilis the total ecdysteroids were approximately 57.3 pg/10 FE, all in the form of a single peak that coeluted with E. D. andersoni VW extract had approximately 6.5 times more immunoreactive material than D. variabilis. The total ecdysteroid content of D. andersoni was 375.1 pg/10 FE. The largest peak coeluted with E (250.6 pg). A small peak (75.5 pg) coeluted with 20E and a very small peak (25.0 pg) with DBE.

Figure 20. Immunoreactive fractions of D. variabilis anterior reproductive tract (ART) extract. Extract was separated by Waters C-18 sep-pak cartidges then by HPLC and 1-min collections were assayed by RIA. Figure shows amounts detected, expressed in picograms (pg) per 10 ticks, superimposed on the HPLC chromatogram. Retention times for ecdysone (E), 20-hydroxyecdysone (20E), 2-deoxyecdysone (DAE) and 2-deoxy-20-hydroxyecdysone (DBE) are shown on the chromatogram (↓). HPLC conditions were the same as described in Figure 18.

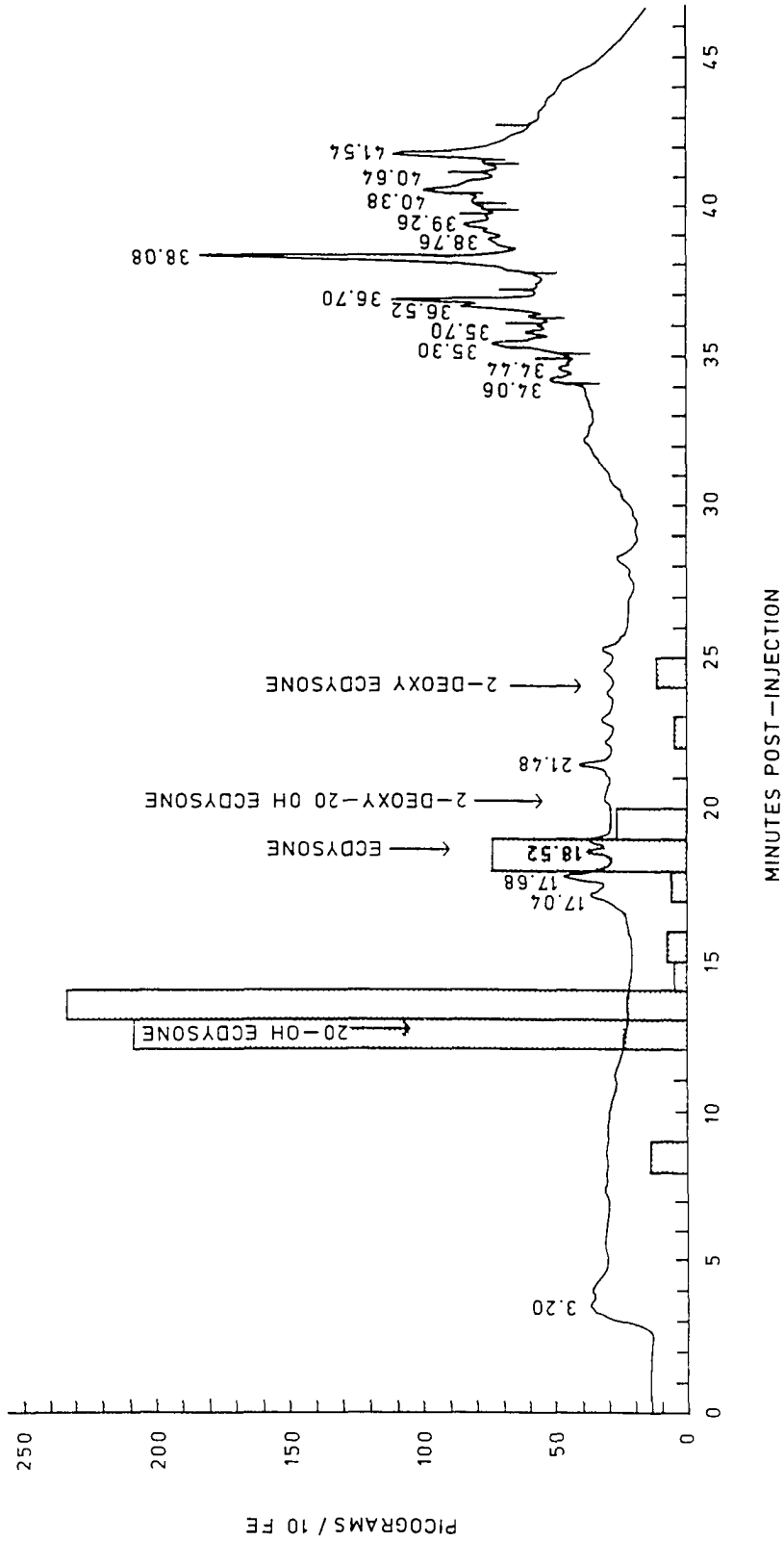


Figure 21. Immunoreactive fractions of D. andersoni anterior reproductive tract (ART) extract. Extract was separated by Waters C-18 sep-pak cartidges then by HPLC and 1-min collections were assayed by RIA. Figure shows amounts detected, expressed in picograms (pg) per 10 ticks, superimposed on the HPLC chromatogram. Retention times for ecdysone (E), 20-hydroxyecdysone (20E), 2-deoxyecdysone (DAE) and 2-deoxy-20-hydroxyecdysone (DBE) are shown on the chromatogram (↓). HPLC conditions were the same as described in Figure 18.

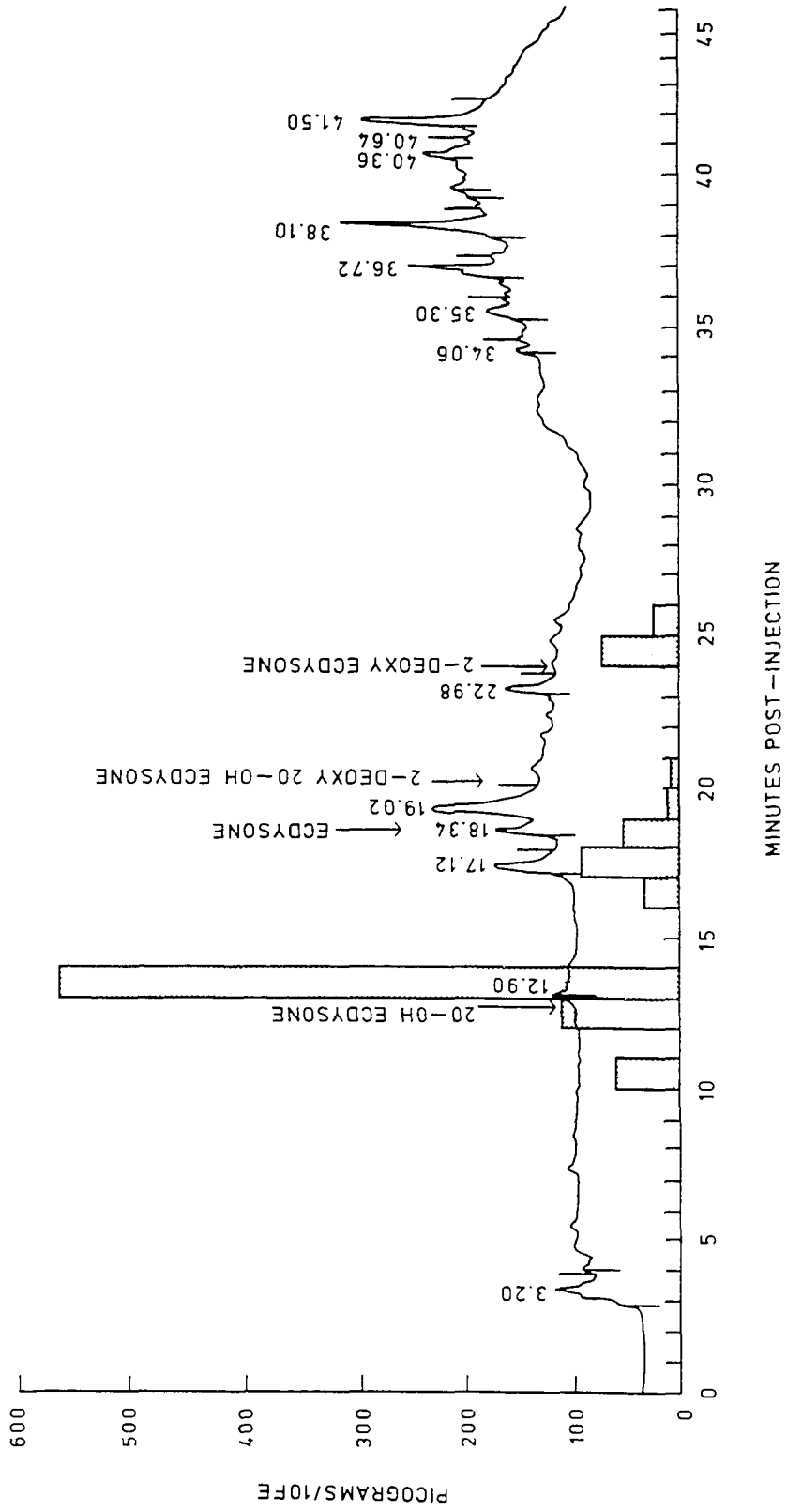


Figure 22. Immunoreactive fractions of D. variabilis vaginal washing (VW) extract. Extract was separated by HPLC and 1-min collections were assayed by RIA. Figure shows amounts detected, expressed in picograms (pg) per 10 ticks, superimposed on the HPLC chromatogram. Retention times for Ecdysone (E), 20-hydroxyecdysone (20E), 2-deoxyecdysone (DAE) and 2-deoxy-20-hydroxyecdysone (DBE) are shown on the chromatogram (↓). HPLC conditions were the same as described in Figure 18.

Figure 23. Immunoreactive fractions of D. andersoni vaginal washing (VW) extract. Extract was separated by HPLC and 1-min collections were assayed by RIA. Figure shows amounts detected, expressed in picograms (pg) per 10 ticks, superimposed on the HPLC chromatogram. Retention times for Ecdysone (E), 20-hydroxyecdysone (20E), 2-deoxyecdysone (DAE) and 2-deoxy-20-hydroxyecdysone (DBE) are shown on the chromatogram (↓). HPLC conditions were the same as described in Figure 18.

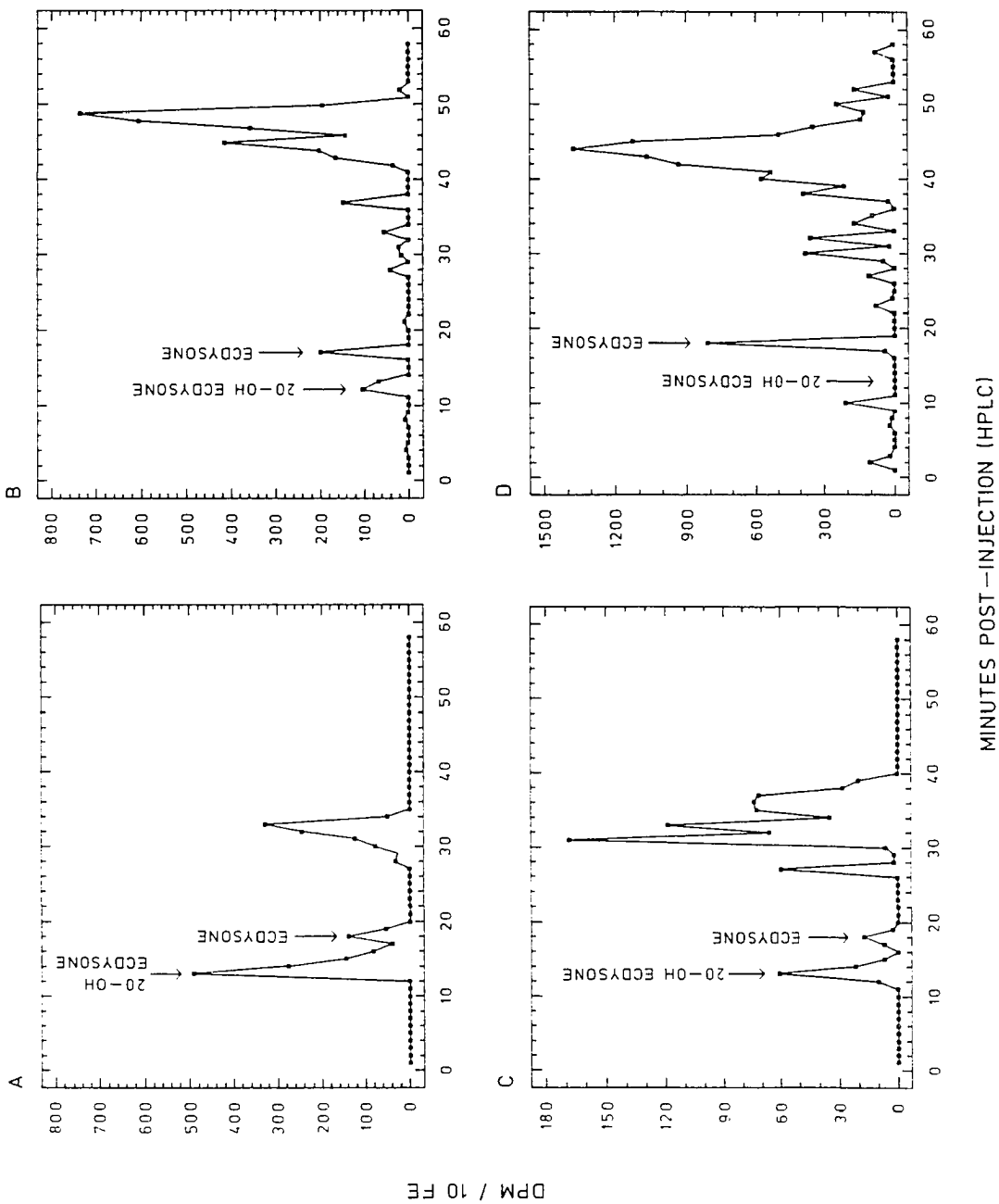
In addition there was a small unknown peak between 15-16 min. Clearly, ecdysteroids were found in larger amounts in the VW extract of D. andersoni then D. variabilis. In both species the majority was in the form of E.

^{3}H Ecdysone Incorporation

Extracts of the ART and HL of ticks injected with ^{3}H-labelled ecdysone were separated by HPLC and counted by LSC to determine the fate of ecdysone in these tissues. The results are presented in Fig. 24. When HL extracts are compared, large amounts of both polar and apolar fractions were found in D. variabilis (Fig. 24A). In D. andersoni (Fig. 24B) however the majority of the ^{3}H labelled activity was found in fractions that are very apolar. D. variabilis apolar fractions eluted within 28 to 34 minutes post-injection, whereas D. andersoni eluted within 42 to 50 minutes, suggesting that different compounds are being made by the incorporation of ^{3}H E in the two species. In D. variabilis more of the ^{3}H E in the HL extract was converted into 20E (765.9 DPM/10 FE) and a small amount remained as E (171.1 DPM/10 FE). In D. andersoni approximately 204.1 DPM remained as E, whereas 168.3 DPM were converted into 20E. In addition D. andersoni had several small peaks at 28-34 and 37-38 min.

The results of ^{3}H activity incorporation into the ART extract of D. variabilis and D. andersoni are presented in Figures 24C and 24D, respectively. When ART extracts are compared the largest amounts of ^{3}H activity occurred in

Figure 24. Distribution of ^{3}H radioactivity of 1-min HPLC collections of extracts from female ticks injected with ^{3}H ecdysone. HPLC conditions same as in Fig. 18. Retention times for ecdysone (18.56) and 20-hydroxyecdysone (12.78) are shown on the graphs (↓). (A) D. variabilis hemolymph (HL) extract, (B) D. andersoni hemolymph (HL) extract, (C) D. variabilis ART extract, (D) D. andersoni ART extract.

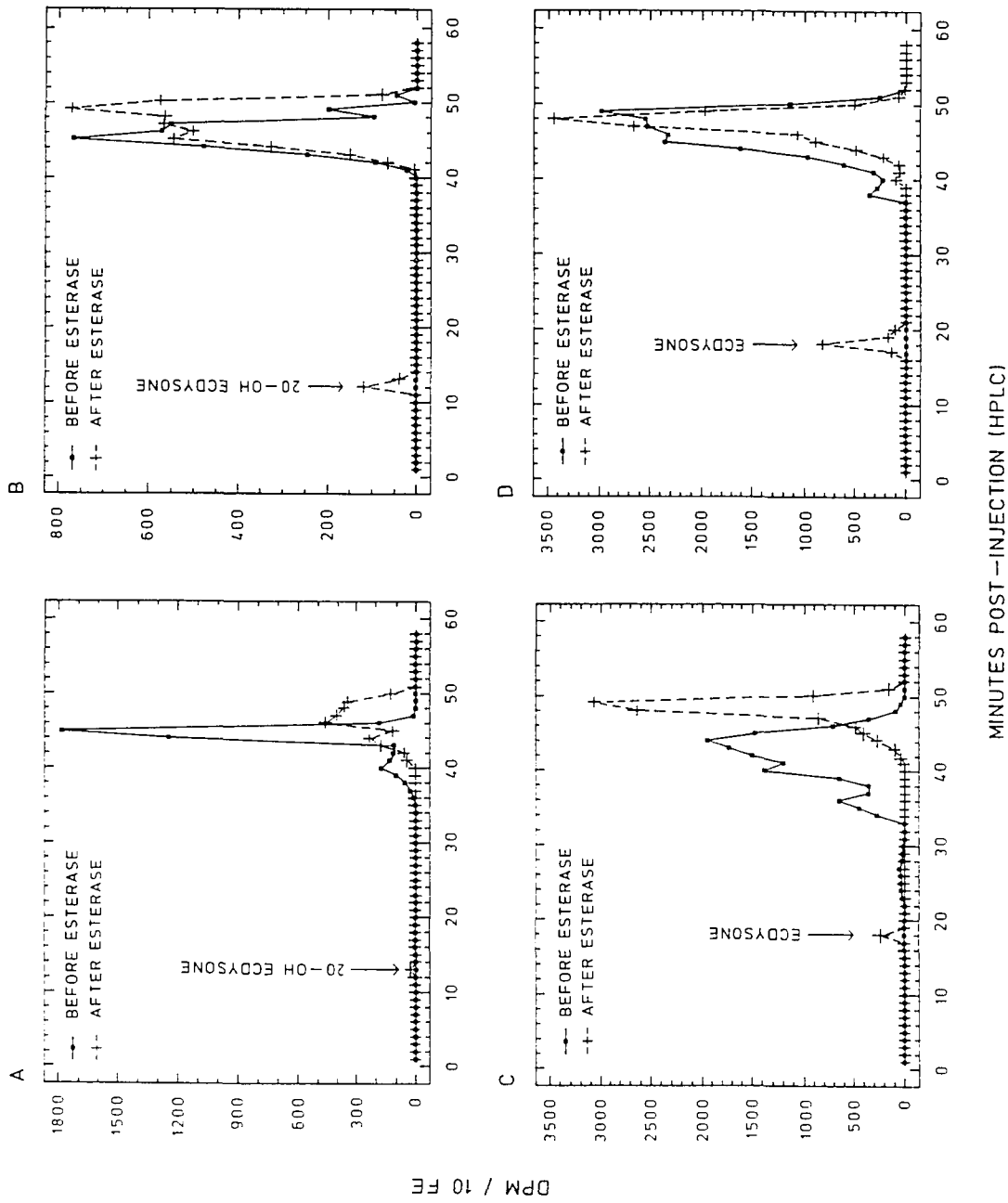


apolar fractions at 31 to 39 minutes post-injection in D. variabilis and 38 to 48 minutes in D. andersoni. The D. andersoni extract again gave fractions more apolar than in D. variabilis. This pattern is the same as was found in HL extracts. In addition to these fractions D. andersoni also had several small sharp peaks from 30 to 35 min. Both D. variabilis and D. andersoni had a large peak at 27 min; the D. andersoni (111.0 DPM/10 FE) peak was about twice as large as the D. variabilis (60.5 DPM/10 FE). In D. variabilis ART extract the largest of the 2 polar peaks (100.0 DPM) coeluted with 20E while the smaller (27.5 DPM) coeluted with E. In the D. andersoni ART extract the largest polar peak (855.8 DPM) coeluted with E. In addition D. andersoni had unkown peaks between 2-3, 10-11 and 23-24 min.

^{3}H Cholesterol Incorporation and Hydrolysis

Figure 25 illustrates the ^{3}H activity detected in ART and HL extracts of D. variabilis and D. andersoni females that were injected with ^{3}H cholesterol. Portions of the extracts were analyzed both before and after being subjected to esterase hydrolysis. The solid lines respresent the extracts before hydroylsis and the dashed lines after hydroylsis. In D. variabilis HL extract (Fig. 25A) the majority of ^{3}H activity before hydrolysis occurred at 44 and 45 min post-injection. This activity coeluted with cholesterol. After hydrolysis the radioactivity shifted and most occurred in a broad fraction 46-51 min.

Figure 25. Distribution of ^{3}H radioactivity of 1-min HPLC collections of extracts from female ticks injected with ^{3}H cholesterol (solid line). Distribution of ^{3}H radioactivity after extract is treated with porcine liver esterase (dashed line). HPLC conditions same as in Fig. 18. Retention times for ecdysone (18.56) and 20-hydroxyecdysone (12.78) are shown on the graphs (↓). (A) D. variabilis hemolymph (HL) extract, (B) D. andersoni hemolymph (HL) extract, (C) D. variabilis ART extract, (D) D. andersoni ART extract.



In addition, a small amount (27.0 DPM/10 FE) coeluted with 20E. The 20E fraction was not seen prior to esterase hydroylsis. In D. andersoni (Fig. 25B) most of the ^{3}H labelled activity was found in the same fraction that coeluted with cholesterol (44-45 min); however, some activity also occurred from 34 to 42 min. After hydrolysis almost all of the activity appeared in a broad nonpolar fraction, 42 to 51 min. In addition a large peak (156.7 DPM) coleuted with 20E.

ART extracts in D. variabilis (Fig. 25C) and D. andersoni (Fig. 25D) showed similar patterns of ^{3}H activity to that seen with the HL extracts. As in the D. andersoni HL extract, before hydrolysis, both species had the majority of their ^{3}H activity between fractions 34 to 48. After hydrolysis the large peaks of activity shifted to 44 to 52 min post-injection. Similarly both D. variabilis and D. andersoni have peaks that coelute with E. A larger peak appears at this point in the D. andersoni ART extract (1168.2 DPM/10 FE) than in the D. variabilis extract (265.6 DPM/10 FE). It appears that both species incorporated some of the ^{3}H cholesterol as conjugated ecdysteroids that were released after hydrolysis to yield 20E in the HL extracts and E in the ART extracts. D. andersoni appears to have done this to a greater degree.

Gas Chromatography

Results of gas chromatography (GC) of ART and HL extracts are presented in Fig. 26 and Fig. 27,

Figure 26. Gas chromatographic traces of ecdysteroids after TMSI derivatization. GC conditions were DB-1 capillary column (0.324 mm x 15 m), column temperature 275° C, injector 285° C and detector 275° C. Carrier gas N_2 at 3-5 ml/min. ECD detector used. (A) authentic ecdysone, (B) blank control, (C) D. variabilis ART extract and (D) D. andersoni ART extract.

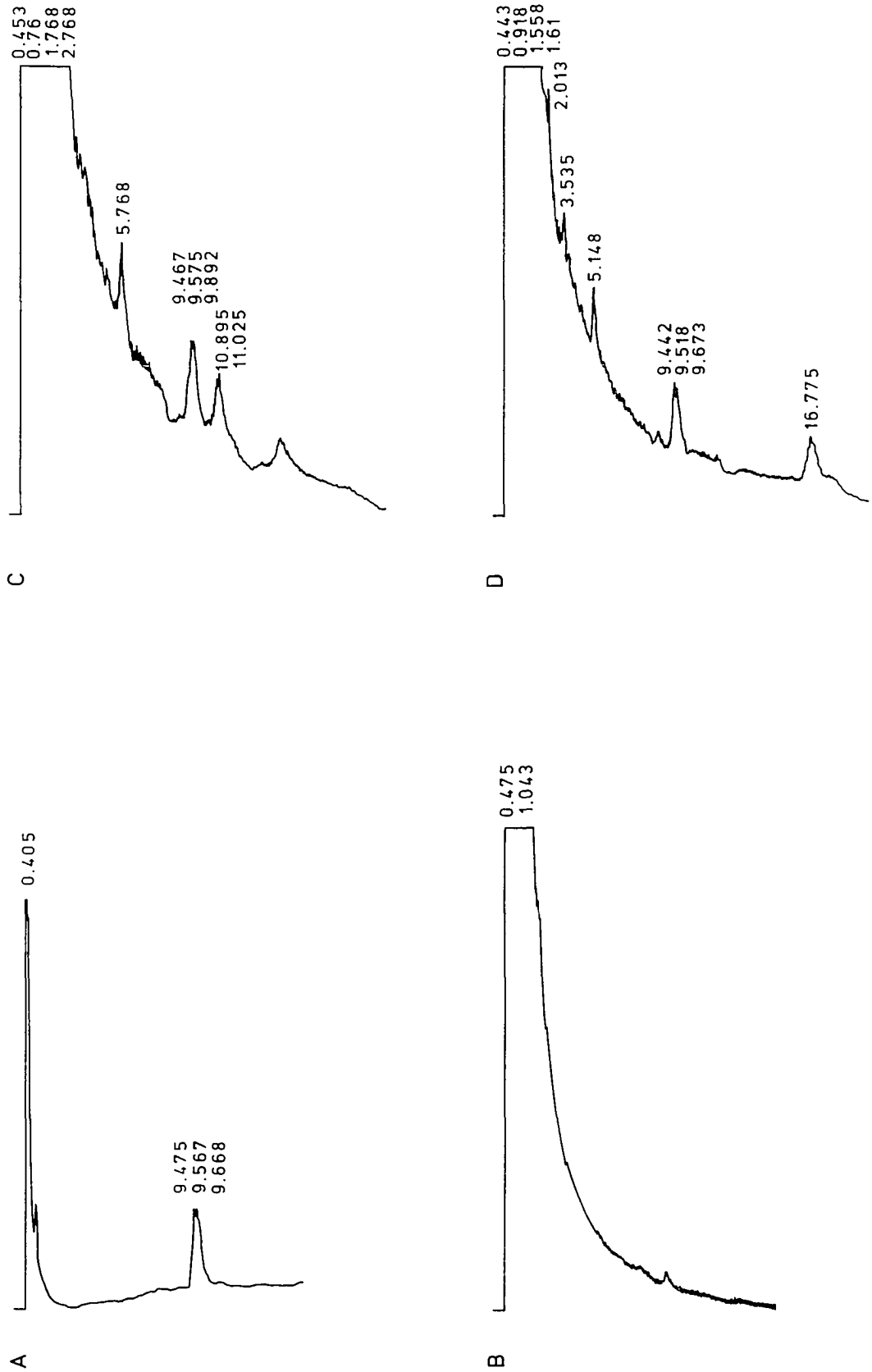
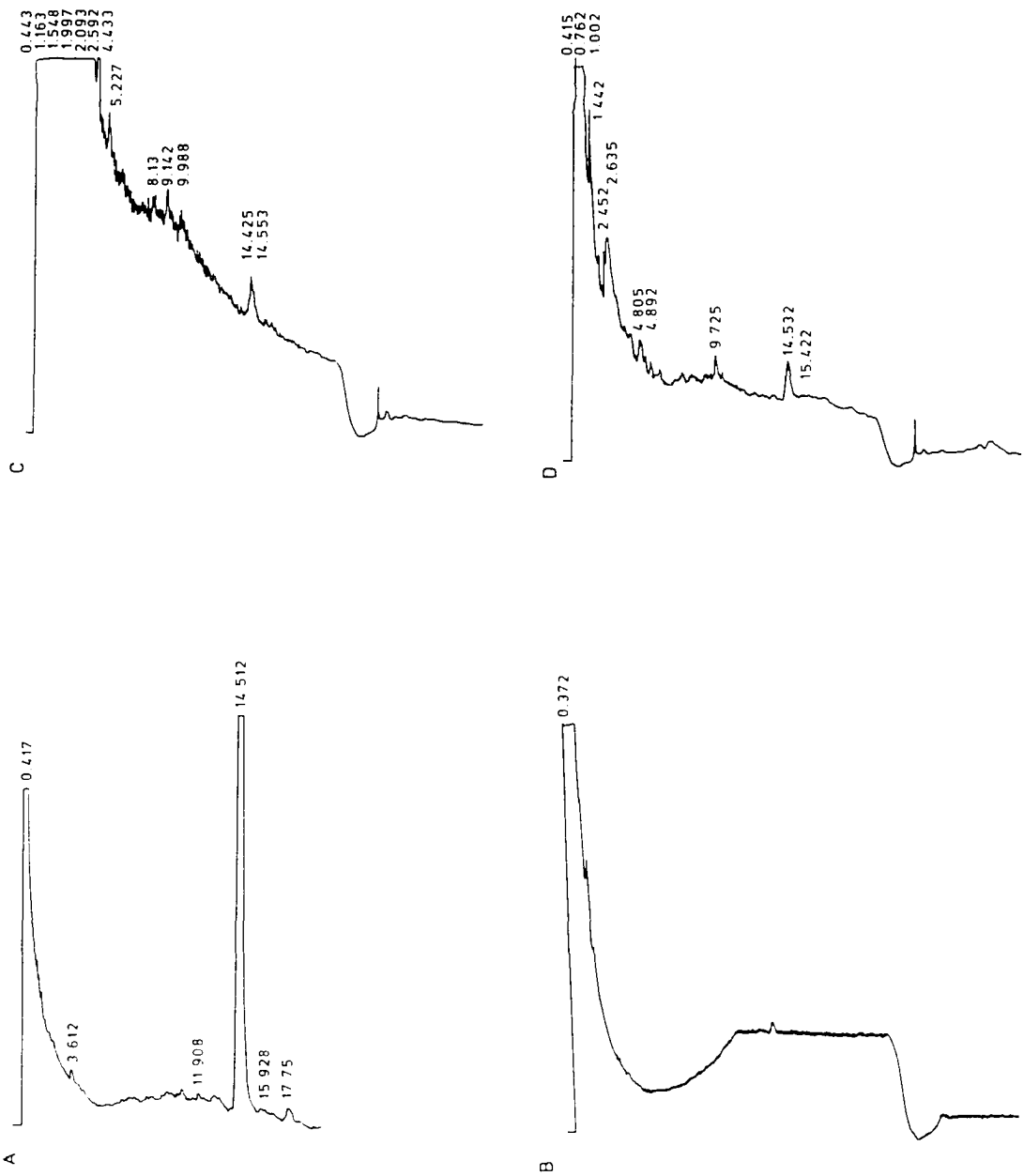


Figure 27. Gas chromatographic traces of ecdysteroids after TMSI derivatization. GC conditions were DB-1 capillary column (0.324 mm x 15 m), thermal gradient with the initial column temperature 250° C constant for 5 min, then raised 5°/min to a temperature of 275° C, constant for 10 min. Injector 285° C and detector 275° C. Carrier gas N_2 at 3-5 ml/min. ECD detector used. (A) authentic ecdysone, (B) blank control, (C) D. variabilis HL extract and (D) D. andersoni HL extract.



respectively. D. andersoni (Fig. 26D) ART extracts showed larger amounts of E than in D. variabilis (Fig. 26C), 0.62 and 0.48 ng/FE, respectively.

HL extracts were analyzed using a thermal gradient from 250° to 275° C in efforts to separate samples for better identification (Fig. 27). Ecdysone was found in the HL in higher amounts in D. andersoni (0.32 ng/ul) (Fig. 27D) than in D. variabilis (0.20 ng/ul) (Fig. 27C). No conclusive evidence of 20E was found in these extracts.

DISCUSSION

The presence of a genital sex pheromone in D. variabilis and D. andersoni has been well established (Sonenshin t al., 1982, 1985b; Allan et al., 1988). GSP facilitates species recognition, prevents interspecific matings, and increases the success of the mating process in these ticks.

The credibility of the "neutered" female bioassay is crucial to the credibility of this study and the assay of GSP activity. The assay is based on the assumption that if there is a pheromone present we should be able to remove the male response by destroying the pheromone source and then restore this response by replacing the pheromone. When a large number of bioassays were done there were a number of false positives that occurred. The neutering procedure was not 100% effective in removing the response, probably because a small fragment of the vestibular vagina remained. Our earlier studies showed that copulations would occur in approximately 25% of the methanol controls. Subsequently, the number of false positives was reduced to <5% by screening the females before bioassay and by using HPLC-grade methanol that had been glass distilled (2x). The neutering process also appears to decrease the feeding efficiency of females, resulting in somewhat smaller

females. The preliminary stages of the courtship behavior were affected, reducing the number of males that proceeded through ventral orientation and probing.

Male responses to ART extracts were restored in both species whereas responses to methanol controls remained near zero. Restoration of the mating response was achieved best at a narrow range of concentrations from 0.5 to 0.1 FE in D. variabilis and 1.0 to 0.1 FE in D. andersoni. These responses followed a dose response curve typical of responses to sex pheromones (Vinson et al., 1975; Hendry et al., 1973). The male responses in D. andersoni were much lower than in D. varaibilis. However, this was also true for the assays of normal (unneutered) females. We were unable to restore the responses with extracts of the ART made from unfed females in either species, showing that pheromone production is induced or increased by feeding. This is consistent with the biology of metastriate ticks in which mating occurs only after feeding. This study confirmed earlier findings that responses of males to neutered females can be restored by the addition of crude ART extracts from part-fed females. Clearly, materials in these extracts are excitatory and thus represent a genital sex pheromone.

If the ART extracts contain a pheromone we should be able to observe the responses of the receptors to the pheromone. The receptors on the cheliceral digits of males responded significantly to crude ART extracts as measured

by the electrophysiological techniques. Responses were obtained at a concentration of 1 FE/ul. Tests with lower concentrations led to ambiguous results. It is likely that the materials in the ART were not completely dissolved in the 0.1 M NaCl solution and thus the stimulus concentration was lower than anticipated. Results could possibly be improved by the use of an organic salt solution to reduce the background responses and the use of small percentages of ethanol to increase the solubility of the extracts. The use of small percentages of ethanol (20%) have been shown to improve the assay by dissolving a plug of material that blocks the pore of the sensilla (Hanson, personal communication). The analysis of electrophysiological data by the computer program developed by Hanson et al. (1986) is difficult because there are a large number of neurons per sensillum in these animals (Sonenshine et al., 1984; Sonenshine et al., 1986b; Waladde and Rice, 1977, 1982). Foelix (1985) has also noted a higher number of neurons per sensillum in arachnids than in comparable insect sensilla. According to Foelix (1985) the more primitive arachnids have higher numbers of neurons per sensillum. This computer program can only distinguish 3 or 4 types of spikes effectively. In many insects this is sufficient because they have few neurons per sensillum. The larger number of neurons in ticks makes the recordings more complicated. However, if the addition of a new compound in the stimulus results in an increased spike frequency, it is

generally assumed that new neurons are responding to the stimilus. Despite the complications posed by the presence of many neurons in the sensillum, my data clearly show that the male receptors do indeed perceive the crude ART extracts, further supporting the evidence that these extracts contain the GSP.

Previous studies showed that we could restore the responses with specific fractions of the ART extract separated by gel permeation (Sonenshine et al., 1985b). Separation was not complete and resulted in activity being spread across several fractions, but it was determined that the GSP consisted of at least two components. More recent studies showed that responses could be restored at least in part, by free fatty acids, specifically stearic and palmitic acids (Allan et al., 1988, in press). Ecdysteroids were also implicated as a component of the GSP (Sonenshine et al., 1985b). To determine the role of ecdysteroids in the GSP it became necessary to first establish their presence in the extracts made from the tissues of the pheromone source in amounts that could not be explained merely as a by-product of hemolymph contamination. The use of radioimmunoassay (RIA) for the determination of ecdysteroids has been established as an excellent method (Morgan & Poole, 1976; Hirn & Delaage, 1980). Presently, RIA is the most widely used assay for the determination of ecdysteroids (Morgan & Wilson, 1980). The sensitivity of the RIA probably exceeds any other known

method for the quantitative assay of natural ecdysteroids (Hirn & Delaage, 1980).

Although RIA is an excellent tool for recognition of ecdysteroids, there are certain disadvantages. RIA requires a long time for production of good antisera, large quantities of expensive ecdysteroids are needed to form the orginal hapten complex and tritiated ecdysone with a high specific activity is needed. In addition, the range of detection is limited and the time required for each analysis is long and tedious. Extraction of a crude extract is also necessary for the concentration of the biological sample for the isolation and determination of the ecdysteroids. Lipids, salts and other substances can interfere with the RIA. The centrifugation of the crude extract removes most lipids. The extract can be further purified by C-18 cartridges (Sep-pak) as described by Lafont et al. (1982).

Antibodies recognize E & 20E as well as closely related metabolites, but do not react with cholesterol and most other steroids. The use of polyclonal antibodies enables the detection of closely related ecdysteroids as a group, but in so doing foregoes the opportunity to recognize and measure individual ecdysteroids. Monoclonal antibodies are the method of choice for specific recognition, but this was not desirable in the current study because the identity of ecdysteroids, if any, found in the GSP was not known. However, polyclonal antibodies

in combination with chromatographic separation can be used to distinguish the specific ecdysteroids (Hirn & Delaage, 1980; Hoffmann & Hetru, 1983). The use of HPLC with a reversed phase column has become widely accepted as the chromatographic method of choice for ecdysteroid analysis. The HPLC has also eliminated many problems that are encountered in TLC and column chromatography (Koreeda & Teicher, 1977; Lafont et al., 1980). The HPLC gradient used here gave good separation of the free ecdysteroids. RIA conclusively demonstrated the presence of ecdysteroids in the VW, the ART and around the genital aperture, providing the male the opportunity to encounter these compounds when he probes the gonopore.

Ecdysteroids occurred in the ART at concentrations higher than what would be expected from hemolymph contamination of the excised tissues. Sonenshine et al. (1985a) showed muscle and other tissues that do not normally contain ecdysteroids gave RIA-positive reactions because hemolymph coagulated around the tissues when they were excised. The hemolymph cannot be removed completely even with repeated washings. The concentrations of ecdysteroids in the ART extracts were much higher than would be expected from just hemolymph contamination in both D. variabilis and D. andersoni. In addition, we were able to extract ecdysteriods by washings of the the vaginal lumen. These washings were not contaminated with hemolymph. Sonenshine et al. (1985a) also found that the amount of

ecdysteroids per mg of tissue in the ART was 4 times higher than per mg of tissue in the muscle.

Further evidence for the presence of ecdysteroids in the anterior reproductive tract of females was obtained by ^{3}H ecdysone injection of ticks. ^{3}H ecdysone was incorporated into the ART of both species, however much greater amounts (10x) were found in the ART of D. andersoni as free ecdysone. In contrast, D. variabilis had larger amounts in the hemolymph with the majority being converted into 20E. The ART of D. variabilis had more 20E then E. Large amounts of radioactivity were found in apolar fractions of both species suggesting that E and possibly 20E were conjugated for storage. The D. andersoni apolar fractions eluted later than the D. variabilis ones, suggesting different conjugates of the ecdysteroids. Other workers have also reported that E and 20E are converted into conjugated ecdysteroids. They reported that the ecdysteroids are conjugated with long chain fatty acids in ticks (Connat et al., 1986; Sonenshine et al., 1986a; Diehl et al., 1985; Wigglesworth et al., 1985) crustaceans, insects and other arthropods (Connat & Diehl, 1986). Conjugated ecdysteroids are separated as apolar metabolites by reversed phase HPLC and are thought to serve to inactivate the moulting hormones and also act as storage products for developing embryos (Wigglesworth et al., 1985; Connat et al., 1984; Crosby et al., 1986).

Tritiated cholesterol was also injected into females during this study to further establish the presence of ecdysteroids in the ART of these ticks. Since insects, ticks and most other arthropods are unable to synthesize the steroid ring structure, they are dependent on an exogenous supply of sterol compounds (e.g. cholesterol). Although the entire biosynthetic pathway of ecdysteroids has not been determined, injection of radiolabelled sterol precursors has been useful in monitoring the biosynthesis of ecdysteroids (Svoboda et al., 1975; Rees et al., 1980; Sonenshine et al., 1986a). Ecdysone was found in the ART extracts and 20E in the HL extracts. They were not detected until the extracts were subjected to esterase hydrolysis, suggesting they were synthesized from cholesterol and then conjugated. The conjugation process probably takes place in the intestinal cells and to a lesser extent in peripheral tissues and occurs very rapidly (Connat et al., 1986). Therefore, we can conclude that new ecdysteroids are being synthesized somewhere in the tick's body tissue and stored in these tissues.

Identification of ecdysteroids by gas chromatography provided additional confirmation of their occurrence in the ART. GC is also considered to be an effective method in the study of ecdysteroids (Koreeda & Teicher, 1977; Morgan & Poole, 1976; Bielby et al., 1980; Poole et al., 1975; Ikekawa et al., 1972; Bielby et al., 1986). The derivatization processes required to produce the TMSI

ethers necessary for ecdysteroid detection can result in variable results because of different reactivities to TMSI of the hydroxyl groups located on the molecule (Poole & Morgan, 1975). Reaction times must therefore be adjusted accordingly. This study showed that E occurred in the HL and ART extracts of both species. The amounts were slightly higher than determined by RIA, but were consistent in that larger amounts of E were found in D. andersoni than D. variabilis. Results with 20E were ambiguous in extract samples because even under rigorous derivatization it was not unquestionably detected in the extracts. Consequently the results confirmed the presence of E but no 20E in the ART and HL extracts. Since 20E is reported to be most aboundant in the HL, a finding confirmed in this study, the failure to detect 20E in HL as well as the ART suggests the GC procedures do not reflect the true status of 20E in these tissues. Nevertheless, these findings bring us once again to the conclusion that ecdysteroids are present.

Having established the presence of ecdysteroids in the vaginal lumen and the anterior reproductive tract of female D. variabilis and D. andersoni we may now address the question of its role in the GSP. To determine this role, if any, we must show that they can be detected by the receptors of males and elicit a behavioral response. The treatment of neutered females with 20E restored the male response to the same level as the crude ART extracts. Ecdysone treatments also partially restored male responses

but were not as effective and were required in higher concentrations than 20E. Moreover, tests of other ecdysteroids and sterols showed that 20E and E were the most effective in restoring the copulatory response. Electrophysiological data also showed that males detected and responded to ecdysteroids. D. variabilis responded almost equally well to 20E and E, whereas D. andersoni responded more strongly to 20E. The lower responses to E may be at least in part a result of its lower solubility in aqueous solutions. Male ticks showed strong response to high concentrations of ecdysteroids (2.0×10^{-2} M), however these responses need to be examined more carefully with improvements in techniques similar to those discussed above to determine whether a dose-response relationship exists with these materials. In summary, evidence from bioassays and electrophysiological assays showed that males of these two species can detect ecdysteroids and that these compounds can restore the male copulatory response as well as the crude extracts in these two species.

The amount of total ecdysteroids found in the ART extracts (RIA) was used to convert the FE of ART extract into ug of ecdysteroids. Ecdysteroid content was found to be 2 or 3 log concentrations lower than the authentic 20E concentration that gave us the strongest response. If we assume the concentrations determined by RIA are correct then the responses to ecdysteroids cannot explain the complete restoration of the copulatory response. This is

also true for the responses to fatty acids. The strongest responses to stearic acid and palmitic acid in D. variabilis were at a concentration of 1 ug. D. andersoni responded more strongly to stearic acid and this was also at a concentration of 1 ug (Allan et al., in press). However, the amounts of total free fatty acids in the vaginal tracts of D. variabilis and D. andersoni were reported to be much lower, 24.8 ng/female and 297.2 ng/female, respectively (Allan et al., 1988). These amounts of fatty acids would not bring about a complete restoration of the copulatory response. Therefore, it is likely that the copulatory response is controlled by a combination of fatty acids and ecdysteroids.

Although most pheromones are single compounds, blends of different compounds or combinations of chemical and physical stimuli are common and initiate different stages of behavioral sequences (Roelofs and Carde, 1977; Roelofs, 1979; Bradshaw et al., 1983) and provide species recognition (Carde et al., 1977; Pickett et al., 1980). Recognition of a conspecific mate occurs in similar ways in ticks. Several of these mechanisms are discussed in reviews by Sonenshine (1984, 1985, 1986). In some species aggregation/assembly pheromones provide a mechanism in which species specific clusters are formed before mating, thereby eliminating the need for species recognition in mate finding (Gladney & Dawkins, 1973; Gladney et al., 1974). However, Taylor et al., (1987) found that assembly

pheromones do not occur in D. variabilis and D. andersoni. Specificity can also be provided by differences in concentrations of the pheromone (Khalil et al., 1983) or to a different blend of 2 or more separate chemicals (Schoeni et al., 1984). Sometimes, physical and chemical criteria are combined to provide the necessary information for species recognition (Andrew and Bull, 1982). Allan et al., (in press) suggest that fatty acids provide species recognition in D. variabilis by differences in concentration of fatty acids. Ecdysteroid concentrations are higher in the ART of D. andersoni than D. variabilis. The relative proportions of E and 20E appear to be slightly different in the two species. When we examine the washings of the vaginal lumina we again see higher concentrations in D. andersoni but we also see differences in composition. D. andersoni contained a small amount of 20E whereas D. variabilis only had E. D. andersoni males similarly responded more strongly to 20E than to E in electrophysiological assays. Not only may ecdysteroid concentration contain species specific information for the males but also relative proportions of 20E and E may be used in determining the species specific response of these ticks. Additional bioassay studies to determine the importance of mixtures of ecdysteroids as well as fatty acids are needed to further elucidate their roles in species recognition in these two species. The HPLC separations of ^{3}H injected samples indicated that the

conjugated ecdysteroids in D. variabilis eluted earlier than those in D. andersoni. This is another possible explanation of species recognition that needs to be investigated. The presence of other compounds in the ART contributing components to the GSP can not be dismissed completely, either. Males also are known to salivate during mating. Therefore, it is also possible that the saliva contains esterases or other enzymes that break the conjugated ecdysteroids and increase the concentrations of fatty acids and/or ecdysteroids available to the male sensillae. In conclusion, we are not completely certain what the males use for species recognition, but fatty acids and ecdysteroids appear to have an extremely important role in this process.

We have shown that ecdysteroids are detected by sensilla of the male and elicit a copulatory response. We have also shown that ecdysteroids are present in the organs that produce the GSP. Additionally in the introduction we established the concept that ecdysteroids are important in controlling a large number of processes in arthropod development and physiology. They are not just moulting hormones as orginally proposed. We have also introduced the idea that they function as a pheromone in crustaceans. Mate finding behavior in crustaceans was first described by Louis Agassiz a century ago. This behavior has been studied in detail by Ryan (1966) and Snow & Neilsen (1966). In addition, Ryan (1966) was the first to provide evidence

that the behavior was released by a pheromone. The pheromone was identified as crustecdysone (20-hydroxyecdysone) by Kittredge et al. (1971). Kittredge & Takahashi (1972) hypothesize that the beginning of sex pheromone communication in the Arthropoda was based on the development of the structures to detect leakage of moulting hormone from females. The development of controlled release of the hormone and the subsequent behaviors elicited by the male then evolved. Allowing 20E to become a sex pheromone in these animals. These complex capabilities became fixed in the genome and were the genetic foundation for mutation and selection to lead to the diverse pheromone systems found in present arthropod communication.

Ticks evolved to become obligate parasites of Reptiles in the late Paleozoic or early Mesozoic era (Hoogstraal & Kim, 1985). As mammals and birds became the dominant vertebrates they replaced the reptiles as hosts. Adaptive radiation of ticks paralleled that of their hosts, but was much slower and more conservative in ticks. Therefore modern ticks are very similar to their ancestors, especially retaining relatively "sluggish physiological processes" (Hoogstraal & Kim, 1985). Although ticks are terrestrial organisms, because they have maintained many ancient processes it is likely that they have retained remanants of the communication system of their aquatic ancestors. Atema (1987) explains that the receptors of

both aquatic and terresterial arthropods are extremely similar. Therefore, the hypothesis that ecdysteroids function as a component of the GSP in these two species of hard ticks is consistent with the evolutionary history of these taxa and is supported by the results of this study.

LITERATURE CITED

Allan, S.A., Phillips, J.S., Taylor, D. and Sonenshine, D.E. (1988) Genital sex pheromones of ixodid ticks: Evidence for the role of fatty acids from the anterior reproductive tract of Dermacentor variabilis and Dermacentor andersoni. J. Insect Physiol. **34**, 315-323.

Allan, S.A., Phillips, J.S. and Sonenshine, D.E. (1989) Species recognition elicited by differences in composition of the genital sex pheromone in Dermacentor variabilis and Dermacentor andersoni (Acari: Ixodidae). J. Med. Entomol. (in press).

Andrews, R.H. and Bull, C.M. (1982) Mating behavior and reproductive isolation of three species of reptile ticks. Anim. Behav. **30**, 514-524.

Atema, J. (1987) Aquatic and terrestrial chemorecptor organs: Morphological and physiological designs for interfacing with chemical stimuli. In Comparative Physiology: Life in Water and on Land (Ed. by Dejours, P., Bolis, L., Taylor, C.R., and Weibel, E.R.) Fidia Research Series Vol. 9. pp. 303-316. Liviana Press, Padova.

Bielby, C.R., Gande, A.R., Morgan, E.D. and Wilson, I.D. (1980) Practical aspects of the preparation and chromatography of the trimethylsilyl ethers of ecdysteroids. J. Chromatog. **194**, 43-53.

Bielby, C.R., Morgan, E.D. and Wilson, I.D. (1986) Gas chromatography of ecdysteroids as their trimethylsilyl ethers. J. Chromatog. **351**, 57-64.

Binnington, K.C. and Obenchain F.D. (1982) Circulatory, nervous and neuroendocrine systems of ticks. In Physiology of Ticks (Ed. by Obenchain, F.D. and Galun, R.) pp. 351-399. Pergamon Press, New York.

Borst, D.W. and O'Connor, J.D. (1972) Arthropod molting hormone: Radioimmune assay. Science **178**, 418-419.

Borst, D.W. and O'Connor, J.D. (1974) Trace analysis of ecdysones by gas-liquid chromatography, radioimmunoassay and bioassay. Steroids **24**, 637-657.

Bradshaw, J.W.S., Baker, R. and Lisk, J.C. (1983) Separate orientation and releaser compounds in a sex pheromone. Nature (London) **304**, 265-267.

Bouvier, J., Diehl, P.A. and Morici, M. (1982) Ecdysone metabolism in the tick Ornithodoros moubata (Argasidae:Ixodoidea). Revue Suisse Zool. **89**:967-976.

Campbell, J.D. and Oliver, J.H. (1984) Membrane feeding and developmental effects of ingested β-ecdysone on Ornithodoros parkeri (Acari: Argasidae). In Acarology VI Vol. 1 (Ed. by Griffiths, D.A. and Bowman C.E.) pp. 393-399. Ellis Horwood Ltd, Chichester.

Cardé, R.T., Cardé, A.M., Hill, A.S. and Roelofs, W.C. (1977) Sex pheromone specificity as a reproductive isolating mechanism among the sibling species Archips argyrospilus and A. mortuanus and other sympatric tortricine moths (Lepidoptera:Tortricidae). J. Chem. Ecol. 3, 71-84.

Connat, J.-L., Diehl, P.A., Dumont, N., Carminati, S., and Thompson, M.J. (1983) Effects of exogenous ecdysteroids on the female tick Ornithodoros moubata: Induction of supermolting and influence on oogenesis. Z. ang. Ent. **96**, 520-530.

Connat, J.-L., Diehl, P.A. and Morici, M. (1984) Metabolism of ecdysteroids during the vitellogenesis of the tick Ornithodoros moubata (Exodoidea, Argasidae): Accumulation of apolar metabolites in the eggs. Gen. Comp. Endocrinol. **56**, 100-110.

Connat, J.-L., Diehl, P.A., Gfeller, H. and Morici, M. (1985) Ecdysteroids in females and eggs of the ixodid tick Amblyomma hebraeum. Int. J. Invert. Reprod. Develop. **8**, 103-116.

Connat, J.-L. and Diehl, P.A. (1986) Probable occurrence of ecdysteroid fatty acid esters in different classes of Arthropods. Insect Biochem. **16**, 91-97.

Connat, J.-L., Lafont, R. and Diehl, P.A. (1986) Metabolism of ^{3}H ecdysone by isolated tissues of the female ixodid tick Amblyomma hebraeum (Ixodoidea:Ixodidae). Mol. Cell. Endocrinol. **47**, 257-267.

Crosby, T., Evershed, R.P., Lewis, D., Wigglesworth, K.P. and Rees, H.H. (1986) Identification of ecdysone 22-long-chain fatty acyl esters in newly laid eggs of the cattle tick Boophilus microplus. Biochem. J. **240**, 131-138.

Dees, W.H., Sonenshine, D.E. and Breidling E. (1984a) Ecysteroids in the American dog tick, Demacentor variabilis (Acari:Ixodidae) during different periods of tick development. J. Med. Entomol. 21, 514-523.

Dees, W.H., Sonenshine, D.E. and Breidling E. (1984b) Ecdysteroids in Hyalomma dromedarii and Dermacentor variabilis and their effects on sex pheromone activity. In Acarology VI (Ed. by Griffiths, D.A. and Bowman, B.J.) pp. 405-413. Ellis Horwood Ltd., Chichester.

Delbecque, J.P., Diehl, P.A. and O'Connor, J.D. (1978) Presence of ecdysone and ecdysterone in the tick Amblyomma hebraeum Koch. Experientia 34, 1379-1381.

Diehl, P.A., Germond, J.E. and Morici, M. (1982) Correlations between ecdysteroid titers and integument structure in nymphs of the tick Amblyomma hebraeum Koch (Acarina:Ixodidae). Rev. Suisse Zool. 89, 859-868.

Diehl, P.A., Connat, J.-L., Girault, J.P. and Lafont, R. (1985) A new class of apolar ecdysteroid conjugates: esters of 20-hydroxy-ecdysone with long-chain fatty acids in ticks. Int. J. Invert. Reprod. Devel. 8, 1-13.

Diehl, P.A., Connat, J.-L. and Dotson, E. (1986). Chemistry, function and metabolism of tick ecdysteroids. In Morphology, Physiology, and Behavioral Biology of Ticks. (Ed. by Sauer, J.R. and Hair, J.A.) pp. 165-193. John Wiley & Sons, New York.

Downer, R.G.H. and Laufer, H. (1983) Endocrinology of Insects. Invert. Endocrinol. Vol. I. 707 pp. Alan R. Liss, Inc., New York.

Dumser, J.B. and Oliver, J.H. (1981) Kinetics of spermatogenesis, cell-cycle analysis, and testis development in nymphs of the tick Dermacentor variabilis. J. Insect Physiol. 37, 743-753.

Ellis, B.J. and Obenchain, F.E. (1984) In vivo and in vitro production of ecdysteroids by nymphal Amblyomma variegatum ticks. In Acarology VI Vol. 1 (Ed. by Griffiths, D.A. and Bowman, C.E.) pp. 400-404. Ellis Horwood Ltd, Chichester.

Foelix, R.F. (1985) Mechano- and chemoreceptive sensilla. In Neurobiology of Arachnids (Ed. by Barth, F.G.) pp. 118-137. Springer-Verlag, New York.

Frazier, J.L. and Hanson, F.E. (1986) Electrophysiological recording and analysis of insect chemosensory responses. In Insect-Plant Interactions (Ed. by Miller, J.R. and Miller, T.A) pp. 285-330. Springer-Verlag, New York.

Germond, J.E., Diehl, P.A. and Morici, M. (1982) Correlations between integument structures and ecdysteroid titers in fifth stage nymphs of the tick, Ornithodoros moubata (Murray, 1877; sensu Walton, 1962). Gen. Comp. Endocrinol. **46**, 255-266.

Gilbert, L.I., Bollenbacher, W.E. and Granger N. (1980) Insect endocrinology: Regulation of endocrine glands, hormone titer, and hormone metabolism. Ann. Rev. Physiol. **42**, 493-510.

Gladney, W.J. and Dawkins, C.C. (1973) Experimental interspecific mating of Amblyomma maculatum and A. americanum. Ann. Entomol. Soc. Amer. **66**, 1093-1097.

Gladney, W.J., Grabbe, R.R., Erst, S.E and Oehler, D.D. (1974) The gulf coast tick: evidence for a pheromone produced by males. J. Med. Entomol. **11**, 303-306.

Hamilton, J.G.C. and Sonenshine, D.E. (1988) Evidence for the occurence of a mounting sex pheromone on the body surface of female Dermacentor variabilis (Say) and Dermacentor andersoni Stiles (Acari:Ixodidae). J. Chem. Ecol. **14**, 401-410.

Hamilton, J.G.C. Sonenshine, D.E. and Lusby, W.R. (1989) Cholesteryl oleate: mounting sex pheromone of the hard tick Dermacentor variabilis (Ixodidae). **Science** (submitted).

Hanson, F.E., Kogge, S. and Clearley, C. (1986) Computer analysis of chemosensory signals. In Mechanisms in Insect Olfaction (Ed. by Payne, T.L., Birch, M. and Kennedy, C.) 364 pp. Clarendon Press, Oxford.

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

Hendry, L.B., Greeny, P.D. and Gill, R.J. (1973) Kairomone mediated host-finding behavior in the parasitic wasp, Orgilus lepidus. Entomol. Exp. Appl. **16**, 471-477.

Hirn, M. and Delaage, M.A. (1980) Radioimmunological approaches to the quantification of ecdysteroids. In Progress in Ecdysone Research (Ed. by Hoffmann, J.A.) pp. 69-82. Elsevier, Amsterdam.

Hodgson, D.A., Lettrin, J.Y. and Roeder, K.D. (1955) Physiology of a primary chemoreceptor unit. Science **122**, 417-418.

Hoffmann, J.A. (1980) Progress in Ecdysone Research. Devel. Endocrinol. Vol. 7, 495 pp. Elsevier/North Holland, Amsterdam.

Hoffmann, J.A. and Hetru, C. (1983) Ecdysone. In Endocrinology of Insects (Ed. by Downer, R.G.H. and Laufer, H.) pp. 65-88. Alan R. Liss, Inc., New York.

Hoffmann, J. and Porchet, M. (1984) Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones. 519 pp. Springer-Verlag, New York.

Hoogstraal, H. and Kim, K.C. (1985) Tick and mammal coevolution, with emphasis on Haemaphysalis. In Coevolution of Parasitic Arthropods and Mammals. pp. 505-568. John Wiley & Sons, New York.

Ikekawa, N., Hattori, F., Rubio-Lightbourn, J., Miyazaki, H., Ishibashi, M., and Mori, C. (1972) Gas Chromatographic separation of phytoecdysones. J. Chromatog. Science **10**, 233-242.

Kerkut, G.A. and Gilbert, L.I. (1985a) Comprehensive Insect Physiology, Biochemistry, and Pharmacology. Endocrinology I Vol. 7, 564 pp. Pergamon Press, Oxford.

Kerkut, G.A. and Gilbert, L.I. (1985b) Comprehensive Insect Physiology, Biochemistry, and Pharmacology. Endocrinology II Vol. 8, 595 pp. Pergamon Press, Oxford.

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

Khalil, G.M., Sonenshine, D.E., Sallam, O. and Homsher, P.J. (1983) Mating regulation and reproductive isolation in the camel ticks, Hyalomma dromedarii and Hyalomma anatolicum excavatum (Ixodoidea:Ixodidae). J. Med. Entomol. **20**, 136-145.

Khalil, G.M., Shaarawy, A.A.A., Sonenshine, D.E. and Gad, S.M. (1984) β-ecdysone effects on the camel tick Hyalomma dromedarii (Acari:Ixodidae). J. Med. Entomol. **21**, 188-193.

Kitaoka, S. (1972) Effect of ecdysone on ticks, especially on Ornithodoros moubata (Acarina-Argasidae). Proc. 14th Int. Congress of Entomology, Australia, p. 272.

Kittredge, J.S., Terry, M. and Takahashi, F.T. (1971) Sex pheromone activity of the molting hormone, crustecdysone, on male crabs (Pachygrapsus crassipes, Cancer antennarius and C. anthonyi). Fish. Bull., U.S. **69**, 337-343.

Kittredge, J.S. and Takahashi, F.T. (1972) The evolution of sex pheromone communication in Arthropods. J. Theor. Biol. 35, 467-471.

Koreeda, M. and Teicher, B.A. (1977) Chemical analysis of insect molting hormones. In Analytical Biochemistry of Insects (Ed. by Turner, R.B.) pp. 207-240. Elsevier Scientific Publishing Co., New York.

Lafont, R., Somme-Martin, G., Mauchamp, B., Maume, B.F. and Delbecque J.P. (1980) Analysis of ecdysteroids by high-performance liquid chromatography and coupled gas-liquid chromatography-mass spectrometry. In Progress in Ecdysone Research (Ed. by Hoffman, J. A.) Devel. Endocrinol. Vol. 7, pp. 45-68. Elsevier/North Holland, Amsterdam.

Lafont, R., Pennetier, J.-L., Andrianjafintrimo, M., Claret, J., Modde, J.-F. and Blais, C. (1982) Sample processing from high-performance liquid chromatography of ecdysteroids. J. Chromatog. **236**, 137-149.

Lafont, R. and Koolman, J. (1984) Edysone metabolism. In Biosynthesis, Metabolism and Mode of Action of Invertebrate Hormones. (Ed. by Hoffmann, J.A. and Porchet, M.) pp. 196-226. Springer-Verlag, Berlin.

Matthews, R.W., Matthews, J.R. (1978) Insect Behavior. 507 pp. John Wiley & Sons, New York.

Morgan, E.D. and Poole, C.F. (1976) The formation of trimethysilyl ethers of ecdysones. A reappraisal. J. Chromatog. **116**, 333-341.

Morgan, E.D. and Wilson, I.D. (1980) Progress in the analysis of ecdysteroids. In Progress in Ecdysone Research (Ed. by Hoffmann, J.A.) Devel. Endocrinol. Vol. 7, pp. 29-44. Elsevier/North Holland, Amsterdam.

Oliver, J.H., Jr. (1972) Cytogenetics of ticks (Acari: Ixodiodea). 6. Chromosomes of Dermacentor species in the United States. J. Med Entomol. 9, 177-182.

Oliver, J.H., Jr. (1974) Observations on hybridization of three species of North American Dermacentor ticks. J. Parasitol. **58**, 380-384.

Pickett, J.A., Williams, I.H., Smith, M.C. and Martin, A.P. (1980) Nasonov pheromone of the honeybee, Apis mellifera L. (Hymenoptera, Apidae). Part I Chemical characterization. J. Chem. Ecol. **6**, 1412-1414.

Poole, C.F. and Morgan, E.D. (1975) Structural requirements for the electron capturing properties of ecdysones. J. Chromatog. **115**, 587-590.

Poole, C.F., Morgan, E.D. and Bebbington, P.M. (1975) Analysis of ecdysones by gas chromatography using electron capture detection. J. Chromatog. **104**, 172-175.

Rees, H.H., Davies, T.G. Dinan, L.N., Lockley, W.J.S. and Goodwin, T.W. (1980). The biosynthesis of ecdysteroids. In Progress in Ecdysone Research (Ed. by Hoffmann, J.A.) Devel. Endocrinol. 7, 125-138. Elsevier/North Holland, Amsterdam.

Roelofs, W.L. (1979) Production and perception of Lepidopterous pheromone blends. In Chemical Ecology: Odour Communication in Animals. (Ed. by Ritter, F.J.) pp. 159-168. Elsevier/North Holland, Amsterdam.

Roelofs, W.L. and Carde, R.T. (1977) Responses of Lepidoptera to synthetic sex pheromone chemicals and their analogues. Ann. Rev. Entomol. **22**, 377-405.

Ryan, E.P. (1966) Pheromone: Evidence in a decapod crustacean. Science **151**, 340-341.

Schoeni, R., Hess, E., Blum, W., and Ramstein, K. (1984) The aggregation-attachment pheromone of the tropical bont tick Amblyomma variegatum Fabricius (Acari:Ixodidae). Isolation, identification and action of its active components. J. Insect Physiol. **30**, 613-618.

Snow, C.D. and Neilsen, J.R. (1966) Premating and mating behavior of the dungeness crab (Cancer magister Dana). J. Fish. Res. Bd. Can. **23**, 1319-1323.

Sokal, R.R. and Rohlf, F.J. (1981) Biometry: The Principles and Practices of Statistics in Biological Research. 2nd edn., 859 pp. Freeman, San Francisco.

Solomon, K.K., Mango, C. K. A. and Obenchain, F. D. (1982) Endocrine mechanisms in ticks. In Physiology of Ticks (Ed. by Obenchain, F. D. and Galun, R.) pp. 399-438. Pergamon Press, New York.

Sonenshine, D.E. (1984) Tick Pheromones. In Current Topics in Vector Research, Vol. 2 (Ed. by Harris, K.F.). pp. 225-263. Praeger Publishers, New York.

Sonenshine, D.E. (1985) Pheromones and other Semiochemicals of the Acari. Ann. Rev. Entomol. 30, 1-28.

Sonenshine, D.E. (1987) Neuroendocrine regulation of sex pheromone-mediated behavior in Ixodid ticks. In Pheromone Biochemistry (Ed. by Prestwich, G.D. and Bloomquist, G.J.) pp. 271-305. Academic Press, Inc., New York.

Sonenshine, D.E., Silverstein, R.M., Plummer, E.C., West, J.R. and McCullough, T. (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., Khalil, G.M., Homsher, P.J. and Mason, S.N. (1982) Dermacentor variabilis and Dermacentor andersoni: Genital sex pheromones. Exp. Parasitol. 54, 317-330.

Sonenshine, D.E., Homsher, P. J., Carson, K. A. and Wang, V. B. (1984) Evidence of the role of the cheliceral digits in the perception of genital sex pheromones during mating in the American dog tick, Dermacentor variabilis (Say) (Acari:Ixodidae). J. Med. Entomol. 21, 296-306.

Sonenshine, D.E., Homsher, P.J., Beveridge, M. and Dees, W.H. (1985a) Occurrence of ecdysteroids in specific body organs of the camel tick, Hyalomma Dromedarii and the american dog tick, Dermacentor variabilis (Acari:Ixodidae) with notes on their synthesis from cholesterol. J. Med. Entomol. 22, 303-311.

Sonenshine, D.E., Silverstein, R.M., Brossut, R., Davis, E.E., Taylor, D., Carson, K.A., Homsher, P.J. and Wang, V. B. (1985b) Genital sex pheromones of ixodid ticks: 1. Evidence of occurrence in anterior reproductive tract of American dog tick, Dermacentor variabilis (Say) (Acari:Ixodidae). J. Chem. Ecol. 11, 1669-1694.

Sonenshine, D.E., Boland, L.M., Beveridge, M. and Upchurch, B.T. (1986a) Metabolism of ecdysone and 20-hydroxyecdysone in the camel tick, Hyalomma dromedarii (Acari:Ixodidae). J. Med. Entomol.

Sonenshine, D.E., Taylor, D. and Carson, K.A. (1986b) Chemically mediated behavior in Acari: Adaptations for finding hosts and mates. J. Chem. Ecol. 12, 1091-1108.

Svoboda, J.A., Kaplanis, J.N., Robbins, W.E. and Thompson, M.J. (1975) Recent developments in insect steroid metabolism. Ann. Rev. Entomol. **20**, 205-220.

Taylor, D., Phillips, J.S., Allan, S.A. and Sonenshine, D.E. (1987) Absence of assembly pheromones in the hard ticks Dermacentor variabilis and Dermacentor andersoni (Acari:Ixodidae). J. Med. Entomol. **24**, 628-632.

Vinson, S.B., Jones, R.L., Sonnet, P.E., Bierl, B.A. and Beroza, M. (1975) Isolation, identification and synthesis of host-seeking stimulants for Cardiochiles nigriceps, a parasitoid of tobacco budworm. Entomol. Exp. Appl. **18**, 443-450.

Waladde, S.M. and Rice, M.J. (1977) The sensory nervous system of the adult cattle tick, Boophilus microplus (Canestrini). Ixodidae. Part III. Ultrastructure and electrophysiology of cheliceral receptors. J. Aust. Entomol. Soc. **116**, 441-453.

Waladde, S.M. and Rice, M.J. (1982) The sensory basis of tick feeding behavior. In Physiology of Ticks (Ed. by Obenchain, F.E. and Galun, R.) Vol. 1 pp. 71-118. Pergamon Press, Oxford.

Whitehead, D.L., Osir, E.W., Obenchain, F.D. and Thomas, L.S. (1986) Evidence for the presence of ecdysteroids and preliminary characterization of their carrier protein in the eggs of the brown ear tick Rhipicephalus appendiculatus (Neumann). Insect Biochem. **16**, 121-133.

Wigglesworth, K.P., Lewis, D. and Rees, H.H (1985) Ecdysteroid titre and metabolism to novel apolar derivatives in adult female Boophilus microplus (Ixodidae). Arch. Insect Biochem. Physiol. **2**, 39-54.

AUTOBIOGRAPHICAL STATEMENT

DeMar Taylor was born on October 19, 1956 in Salina, Utah. He received his A.S. in Biology from Snow College in 1978, his B.S. in Zoology from Brigham Young University in 1980 and earned his M.S. in Entomology from Brigham Young University in 1983. He has authored or co-authored the following articles:

Taylor, D., J.S. Phillips, S.A. Allan and D.E. Sonenshine. 1987. Absence of Assembly Pheromones in the hard ticks, Dermacentor variabilis (Say) and Dermacentor andersoni Stiles (Acari: Ixodidae). J. Med. Entomol. 24(6):628-632.

Allan, S.A., J.S. Phillips, D. Taylor, D.E. Sonenshine. 1988. Genital sex pheromones of Ixodid ticks: evidence for the role of fatty acids from the anterior reproductive tract in mating of Dermacentor variabilis and Dermacentor andersoni J. Insect Physiol. 34(4):315-323.

Carson, K.A., D.E. Sonenshine, L.M. Boland and D. Taylor. 1987. Localization of Acetylcholinesterase in the synganglion of the American dog tick, Dermacentor varibilis. Cell & Tissue Res. 249:615-623.

Sonenshine, D.E., D. Taylor and K.A. Carson. 1986. Chemically mediated behavior in Acari: Adaptations for finding hosts and mates. J. Chem. Ecol. 12:1091-1108.

Sonenshine, D.E., D. Taylor and G. Corrigan. 1985. Studies to evaluate the effectivenesss of sex pheromone impregnated formulations for control of populations of the American dog tick Dermacentor variabilis (Say) (Acari:Ixodidae). Exptl. & Appl. Acarology 1:23-34.

Sonenshine, D.E., R.M. Silverstein, R. Brossut, E.E. Davis, D. Taylor, K.A. Carson, P.J. Homsher, and V.B. Wang. 1985. The genital sex pheromones of Ixodid ticks; 1. Morphologic, experimental and chemical evidence of their occurrence in the anterior reproductive tract of the American dog tick, Dermacentor variabilis (Say). J. Chem. Ecol. 11:1669-1694.

Sonenshine, D.E., R.M. Silverstein, J.R. West, S. Bennett, and D. Taylor. 1985. Studies on the possible role of catecholamines in the regulation of sex pheromone activity in the American dog tick, Dermacentor variabilis (Say). J. Chem. Ecol. 11:363-382.

He held the following positions:

Research Assistant: Dr. Daniel E. Sonenshine, Old Dominion University, July 1983-Feb. 1989.

Range Science Technician. USDA Soil Conservation Service. June 1987-Aug. 1987.

Research Assistant: Dr. Armand T. Whitehead, Brigham Young University, Jan. 1983-May 1983.

Research Assistant: Dr. Gary M. Booth, Brigham Young University, Summer 1982.

Graduate Teaching Assistant: Department of Zoology, Brigham Young University.

He has membership in the following professional and honor societies:

Entomological Society of America
International Society of Chemical Ecology
American Association for Advancement of Science
International Chemoreception Workshop on Insects
Sigma XI (Tidewater Virginia Chapter)

He received the following awards:

Doctoral Fellowship, Old Dominion University, 4 yrs.
Japanese Language Study Fellowship, NSF, 1 yr.