CHARACTERIZATION OF ORAL SECRETION AND SALIVARY GLAND HOMOGENATE EXTRACTS OF *AMBLYOMMA AMERICANUM* (L.) (ACARINA: IXODIDAE)

By

ROBERT WELLS BARKER

Bachelor of Science
Northeastern State College
Tahlequah, Oklahoma
1967

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
DOCTOR OF PHILOSOPHY
May, 1973

CHARACTERIZATION OF ORAL SECRETION AND SALIVARY GLAND HOMOGENATE EXTRACTS OF *AMBLYOMMA AMERICANUM* (L.) (ACARINA: IXODIDAE)

Thesis Approved:

J. Alexander Hair
Thesis Adviser
George V. Odell
John R. Sauer
Joseph L. Buckner
B. N. Durham
Dean of the Graduate College

PREFACE

This study is concerned with the characterization of female lone star tick [Amblyomma americanum (L.)] oral secretions. The primary objectives were to develop an artificial method to stimulate and collect oral secretion from partial and engorged ticks. Several chromatography techniques were utilized to aid identification of proteins, amino acids, molecular weights, etc. Various other micro-analytical procedures were used in the biochemical analyses.

My most sincere appreciation is extended to my major adviser, Dr. Jakie Alexander Hair, for his financial support and guidance throughout this study and his assistance in the preparation of this manuscript.

I would also like to thank Doctors Ralph G. Buckner, George V. Odell and John R. Sauer for their advise and guidance on various phases of this study.

I am also indebted to Dr. Tak Chan, Mrs. Kathy Durham, Mr. Collis Geren, Miss Ann Haas, Mrs. Sue Ann Hudiburg and Dr. Richard Wright for their assistance in the laboratory.

I am especially grateful to my parents for their encouragement and support throughout this study and may all my success be theirs.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

According to Leclercq (1969), haematophagous (bloodsucking) insects and arachnids play an important part in human and veterinary medicine because they are capable of transmitting numerous pathological conditions to the host animals which they parasitize. Leclercq (1969) indicates that most haematophagous arthropods are Diptera, belonging to the class insecta. Among other haematophagous arthropods are ticks, mites, spiders, scorpions, and related forms, which belong to the class Arachnidia. James and Harwood (1969) indicate that "among the species of arachnids are some of the most important parasites and vectors of pathogens to man and beast ...", and they further cite the specific importance of ticks in this respect. With few exceptions, ticks are obligatory haematophagous ectoparasites, since blood is the principal food and is indispensible to their survival (Leclercq 1969).

Although their recorded antiquity dates to the time of Aristotle, in the fourth century B. C., both Ixodidae (hard-bodied) and Argasidae (soft-bodied) ticks have stimulated considerable scientific investigation in more recent times. The literature pertaining to ticks is voluminous and often published in obscure journals which make a review of the present knowledge difficult. However, Arthur (1962) and Balashov (1972) have good reviews on the present knowledge of many tick species. Although much has been elucidated about ticks, it is

evident from these reviews that many aspects of tick biology are far from complete.

This study involves one tick species (Amblyomma americanum) which belongs to the family Ixodidae. The most frequently used common names for this tick include: (1) the wood tick; (2) spot-back tick; and (3) the lone star tick. The yellowish white spot located on the posterior scutum occurs only in the females of this specie. This spot can aid identification of engorging females of this specie, since most engorged female tick species have a somewhat similar appearance to the novice.

Amblyomma americanum inhabits forest covered regions throughout the southeastern United States and feeds on a wide range of hosts (Bishopp and Trembley 1945). The number of suitable hosts in eastern Oklahoma and large acreages of suitable tick habitat are factors contributing to the severe tick problem in Oklahoma. In addition to causing annoyance and transmitting diseases to man, ticks also pose a problem to domestic and wildlife management programs in these regions. Bolte et al. (1970) reported on this tick's involvement in mortality of neo-natal white-tailed deer (Odocoileus virginianus) under field conditions, and Barker et al. (1973) conducted laboratory investigations to further clarify the role of A. americanum in mortality of young fawns.

Because of its potential role in disease transmission to man as well as domestic and wild animal populations, the bite of this tick should be considered significant. Several investigators have reported on the significance of ticks in the dissemination of disease and related pathological conditions in a wide variety of vertebrate hosts

(Arthur 1962, 1966; Riek 1957, 1959, 1962, 1964, and 1966; Ristic 1960, 1965, and 1966; Hoogstraal 1966, 1967; Balashov 1972).

The present study was designed to investigate the effect a particular stimulation method might have on the composition of oral secretion produced by engorged or semi-engorged ticks, to characterize female A. americanum oral secretion at different engorgement stages, and identify some chemical components which may have pathological significance.

CHAPTER II

LITERATURE REVIEW

The mechanisms of pathogen transmission from ixodid ticks to their hosts are varied and can be accomplished by: (1) defecation, (2) ingesting the infected tick, (3) crushing the infected tick on an open wound or (4) by the transfer of gland secretions, principally the salivary and coxal glands (James and Harwood 1969). Purnell and Joyner (1968), Levine (1967) and Reichenow (1940) reported on salivary gland involvement with the transmission of the East Coast Fever organism (Theileria parva) by Rhipicephalus appendiculatus. Salivary glands of one argasid tick, Ornithodoros moubata, have also been incriminated by Burgdofer (1951) as a temporary reservoir for the spirochete which causes relapsing fever.

In addition to salivary gland associated pathogen transmission, some investigators (i.e., Nuttall and Strickland 1908 and Tatchell 1968) have reported anticoagulant and hemolytic activity in tick saliva. However, anticoagulants have not been unequivocally demonstrated in all ixodid ticks (Roshdy 1972) and haemolysins are now believed to be derived principally from the gut epithelial cells of ticks (Hughes 1954). Since these early investigations, many competent researchers have continued to reveal interesting aspects of salivary gland structure, function, and role in disease transmission. Tatchell (1967) recently reported on the vital role of certain salivary gland alveoli

in eliminating excess water as a means of concentrating the blood meal and maintaining an electrolyte and osmotic balance in the cattle tick Boophilus microplus. The presence of cytolysins in tick secretions have been reported by Cowdry and Danks (1933) and slow acting hemolysins in tick saliva were reported by Pavlovsky and Chodukin (1929).

Another important pathological condition associated with tick parasitism is tick paralysis. Reported cases of this still mysterious disease appeared in the early part of this century. More recent reports (Oxer 1948, Gregson 1957, 1958, 1966, Murnaghan 1958, 1960a, b, Moorhouse 1966, and Cherington and Snyder 1968) related to tick paralysis suggest that some orally secreted component (toxin) may be involved with the onset of this ascending flaccid paralysis.

Other techniques have been utilized to determine the nature of salivary secretions in arhtropods. Microscopic examination of histological sections of tick salivary gland alveolar cells stained with specific biological stains have revealed the classes of biological materials which are present (Fairbairn and Williamson 1956, Roshdy 1972, and Kirkland 1971). A review of the early histological works on tick salivary glands is presented by Till (1959, 1961), and Balashov (1972).

CHAPTER III

EXPERIMENTAL PROCEDURE

Ticks used in this study were reared at the Oklahoma State University Medical Entomology Laboratory. Domestic rabbits served as hosts for immature tick stages and adult ticks were fed on stanchioned Shetland ponies. Female ticks that were chosen for experimental tests were less than 3 weeks of age and had been held at ca 85% relative humidity at 25^{o}C with a photoperiod of 12 hrs. daylight and 12 hrs. darkness.

Host Inoculation

Ponies were infested with equal numbers of male and female A. americanum and ticks were allowed to engorge. Foam rubber capsules, 5 inch sq. and 1 inch deep were glued to the dorsal midline of the thoracic and lumbar regions of the ponies using formica contact cement. To facilitate tick movement inside the capsule a portion of the capsule's center area was cut away with scissors. Ticks were introduced into the capsule through a small slit in the foam rubber and after the ticks began engorging a larger slit was made in the capsule to make periodic removal of ticks easier.

Oral Secretion Collection

Three methods of artificial stimulation of ticks were compared in

an effort to determine the most suitable method for oral secretion collection. Pilocarpine nitrate was prepared for injection at a concentration of 10% in a 6% solution of sodium chloride (Purnell et al. 1969). Before injection, a 100 µl syringe with a 27-gauge needle was mounted in a micro-applicator and prechecked for constant volume delivery rate. Five µl of the pilocarpine solution per tick was found to be the minimum amount that would elicit adequate secretion in A. americanum and this volume was used for anal injections throughout.

For infra-red heat stimulation a 250-watt Sylvania heat lamp was positioned at least 45cm above ticks confined to tape. One or 2 min. of exposure to infra-red heat of 35-40°C was usually required before the ticks would begin secreting. Care was taken to expose the ticks for the minimum length of time that would insure secretion. To prevent evaporation of the secretion following stimulation and collection, capillary tubes were manipulated by hand near the mouthparts only long enough to collect the secretions and were then stored at -19.0°C in closed petri dishes until analysis.

An audio generator model IG-72 (The Heath Co., Benton Harbor, Mich.) served as a power source for stimulating ticks by electrical shock. A frequency of 100 cycles at 8-10 volts proved the most suitable and was used throughout. Female ticks confined to tape were stimulated individually by inserting two 20-gauge wire electrodes through the cuticle into the hemolymph - one on each side of the tick just posterior to the hind leg.

Salivary Gland Homogenates

With the aid of a dissection microscope, salivary glands were

removed from 25 unfed female ticks while they were immersed in cold Ringer's solution. They were then homogenized in 0.25 ml of cold Ringer's solution using a 6 ml ground glass tissue homogenizer. The homogenate was centrifuged at 6000 rpm for 15 min. and the supernatant was drawn into microcapillary tubes and the ends sealed with plasticine for short termed refrigerated storage at -19°C.

Electrophoresis

Proteins from salivary gland homogenates and oral secretions were separated according to Ornstein (1964) in 7% polyacrylamide gels (pH 8.9) as described in the Canalco Disc Gel Electrophoretic Instruction manual. Tube dimensions of 2.8 mm i.d. x 10 cm in length was the only technique modification. Stacking gels measured 1.5 cm, while separating gels were 6.5 cm in length. All electrophoresis reagents were purchased from Canalco, Rockville, Maryland. Samples, buffered at pH 8.3, contained 10% sucrose and tracking dye. Gels were run at room temperature at 1 mA/tube until the tracking dye was in the stacking gel as a thin band and 2 mA/tube until the tracking dye was 1-3 cm from the bottom of the gel. Gels were stained with 1.0% Coomassie blue (Charambach et al. 1967) for approximately 1.5 hr and destained by diffusion in 7% acetic acid. Stained gels were photographed for future reference with a Canalco Phoreto-phot photographic unit which incorporates a Polaroid-Land automatic camera. A Beckman DU spectrophotometer with a Gilford Model 2410 gel scanning attachment was used to scan stained gels at 550 nm and a slit width of 0.2 mm.

Electrolyte Analysis

The oral secretion collected from ticks that had fed 10 days or longer was subjected to electrolyte analysis within 24 hrs. following stimulation. The concentrations of sodium, potassium, and chloride were determined to the nearest mM/L. A Beckman 440 atomic absorption spectrophotometer was used for assays on the two former ions and a Fiske/Marius micro-chlor-o-counter was used for chloride assays.

Amino Acid Analysis

Analysis of free non-protein amino acids and other amino compounds of salivary gland homogenates (4 replicates) and oral secretions (6 replicates) were separated in the Beckman Model 120C by the Automatic Amino Acid Analyzer procedure of Moore & Stein (1963). Prior to analysis, proteins were precipitated from the sample by the addition of 2-3 ml of 95% ethanol. The sample was centrifuged for 5-10 min. and the ethanol supernatant was separated from the amino acids with a rotary evaporator. Samples were held at -37^{o}C until analysis.

Protein Concentration

Protein concentration of the salivary gland homogenate was assayed according to the method of Lowry et al. (1951) using bovine serum albumin as a standard. Maximum absorbancy (λ max) and the A_{max} ratio of the protein content was determined with a Beckman DB recording spectrophotometer from 260 to 280 nm.

Biological Activity

Sheep erythrocytes (RBC) were suspended in Alsever's solution, washed 3 times in neutral NaCl and a 2.5% suspension (2.5 ml of packed RBC's in 100 ml saline) was made. The hemolytic activity of soluble protein extracts from homogenized salivary glands was determined by modifying the technique of Higginbotham and Karnella (1970) and mixing 200 $\mu\ell$ of the extract with diluted (1/100) saline washed rabbit RBC's. The same procedure was followed to measure the hemolytic activity of oral secretion. The control consisted of saline suspended RBC's only. At the end of 30 min. both tubes were centrifuged at ca 2000 rpm for 5 min. The degree of hemolysis in the tubes was determined by reading the optical density of each supernatant at 540 nm.

Osmolarity Determinations

Osmolarity determinations of tick oral secretions and hemolymph were made with a Clifton Technical Physics Nanoliter Osmometer accurate to the nearest $\pm$ 0.001^{o}C (Frick and Sauer 1973). Less than 0.1 $\mu\ell$ of sample was required from an individual tick for each assay.

Enzyme Assay

Hyaluronidase was the only enzyme assayed for in this study. The presence of hyaluronidase in tick oral secretions and salivary gland homogenates was determined by measuring the ability of hyaluronic acid to form turbidity with an acid albumin solution (Tolksdorf et al. 1949; Kass and Seastone 1944). The turbidity of the hyaluronic acid and an acid albumin complex is used to determine hyaluronic acid concentration

which can be related to enzyme activity. In this assay one turbidity reducing unit decreases the turbidity producing capacity of 0.2 mg of hyaluronic acid to that of 0.1 mg of hyaluronic acid in 30 min. at 37°C.

Bovine testicular hyaluronidase (HSEP grade, Worthington) was used as a reference enzyme. All assays were conducted at room temperature. The assay was conducted as follows: Infra-red heat collected oral secretion (0.5 ml) from engorging ticks and salivary gland homogenates (0.5 ml) from unfed female ticks were added to 0.5 ml of hyaluronic acid (Worthington) in a concentration of 0.4 mg/ml in a 0.1 M monosodium phosphate in 0.15 M sodium chloride buffer, pH 5.3. After 10 min. at 37°C, 9 ml of acid albumin [2.5 mg fraction V albumin powder (Sigma)/L., 0.5 M sodium acetate buffer, pH 4.2] was added to each sample and read 10 min. later at 540 nm. Absorbancy of unknowns was compared to a blank (1 ml of buffer and 9 ml of acid albumin) and plotted against previously determined enzyme standards (bovine testes hyaluronidase, HSEP-Worthington, 3000 U.S.P. units/mg, 0.01-0.04 mg/ml in buffer) as mg hyaluronic acid versus absorbancy.

Molecular Weight Determination

Several attempts were made to determine the molecular weights of oral secretion and salivary gland homogenate proteins. Three different techniques were employed and included: (1) gel filtration, (2) thin-layer gel filtration (TLG), and (3) SDS-polyacrylamide electrophoresis.

Determination of molecular weights of proteins by gel filtration described by Andrews (1964) was attempted using Bio-Gel^{R} (Bio Rad Laboratories, Richmond, Calif.), a porous polyacrylamide gel instead of

Sephadex. Three different pore sizes were used and included: P-30, P-60, and P-100. Gels were prepared for use according to the manufacturers' recommendations and packed in each of 3 silanized glass columns with an internal diameter of 1.5 cm and 48 cm in length. The column was then thoroughly flushed with 0.02 M NH_4HCO_3 buffer (pH 7.8) before the following standards were eluted through the columns: (1) 3 mg Ovalbumin: (2) 3 mg Cytochrome C; (3) 1 mg Chymotrysinogen A; and (4) ½ mg Blue Dextran.

One ml fractions were collected per tube using a Buchler Fractomat Automatic Fraction Collector^R which was housed in a pH Unitherm^R refrigeration unit and held at 38^{o}C. Absorbancy readings were made with a Beckman DB recording U-V spectrophotometer at 280 nm.

Thin-layer gel filtration (Radola 1968) was also used to determine protein molecular weight. Sephadex G-25 and Bio-Gel P-60 were used for separation of samples. After proper hydration of the gels, plates were spread and placed in a TLG tank and buffer-gel bridges were connected. Standards were the same as in column chromatography, excluding Blue Dextran, and all separations were conducted at room temperature. The volume of oral secretion and homogenate protein materials used ranged from 20 to 200 µℓ. Coomassie blue dye was used to locate proteins following separation and destaining was done in 7% acetic acid.

The final method used for molecular weight determination was reported by Shapiro et al. (1967). Prior treatment of electrophoretic separated and lyophilized oral secretion and homogenate proteins with sodium dodecyl sulfate (SDS) and 2-mercaptoethanol, followed by deoxygenation with nitrogen gas, allows proteins to be separated on the basis of molecular weights since charge differences are reduced

by SDS treatment. Proteins were electrophoresed for 3½ hrs. then stained with Coomassie blue and molecular weights were determined by relative mobility comparisons with standard proteins.

pH Determination

The pH of oral secretions and salivary gland homogenate extracts was determined by applying microliter (μℓ) quantities to strips of pH Hydrion paper (range 6-8) (Micro-essential Laboratories, Inc., Brooklyn, N. Y.).

CHAPTER IV

RESULTS

Stimulation Techniques

In preliminary studies, adults were removed from their host daily and subjected to the 3 stimulation techniques mentioned previously. Because difficulties were encountered in obtaining adequate volumes of secretion from ticks for electrolyte analysis during the early stages of feeding (days 1, 2), this phase of the research was terminated. Particulate contamination and discoloration of secretions were common from ticks weighing less than 50 mg; therefore, secretions from ticks weighing 50 mg or more were used.

Higher average percentages of secreting ticks were observed after ticks were stimulated by electrical shock and infra-red heat than after pilocarpine injection (Table I). The volume of secretion collected was dependent on the time that elapsed between removal of ticks from the host and stimulation. Failure to stimulate within the first 12 hr after removal from host animals usually resulted in very low volumes of secretion being produced.

Quantity of Oral Secretion

The quantities of oral fluid obtained using the 3 methods of stimulation are shown in Table I. It was not possible to obtain oral secretions from all ticks stimulated by pilocarpine injection and the

TABLE I

ORAL SECRETION OBTAINED BY STIMULATING A. AMERICANUM FEMALES WITH 3 TECHNIQUES

Method of Stimulation	Time on Hosts (Days)	Tick Weights (MG)	No. of Ticks Stimulated	% Secreting	Average Quantity of Secretion/Tick µl
Pilocarpine	3-8	20-40	10	50	2.3
"	7-9	40-112	19	42	2.6
"	8-10	45-185	5	60	5.0
"	8-10	40-190	30	43	1.6
"	9-11	115-540	15	66	4.0
"	10-11	200-645	24	86	9.0
Infra-red heat	7-9	175-820	13	100	2.7
"	11-13	190-890	34	99	10.0
"	11-13	210-900	8	100	15.0
Electrical shock	5-9	30-425	27	85	2.0
"	10-11	500-575	40	85	3.5

greatest success was obtained from ticks that were in final stages of engorgement and had weights between 200-645 mg. The highest volumes (avg. = 9.0 µℓ) were collected from ticks that had been on the host for at least 10-11 days.

Almost all ticks stimulated by infra-red heat produced some oral secretion. The volume secreted was also generally higher, with an average of 15.0 µℓ being produced from ticks that had been on the host 11-13 days and that had engorgement weights ranging from 210-900 mg.

The lowest volumes of secretion were obtained from ticks stimulated by electrical shock. The percentage of ticks producing secretion after this treatment was higher than that after pilocarpine injection and lower than that following infra-red heat stimulation.

Protein Electrophoresis

When disc polyacrylamide gel electrophoresis of oral secretion proteins from female A. americanum of 60, 135, 370, 550, and 700 mg engorgement weight were compared (Figure 1), there was an apparent reduction in the number and staining intensity of proteins from engorging ticks of 370 and 550 mg of weight. This may suggest reduced secretion of intracellular synthesized salivary gland components when A. americanum reach an engorgement weight of 350 to 450 mg of weight. This may further indicate that tick secretions are composed primarily of excess fluids resulting from hemoconcentration of the ingested blood meal at greater engorgement weights. Although protein bands were intensely stained as shown in the first 3 gels, this should not be interpreted as a quantitative measurement of proteins, since the

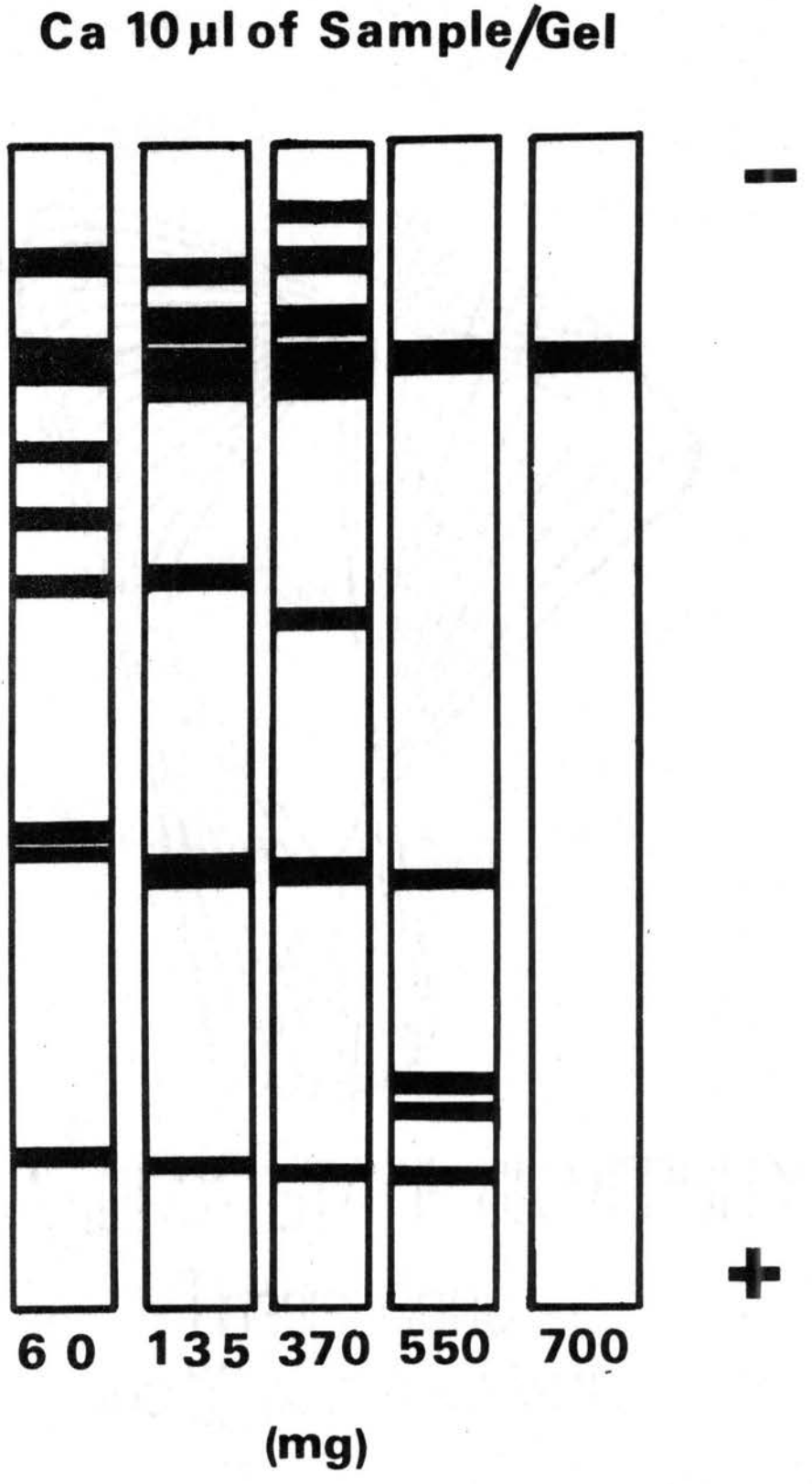

Figure 1. Protein Components in Oral Secretion from Ticks of Different Weights

ability of proteins to combine with dyes varies. It is apparent from Figure 1, that the number of oral secretion protein bands were somewhat different with respect to the engorged state of female ticks studied. However, most of the slower migrating proteins were present at each engorgement stage, whereas, more variation was observed in the faster migrating proteins of low molecular weight. When proteins were electrophoresed in gels without ammonium persulfate (Figure 2) the slower migrating basic proteins were evident in both samples, but oral secretion exhibited only one acidic protein. In Figure 2 it should be noted that oral secretion was collected from female ticks weighing ca 500 mg and these results would compare only to gel number 4 of Figure 1.

The stained gels of both electrophoretic methods (with and without ammonium persulfate) were scanned at 550 nm at 1 cm/min. At this low speed a better separation of proteins could be achieved. Figures 3-7 show the protein peaks present in oral secretion of ticks at the previously mentioned engorgement weights (Figure 1). Due to the possible effect of ammonium persulfate, caution should be taken in the interpretation of these results.

Figures 8 and 9 are gel scans of the proteins separated in Figure 2. Soluble proteins obtained from homogenized salivary glands of unfed ticks exhibited at least 10 different protein peaks (Figure 8), as compared to at least 5 peaks (Figure 9) in oral secretion from ticks weighing ca 500 mg. At the pH used to separate these proteins, the soluble proteins from salivary gland homogenates exhibited 5 basic and 1 acidic proteins of oral secretion (Figure 9).

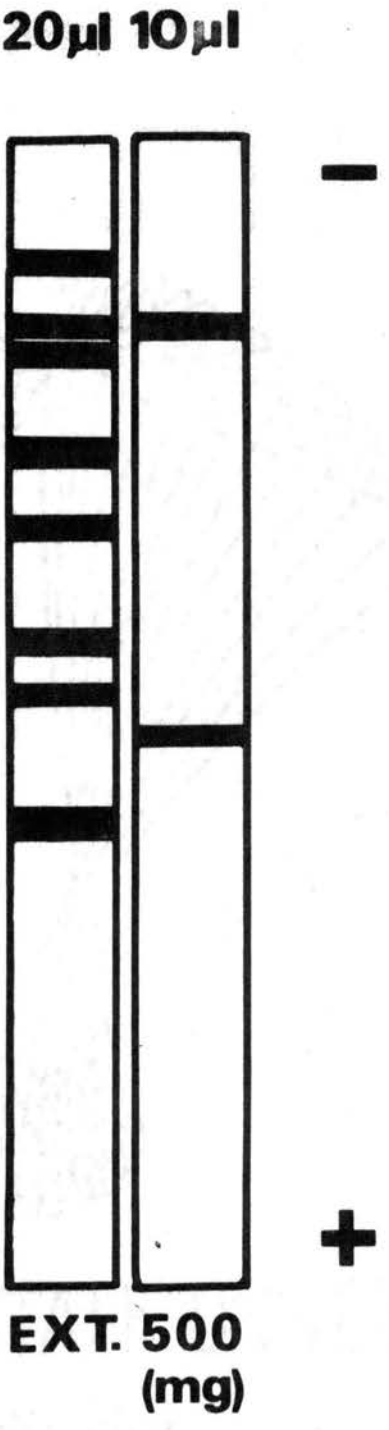

Figure 2. Protein Components in Tick Salivary Gland Homogenate Extract and Oral Secretion

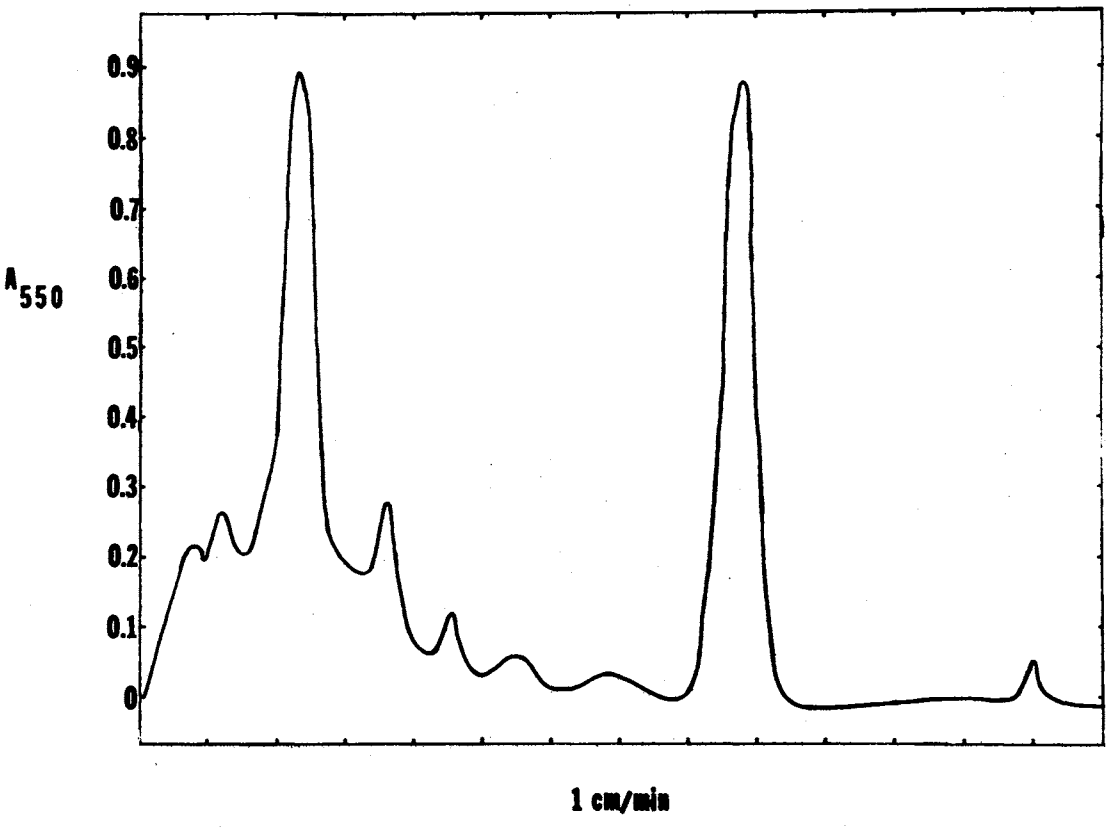

Figure 3. Oral Secretion Proteins from Female A. americanum (ca 60 mg) in Stained Gel Containing Ammonium Persulfate. Scanned at 550 nm and a slit width of 0.2 mm, in a Gilford Linear Transport Model 2410

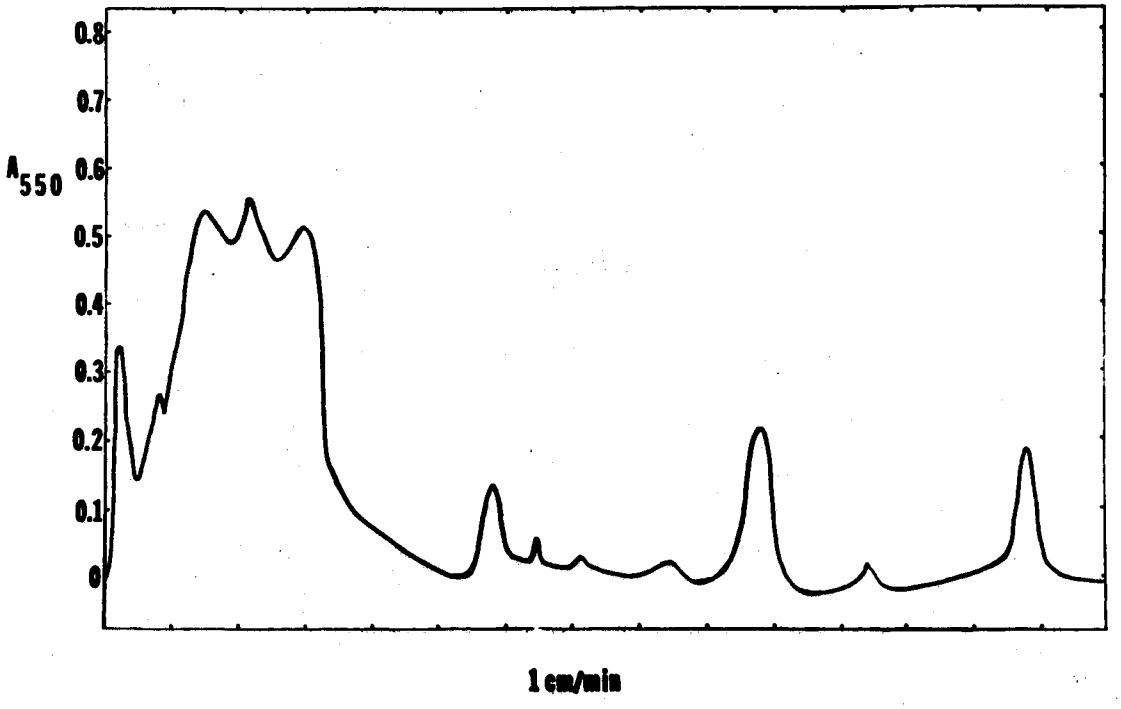

Figure 4. Oral Secretion Proteins from Female A. americanum (ca 135 mg) in Stained Gel Containing Ammonium Persulfate. Scanned at 550 nm and a slit width of 0.2 mm, in a Gilford Linear Transport Model 2410

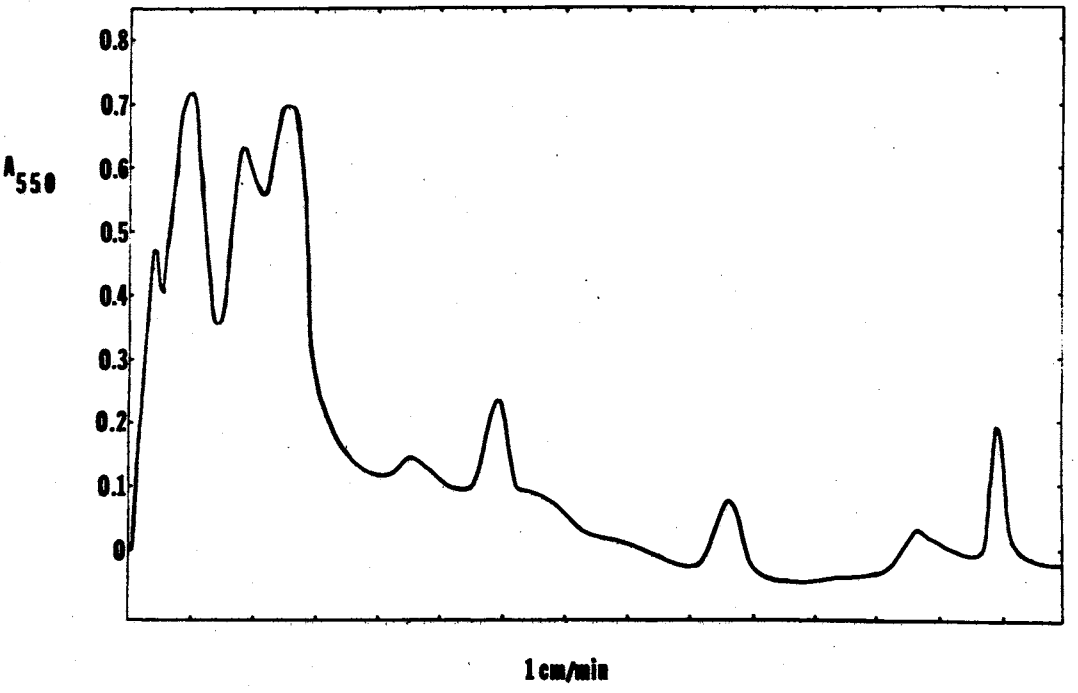

Figure 5. Oral Secretion Proteins from Female A. americanum (ca 370 mg) in Stained Gel Containing Ammonium Persulfate. Scanned at 550 nm and a slit width of 0.2 mm, in a Gilford Linear Transport Model 2410

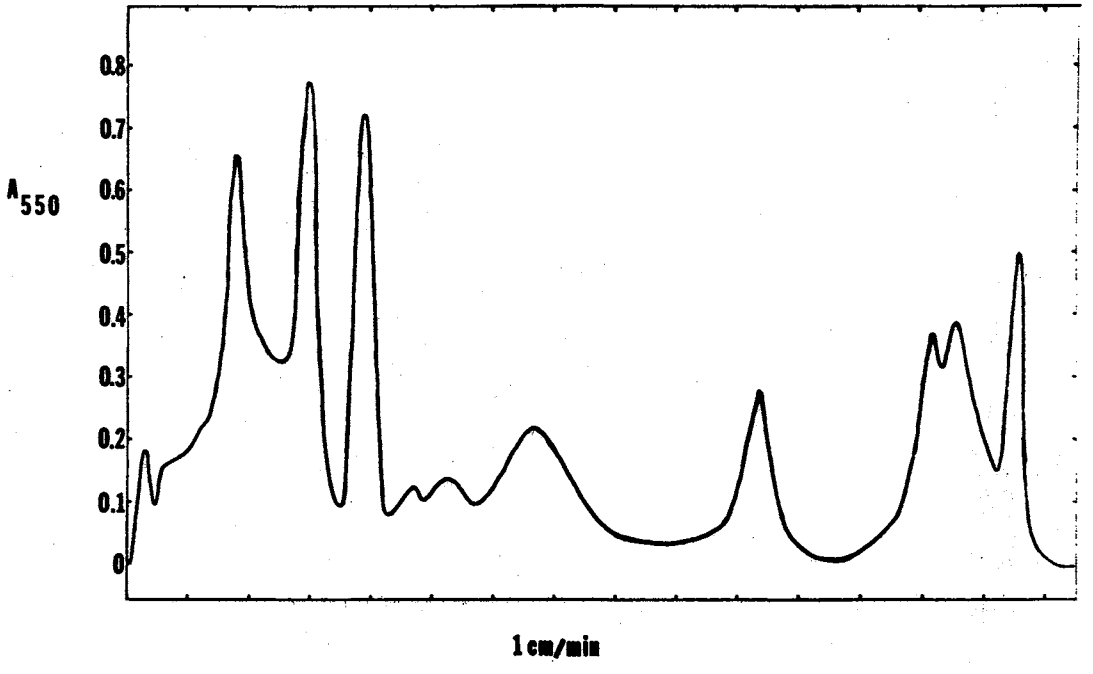

Figure 6. Oral Secretion Proteins from Female A. americanum (ca 550 mg) in Stained Gel Containing Ammonium Persulfate. Scanned at 550 nm and a slit width of 0.2 mm, in a Gilford Linear Transport Model 2410

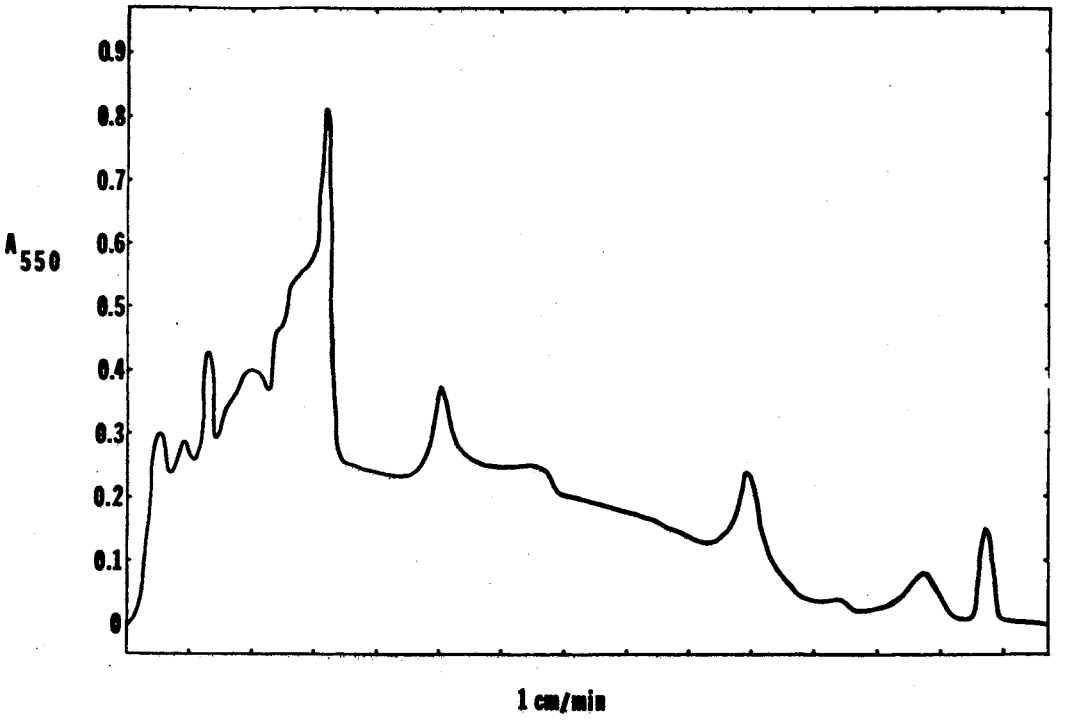

Figure 7. Oral Secretion Proteins from Female A. americanum (ca 700 mg) in Stained Gel Containing Ammonium Persulfate. Scanned at 550 nm and a slit width of 0.2 mm, in a Gilford Linear Transport Model 2410

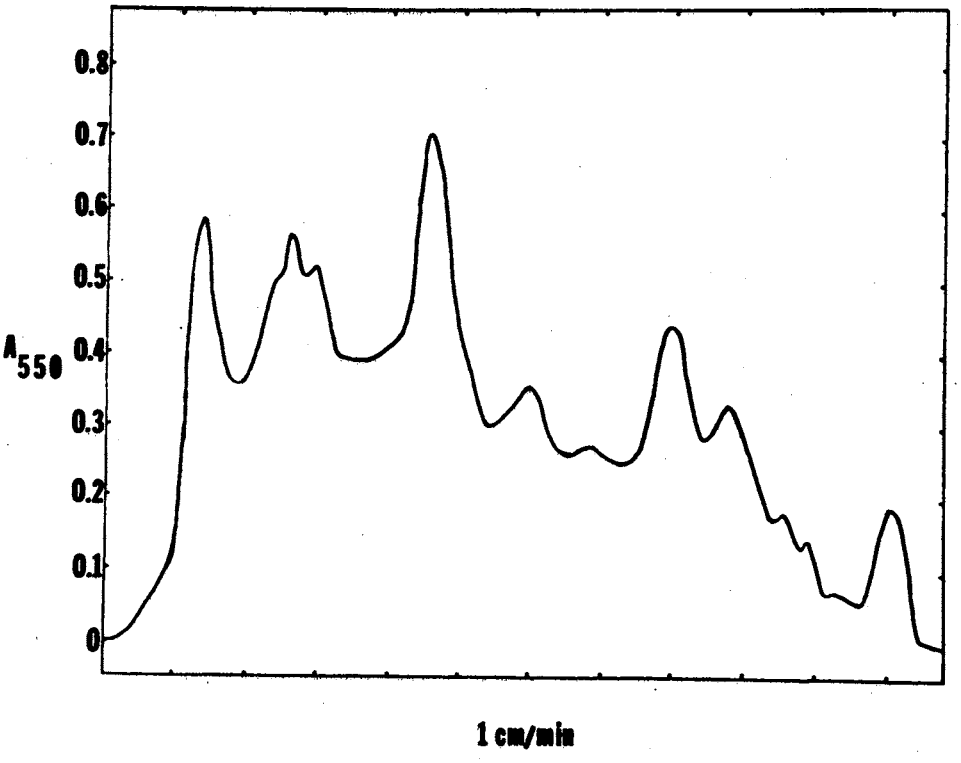

Figure 8. Homogenized Protein Extract from Unfed Female A. americanum in Stained Gel Without Ammonium Persulfate. Scanned at 550 nm and a slit width of 0.2 mm, in a Gilford Linear Transport Model 2410

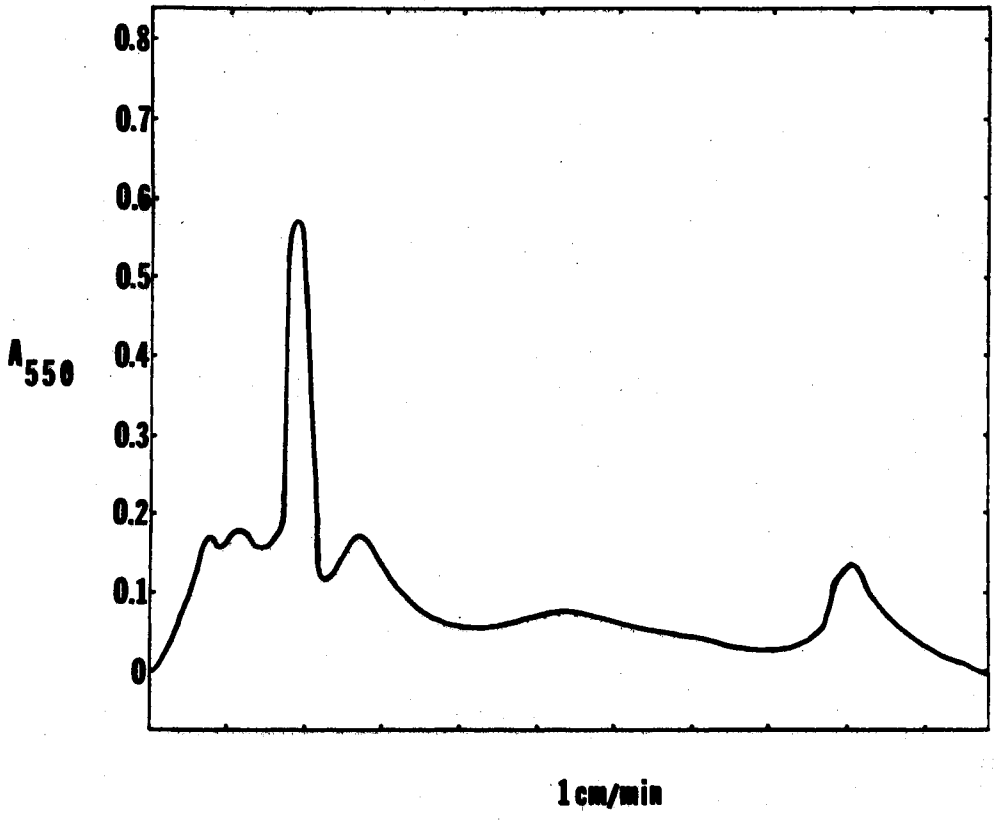

Figure 9. Oral Secretion Proteins from Female A. americanum (ca 500 mg) in Stained Gel Without Ammonium Persulfate. Scanned at 550 nm and a slit width of 0.2 mm, in a Gilford Linear Transport Model 2410

Ion Concentrations

The results of electrolyte analysis of female A. americanum oral secretion, collected by each of the 3 stimulation techniques are shown in Table II. The average concentrations of Na^+, K^+ and Cl^- in the secretions obtained by infra-red heat stimulation were 85.0, 16.0, and 297.0 mM/liter respectively. The average values of the same ions following pilocarpine injection were 430.0, 121.0, and 215.0 mM/liter. The secretions produced after electrical shock had average Na^+, K^+ and Cl^- concentrations of 218.0, 49.0 and 196.0 mM/liter.

Amino Acid Components

Results of amino acid analysis of female tick oral secretions and salivary gland homogenates are shown in Table III. Sixteen amino acids were identified in the oral secretion and 12 in the homogenates. Quantitatively, ammonium ion (NH_4) was the most abundant amino compound detected in oral secretion (0.1795 µℓ/ml), whereas, Arginine was found to be in slightly higher concentrations (0.1084 µℓ/ml) than NH_4 (0.7380 µℓ/ml) in salivary gland homogenates. Histidine was absent in salivary gland homogenates but was present in oral secretions. Other amino compounds which were detected in concentrations below 0.005 µℓ/ml of oral secretions were taurine, urea and ornithine. These compounds were absent in salivary gland homogenates.

Protein Concentration

According to the Lowry protein assay, salivary gland homogenates from unfed female A. americanum contained 2.67 µg of protein/µℓ. The

TABLE II

RESULTS OF ELECTROLYTE CONCENTRATION ASSAYS OF ORAL SECRETIONS FROM ENGORGING FEMALE A. AMERICANUM

Stimulation Method	Electrolyte (mM/l)					
	No. Ticks	Na±S.D.[a]	No. Ticks	K±S.D.	No. Ticks	Cl±S.D.
Infra-red heat	15	85.0±7.4	12	16.0±4.6	15	297.0±61.9
Pilocarpine	19	430.0±8.7	16	121.0±7.2	19	215.0±37.3
Electrical shock	10	218.0±4.4	5	49.0±2.4	15	196.0±80.1

[a] With the exception of Cl^- ion concentrations (NS) by pilocarpine and electrical stimulation, significant differences at 0.05 level exist between stimulation methods in all 3 columns.

TABLE III

FREE AMINO ACIDS AND AMINO COMPOUNDS IDENTIFIED IN FEMALE SALIVARY GLAND HOMOGENATES AND ORAL SECRETIONS OF A. AMERICANUM*

Amino Acids and Related Compounds	Concentration (μM/ml) Homogenate** (4 replicas)	Secretion (6 replicas)
Lysine	0.0051	0.1795
Arginine	0.1084	0.1788
Histidine		0.0446
Ammonium ion	0.0738	0.1885
Aspartic acid		0.0209
Threonine	0.0078	0.0262
Serine	0.0047	0.0235
Glutamic acid	0.0140	0.0159
Proline		Present
Glycine	0.0037	0.0324
Alanine	0.0046	0.0769
Valine	0.0032	0.0461
Methionine	0.0018	0.0060
Isoleucine	Present	0.0032
Leucine	Present	0.0259
Tyrosine	Present	0.0165
Phenylalanine		0.0169
Taurine		Present
Ornithine		Present
Urea		Present

* Oral secretion collection by infra-red heat stimulation

** 25 paired salivary glands/0.25 ml Neutral saline

protein concentration in oral secretion collected from ticks was too dilute for accurate determinations using the Lowry method.

Biological Activity

Hemolytic activity of oral secretion and salivary gland homogenates on sheep erythrocytes could not be detected at the end of 30 min. by colorimetric procedure.

Osmolarity of Oral Secretion and Hemolymph

The average freezing point ($-\Delta^{\circ}C$) of the oral secretions produced after stimulation with infra-red heat was -1.01 and -0.89 following stimulation with electrical shock (Table IV). These 2 means are significantly different at the $P<0.05$ level (T-test). The average $-\Delta^{\circ}C$ of the secretion produced after injecting pilocarpine into the tick was -0.93, but it was not possible to show any significant difference between it and the freezing points of the other secretions. Because of added heat, the slightly higher solute concentration in the secretion produced after stimulation with infra-red heat may have been due to greater evaporation during collection.

The average freezing point depression ($-\Delta^{\circ}C$) of female A. americanum oral secretion produced after stimulation with infra-red heat was -1.01 ±0.09 and the average hemolymph ($-\Delta^{\circ}C$) was -0.76 ±0.06 (Table V). Because the temperature involved with heat stimulation may reach ca $45^{\circ}C$, slightly higher solute concentration in the oral secretions may be due to evaporation.

TABLE IV

FREEZING POINT DEPRESSION (-Δ°C) OF TICK ORAL SECRETION PRODUCED BY DIFFERENT METHODS OF STIMULATION

Tick Wt. (MG)	Freezing Point Depression (-Δ°C) Infra-red Heat	Electrical Stimulation	Pilocarpine Inj.
50	-1.44	-1.71	
75		-0.86	
100	-1.05	-1.02	-0.96
160	-0.85		
170			-1.00
175		-0.68	
225			-0.66
275	-0.84		
280		-0.88	
350			-1.28
350		-0.69	
400		-0.92	
400	-1.35		
450		-0.66	
450	-0.81		
472	-1.14		
480			-0.80
530	-0.69		
575			-0.87
600		-0.68	
650	-0.99	-0.81	
	$\bar{x}$ = -1.01 ± 0.09[a]	$\bar{x}$ = -0.89 ± 0.11[b]	$\bar{x}$ = -0.93 ± 0.09[a b]

a b
Means $\bar{x}$ followed by the same letter are not significantly different at the $P<0.05$ level (t-test)

TABLE V

COMPARISON OF FREEZING POINT DEPRESSION (-Δ°C) BETWEEN
A. AMERICANUM ORAL SECRETIONS* AND HEMOLYMPH

Experiment No.	Average Freezing Point Depression (-Δ°C)			
	No. Samples	Oral Secretion	No. Samples	Hemolymph
1	2	-1.44	2	-0.77
2	2	-1.05	2	-0.65
3	2	-0.85	2	-0.74
4	2	-0.84	2	-0.72
5	2	-1.35	2	-0.68
6	2	-0.81	2	-1.04
7	2	-1.14		
8	2	-0.69		
9	2	-0.99		
	$\bar{x}$ = -1.01 ± 0.09**		$\bar{x}$ = -0.76 ± 0.06	

* Infra-red heat stimulation

** Significant at 0.05 level

Enzyme Assay

Table VI shows hyaluronidase activity results. Oral secretion used for this determination was collected from female ticks which weighed ca 400 mg and therefore, these values may not be representative of concentrations in oral secretion of ticks at earlier or later stages of engorgement.

Molecular Weights of Proteins

Successful molecular weight estimations were obtained only by the SDS-polyacrylamide electrophoretic technique. Column chromatography and Thin-layer gel filtration apparently diluted the proteins to an undetectable concentration level. Figure 10 shows the relative mobility (cm from top of gel to mid-point of band) of known proteins plotted as a function of molecular weight. From the mobility of the unknown samples, their molecular weights were extrapolated from the calibration graph. The 3 unknowns (homogenized salivary gland extract) plotted in Figure 10 were proteins from unfed A. americanum females.

The pH of engorging A. americanum oral secretions was consistently within a 7.6 to 8.0 range and this was also true of homogenized gland extracts. This range approximates neutrality which is the optimum pH for most enzymes (Williams and Lansford, 1967).

Table VII summerizes the properties of A. americanum oral secretion and homogenized salivary gland extracts.

TABLE VI

HYALURONIDASE ACTIVITY IN A. AMERICANUM ORAL SECRETION AND SALIVARY GLAND HOMOGENATES

Samples (A_{540})	Oral Secretion	Gland Homogenate**
1	0.130	0.295
2	0.155	0.315

* Blank contained buffer and acid albumin

** 25 paired salivary glands/0.25 ml neutral saline

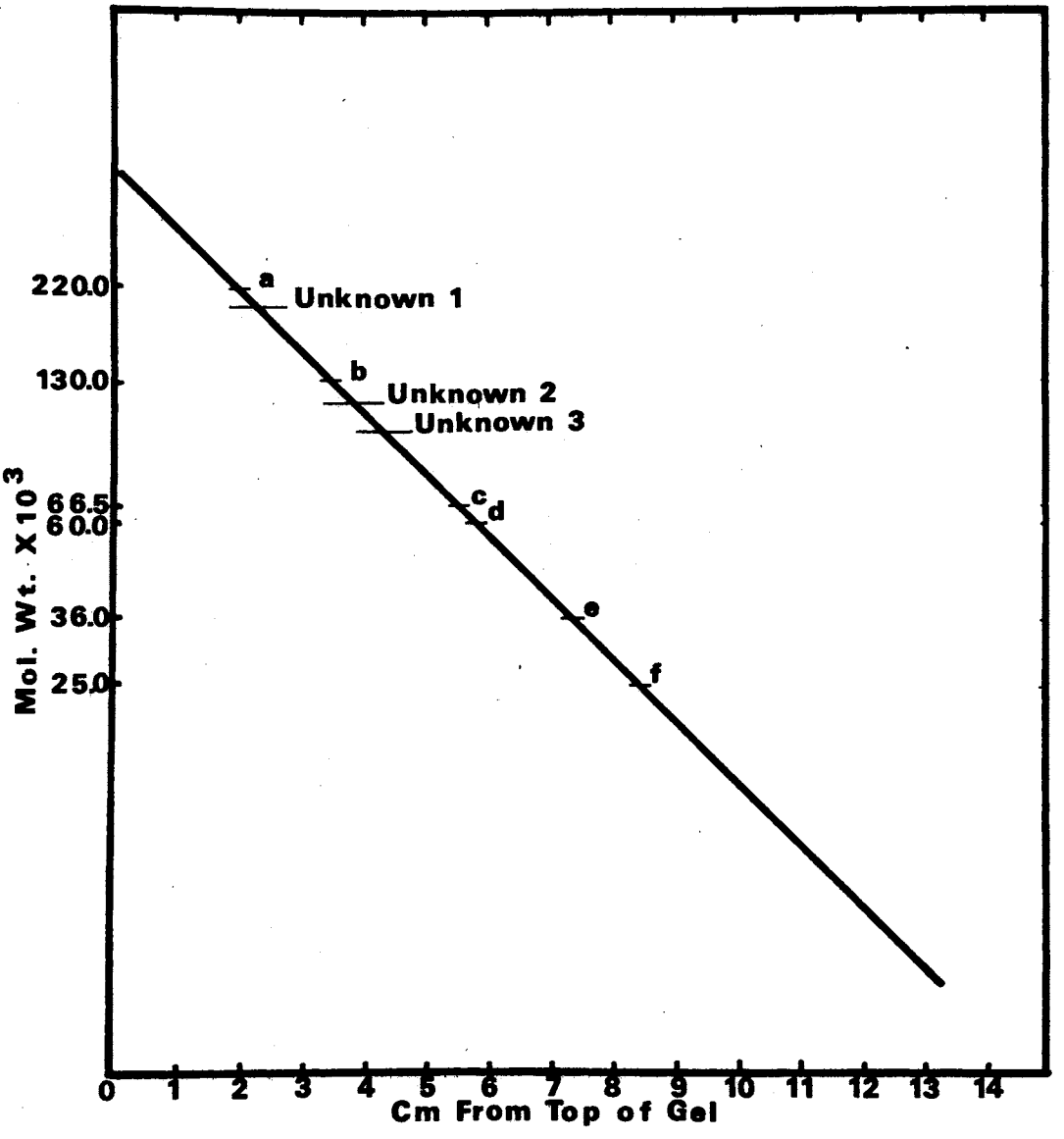

Figure 10. Molecular Weight Estimation of A. americanum Salivary Gland Homogenate Extracted Proteins by SDS-Electrophoresis in 5% Gel at 8 mA/gel for 3½ hours (a) Myosin, (b) Beta Galactosidase, (c) Bovine Serum Albumin, (d) Catalase, (e) Lactate Dehydrogenase, and (f) Chymotripsinogen A

TABLE VII

PROPERTIES OF A. AMERICANUM* ORAL SECRETION AND HOMOGENIZED SALIVARY GLAND EXTRACTS

pH	7.6-8.0†
Protein Concentration	2.67 μg/μℓ**
λ max.	260. nm**
$A_{280/260}$ Ratio	0.876**
Disc Gel Electrophoresis†† Components	(ca. 4-6 basic, ca. 1-4 acidic)† (ca. 4-5 basic, ca. 5 acidic)**
Protein Mol. Wt. Estimation	ca. 220 x 10^3, 125 x 10^3, 120 x 10^3**
Free Amino Acids and Amino Compounds (6 Major)	(NH_3, Lys, Arg, Ala, Val, and His)† (Arg, NH_3, Glu, Thr, Lys, and Ser)**
Osmolarity (-Δ°C)	$\bar{x}$ = -1.01 ± 0.09†
Enzymes Present	Hyaluronidase†
Hemolytic Activity	Slight to None† §
Electrolytes (Major)	Na^+, K^+, Cl^-†

* Female A. americanum

** Values determined on homogenant extracts

† Values determined on oral secretion

† Identified in oral secretions and extracts
†

§ Saline washed ovine erythrocytes

†† Separation with ammonium persulfate present

CHAPTER V

DISCUSSION

It is apparent from these results that female A. americanum oral secretions and salivary gland homogenantes have a complex composition and that components of oral secretion in engorging ticks are dynamic. Hajjar (1971) noted great variability in developmental events of nymphal argasid and ixodid ticks reared under the same conditions. This fact complicates the study of biochemical and physiological states in ticks and probably other arthropods as well.

The stimulation technique used to induce A. americanum to secrete was similar to that of Gregson (1957) for Dermancentor andersoni. Although Tatchell (1967) reported collection of secretions from Boophilus microplus identical to those collected from D. andersoni by Gregson, Barker et al. (1973) demonstrated qualitative and quantitative differences between oral secretion collected from A. americanum using 3 different stimulation techniques. Therefore, interpretation of results should include consideration of methodology effects.

It is important to make a distinction between oral secretion and saliva because orally secreted substances may not have been synthesized in salivary gland cells, as is the case of true saliva. Balashov (1972) states that, "unfed or completely engorged ticks do not discharge saliva", however the fluids we collected from completely engorged ticks contained small quantities of protein of unknown origin and the $-\Delta^{\circ}C$

values of these secretions indicated high solute content. Amino acids, peptides, nucleotides and other protein complexes could account for the elevated -Δ^{o}C values. Balashov (1972) also states that, "data on chemical composition of tick saliva is scant and has generally involved the identification of anticoaqulants, cytolytic components and histochemical identification of proteins, lipids and carbohydrates."

Infra-red heat was a very efficient stimulus and consistently induced ticks of similar engorgement weights to secrete greater volumes of oral secretion than did the other stimulation techniques. Greater volumes of oral secretion were collected when female ticks were stimulated immediately after removal from the host regardless of their stage of engorgement. This was also observed to occur in semi-engorged female Ixodes ricinus and Hyalomma asiaticum (Balashov 1972).

There are probably disadvantages to infra-red heat stimulation (i.e. possible evaporation) which could be avoided but, few techniques are flawless when microquantities of biological fluids are subjected to micro-assay techniques.

Oral secretion proteins collected from engorging female A. americanum of vastly different engorgement weights and electrophoresed were similar, with respect to the number and migration similarities of major proteins. It is possible that some tick oral secretion proteins were not synthesized by salivary gland cells, but rather originated from metabolic catabolism of the ingested blood meal. It should be reemphasized that oral secretion is not synonomus with salivary secretion although the former may contain the latter. The fact that regurgatation occurs in some naturally feeding ixodid ticks (Gregson 1967) and also was observed in oral secretions collected by infra-red

heat stimulation, the possibility of non-saliva protein identification exists. However, I feel that regardless of protein origin (i.e., saliva, regurgitated blood and/or metabolic waste products associated with natural tick feeding) it would be speculative to suggest that salivary proteins are the only components of consequence to host lesion formation or tick induced toxicosis.

Leclercq (1969) reported that the toxic secretion of ticks is their saliva and it should not be considered as a true venom. Venomous arthropods such as the hymenopterous insects and scorpions possess rapid acting venom which is stored in venom glands, without autointoxication, and is resynthesized so as to maintain a constant supply. Beard (1963) also gives an excellent review of insect toxins and venoms. Evidence of a tick venom synonomus with poisonous material identified in insects and other arthropods is remote and not considered to be of major consequence of A. americanum parasitism, even though several of the same chemical compounds (histidine, phenylalanine and hyaluronic acid) present in bee venom (Fischer and Dorfel 1953; and Neumann and Hahermann 1954) were identified in oral secretion from A. americanum.

Several investigators (i.e., Gregson 1970; Tatchell and Moorhouse 1968; Roberts 1968; and Trager 1939) have eluded to the antigenic properties of tick secretions involving acquired sensitivity in human and bovine hosts. Whole tick saliva and crushed tick cement feeding cones were shown to have antigenic properties (Gregson 1970). However, information concerning the antigenic nature of individual oral secretion proteins from ticks is relatively non-existent and deserves investigation. Other investigators (Allen and West 1966; and

Benjamini et al. 1963) have reported on skin-sensitizing components of mosquito (Aedes aegypti) and flea (Ctenocephalides felis felis) oral secretions. Williamson (1956) identified numerous amino acids in tsetse fly saliva, and 13 of the same were identified in this study.

The exact nature of tick induced paralysis which occurs in man and domestic animals is unknown, it is interesting that amino acid analysis of female A. americanum oral secretion revealed relatively high concentrations of ammonium ion (NH_4). Williams and Lansford (1967) indicate that, "ammonia (NH_3) is a gas which exists in tissue fluids near neutral pH predominately as the monovalent ammonium ion (NH_4^+)". Ammonium is potentially quite toxic to vertebrates especially if hepatic functions fail to reduce its concentration in portal blood. Williams and Lansford also state that, "raising the pH of the blood increases the toxicity of ammonium salts in animals, presumably because it favors formation of ammonia (NH_3) which more readily diffuses from the blood into the brain than the ammonium ion. However, the precise way in which excess ammonium produces central nervous system symptomology is not understood." Considering the tremendous numbers of A. americanum found on some wild and domestic animals (Bolte et al. 1970) during peak seasonal tick abundance, the quantity of (NH_4) returned to host tissues during normal tick feeding could conceivably have a detrimental effect on neo-natal and adult host animals. However, another possibility that must be considered when attempting to account for the relatively high NH_4 content in tick oral secretion would be in vitro methodology. Ion exchange separation involved with automated amino acid analysis of free amino acids may result in degradation of amino acids and the release of ammonia groups.

Tatchell (1969) has reported on the slightly hyperosmotic (as compared to hemolymph) saliva secreted by Boophilus microplus. The same phenomenom appears to be true of engorging A. americanum as shown by this study.

Recent experiments by Gregson (1967) and Tatchell (1967a; 1969) have shown clearly that feeding ixodid ticks eliminate excess water via the salivary glands during the final phases of feeding. Tatchell (1969) has also shown that the secretion functions in ion regulation. The latter role appears analagous to that of the combined Malphigan tubule rectal complex in most insects (Ramsay 1971, Maddrell 1969, Berridge 1967). This postulated function has been supported by recent morphological evidence (Kirkland 1971) showing extensive infoldings of the plasma membrane (a constant feature of fluid transporting epithelia, Diamond and Tormey 1966a, b; Diamond and Bossert 1967, 1968) of the type I alveoli in the salivary glands of the rabbit tick, Haemaphysalis leporis palustris.

Since this model proposes a secretion of fluid isosmotic or slightly hyperosmotic to the hemolymph (Berridge and Oschman 1969) it is not too surprising that the osmolarity of the fluid secreted by A. americanum is nearly constant regardless of the time or technique of collecting fluid. This is further strengthened by the work of Shih et al. (1972) showing that A. americanum has considerable powers of hemolymph osmoregulation.

The concentration of individual solutes (ions) identified in A. americanum oral secretions was interesting. The high concentrations of chloride in the secretion obtained by infra-red heat stimulation may come about because chloride ions make up approximately two-thirds of

the osmotically active constituents of mammalian plasma (Lees 1946). Chloride ions probably pass from the gut out into the hemolymph down a concentration gradient and water follows passively (concentrating the blood meal). Because of these events, ionic and water balance stress is placed on the tick (Kirkland 1971). The stress is overcome by the formation of local osmotic gradients within basal infoldings of type I alveoli of the salivary glands brought about in part by active transport of solutes (many of which may be chloride) forming the nearly isomotic oral secretion with a high concentration of chloride.

It is interesting that the concentrations of Na^+ and K^+ were low in the oral secretion following infra-red heat stimulation. A recent report by Shih et al. (1972) indicates that Na^+ plays an important role in hemolymph volume regulation during desiccation of unfed adults. These authors reported that during severe desiccation the total body water decreased but the volume of hemolymph remained constant at the same time the concentration of Na^+ rose sharply. It may be that the infra-red heat method (which would be desiccating) causes a similar reabsorption of Na^+ into the hemolymph (for the purpose of volume regulation) resulting in a low concentration of Na^+ in the oral secretion.

It must be reemphasized that with either of these methods of stimulation the total concentration of solutes in the oral secretion is about the same; however, the nature of these constituents varies considerably. From the above results one should find the highest concentrations of organic solutes (proteins, amino acids, etc.) in the secretion after employing the infra-red heat method of stimulation.

These facts are of considerable importance to researchers attempting to chemically assess the nature and significance of tick oral secretions and their potential effect on the host.

The presence of several proteins in unfed female A. americanum salivary gland homogenate extracts and in oral secretions at various engorgement stages would suggest that other enzymes normally associated with haematophagous arthropods, but not identified in this study may be present. Geczy et al. (1971) reported the presence of a macromolecule with esterase activity in fractions of Boophilus microplus saliva. The relatively slow feeding behavior of A. americanum [and most ixodid adults (Arthur 1962)], as compared to some of the other haematophagous arthropods would indicate that slow acting protein materials, possibly of a toxic nature, could be involved with lesion formation resulting from host tissue responses.

Riek (1957) indicated that onset of tick (Ixodes holocyclus) paralysis in host animals is seldom seen until 4 days of feeding has been completed and that the toxin he identified was distinct from the toxin which causes tick-induced paralysis.

Because A. americanum homogenized salivary gland extracts had a UV A_{max} of 260 nm, this may suggest the presence of a nucleotide or cofactor. The ratio of optical density at 280 nm to optical density at 260 nm of homogenized extract was 0.878. This indicates that the percent of nucleic acid present was ca 5.0%.

CHAPTER VI

SUMMARY

Amblyomma americanum oral secretions were collected by infra-red heat stimulation and partially identified by several techniques. The protein concentration of *A. americanum* homogenized salivary glands was less than 3% and thus, identification techniques that required large volumes of oral secretions (1 ml or more) were impractical. Electrophoretic separation of oral secretions and salivary gland homogenate extracts revealed fewer proteins in polyacrylamide gels without ammonium persulfate, although the major protein bands were present in both. Based on protein staining of simultaneously separated oral secretions (pooled) from ticks of different engorgement weights, a reduction in protein numbers were observed between engorging ticks of 370 to 550 mg of weight. This may suggest reduced secretion of synthesized salivary gland components when *A. americanum* reach an engorgement weight of 350 to 450 mg and may further indicate that tick secretions are composed primarily of excess fluids resulting from hemoconcentration of the ingested blood meal at greater engorgement weights. Gel scanning facilitated visual observations for detecting stained gel column components, but, quantitation of proteins with any degree of confidence was not possible with these graphs. At least one major protein component migrated the same distance in homogenate extracts and oral secretions and was assumed to be the same protein.

Free amino acid analysis of oral secretion revealed the presence of 20 amino acids or related compounds. Many are known metabolites in nitrogen metabolism and protein catabolism.

Electrolyte analysis of oral secretion revealed the following concentration in decending order: Cl^-, Na^+, and K^+. Chloride ions made up more than 70% of the ions in oral secretion collected by infra-red heat stimulation. This method of stimulation may have influenced the low sodium concentration because of its reported retension in adult ticks subjected to desiccation. Potassium being primarily an intracellular ion would naturally be in low concentrations.

Osmolarity determinations made on oral secretion and hemolymph from engorging ticks revealed a slightly higher solute content in oral secretions thus, indicating salivary gland involvement in hemolymph osmotic regulation.

Unfractionated A. americanum homogenized salivary gland extracts failed to show hemolytic activity within 30 min. at room temperature, which suggested very little or no hemolytic components were present in the extracts.

Using SDS-polyacrylamide electrophoresis, molecular weight estimations were made for 3 proteins in salivary gland homogenate extracts. This method proved most successful for working with small volumes of material.

Estimation of proteins in salivary gland homogenate extracts by UV-absorption revealed a strong absorption at 260 nm and indicates that more nucleic acid was present than protein.

SELECTED BIBLIOGRAPHY

Allen, J. R. and A. S. West. 1966. Some properties of oral secretion from Aedes aegypti (L.) Exp. Parasitol. 19, 124-131.

Andrews P. 1964. Estimation of the molecular weights of proteins by Sephadex gel-filtration. Biochem. J. 91, 222-233.

Arthur, D. R. 1962. Ticks and Disease. Pergamon Press, New York 445 p.

Arthur, D. R. 1966. The ecology of ticks with reference to the transmission of protozoa. In Biology of Parasites. Soulsby, E. J. L. (ed.) Academic Press, New York. p. 61-84.

Balashov, Yu. S. 1972. A translation of bloodsucking ticks (Ixodoidae) Vectors of diseases of man and animals. Entomol. Soc. Amer. Misc. Pub. 8, 161-376.

Barker, R. W., E. Burris, J. R. Sauer and J. A. Hair. 1972. Composition of tick oral secretions obtained by three different collection methods. J. Med. Entomol. 10, 198-201.

Barker, R. W., A. Hoch, R. G. Buckner and J. A. Hair. 1973. Haematological changes in white-tailed deer fawns infested with Theileria infected ticks. (Submitted to J. Parasitol.)

Beard, R. L. 1963. Insect toxins and venoms. In Ann. Review of Entomol. 8, 1-18.

Benjamini, E., B. F. Feingold, J. D. Young, L. Kartman and M. Shimizu. 1963. Allergy to flea bites. IV. In vitro collection and antigenic properties of the oral secretion of the cat flea, Ctenocephalides felis felis (Bouche). Exp. Parasitol. 13, 143-154.

Berridge, M. J. 1967. Ion and water transport across epithelia. In Insects and Physiology. J. W. L. Beament and J. E. Treherne (ed.) Amer. Elsevier Pub. Co., Inc., New York. pp. 329-347.

Bishopp, F. C. and H. L. Trembley. 1945. Distribution and hosts of certain north american ticks. J. Parasitol. 31, 1-54.

Bolte, J. R., J. A. Hair and J. Fletcher. 1970. White-tailed deer mortality following tissue destruction induced by lone star ticks. J. Wildl. Mgt. 34, 546-552.

Burgdorfer, W. 1951. Analyse des Infektionsverlaufes bei Ornithodorus moubata (Murray) und de naturlichen Ubertragung von Spirochaeta duttoni. Acta Trop. 8, 193-262.

Cherington, M. S. and R. D. Synder. 1968. Neurophysiologic studies in tick paralysis. N. Engl. J. Med. 278, 95-97.

Chrambach, A., R. A. Reisfeld, M. Wyckoff, and J. Zaccari. 1967. A procedure for rapid and sensitive staining of protein fractionated by polyacrylamide gel electrophoresis. Anal. Biochem. 20, 150-154.

Cowdry, E. V. and W. B. C. Danks. 1933. Studies on East Coast fever. II. Behavior of the parasite and the development of distinctive lesions in susceptible animals. Parasitology. 25, 1-63.

Diamond, J. M. and J. McD. Tormey. 1966a. Role of long extracellular channels in fluid transport across epithelia. Nature (Lond.). 210, 817-820.

Diamond, J. M. and J. McD. Tormey. 1966b. Studies on the structural basis of water across epithelial membranes. Fed. Proc. 25, 1458-1463.

Diamond, J. M. and W. H. Bossert. 1967. Standing-gradient osmotic flow. A mechanism for coupling of water and solute transport in epithelia. J. Gen. Physiol. 50, 2061-2083.

Diamond, J. M. and W. H. Bossert. 1968. Functional consequence of ultra-structural geometry in 'backwards' fluid-transporting epithelia. J. Cell. Biol. 37, 694-702.

Fairbairn, H. and J. Williamson. 1956. The composition of Tsetse-fly saliva. I. A histochemical analysis. Ann. Trop. Med. Parasitol. 50, 322-333.

Fischer, F. G. and H. Dorfel. 1953. Das Gift de honigbiene. II. Die aminosauren zusammensetzung der bienengift-fraktionen. Biochem. Z., 324, 465-475.

Frick, J. H. and J. R. Sauer. 1973. Examination of a biological cryostat/nanoliter osmometer for use in determining the freezing point of insect hemolymph. Ann. Entomol. Soc. Amer. (In Press).

Geczy, A. F., M. A. Naughton, J. B. Clegg and R. W. Hewetson. 1971. Esterase and carbohydrate splitting enzyme in the saliva of the cattle tick, Boophilus microplus. J. Parisitol. 57, 437-438.

Gregson, J. D. 1957. Experiments on oral secretion of the Rocky Mountain Wood tick, Dermacentor andersoni Stiles (Acarina: Ixodidae). Can. Entomol. 89, 1-5.

Gregson, J. D. 1967. Observations on the movement of fluids in the vicinity of the mouth parts of naturally feeding Dermacentor andersoni Stiles. Parasitology. 57, 1-8.

Gregson, J. D. 1958. Host susceptibility to paralysis by the tick Dermacentor andersoni Stiles (Acarina: Ixodidae). Can. Entomol. 90, 421-424.

Hajjar, N. P. 1971. Biochemical and physiological studies of certain ticks (Ixodoidea). Selection of physiological states for biochemical analysis of fluids. J. Med. Entomol. 8, 643-647.

Higginbotham, R. D. and S. Karnella. 1970. The significance of the mast cell response to bee venom. J. Immunol. 106, 233-240.

Hoogstraal, H. 1966. Ticks in relation to human diseases caused by viruses. In Ann. Rev. Entomol. 11, 261-308.

Hoogstraal, H. 1967. Ticks in relation to human diseases caused by Rickettisa species. In Ann. Rev. Entomol. 12, 377-420.

James, M. T. and R. F. Harwood. 1969. Herms's Medical Entomology. Collier-MacMillian Ltd., London, 6th ed.

Kass, E. H. and C. V. Seastone. 1944. The role of the mucoid polysaccharide (hyaluronic acid) in the virulence of group A hemolytic streptococci. J. Exp. Med. 79, 319-330.

Leclercq, M. 1969. Entomological parasitology - The relation between entomology and the medical science. Pergamon Press, New York. p. 158.

Lees, A. D. 1946. Chloride regulation and the function of the coxal glands in ticks. Parasitology. 37, 172-184.

Levine, N. D. 1967. Protozoan parasites of domestic animals and of man. Burgess Pub. Co., Minneapolis. p. 411.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265-275.

Maddrell, S. H. P. 1969. Secretion by the malipighian tubules of Rhodnius. The movements of ions and water. J. Exp. Biol. 51, 71-79.

Moore S. and W. H. Stein. 1963. Chromatographic determination of amino acids by the use of automatic recording equipment. Methods in Enzymol. 193, 265-275.

Moorhouse, D. E. 1966. The onset of paralysis caused by the scrub-tick Ixodes holocyclus Neumann. Aust. Vet. J. 42, 311.

Muranghan, M. F. 1958. Neuro-anatomical site in tick paralysis. Nature (Lond.) 181, 131.

Muranghan, M. F. 1960a. Site and mechanism of tick paralysis. Science, 131, 418-419.

Muranghan, M. F. 1960b. Conduction block of terminal somatic motor fibers in tick paralysis. Can. J. Biochem. Physiol. 38, 287-295.

Nuttall, G. H. F. and C. Strickland. 1908. On the presence of an anticoagulin in the salivary glands and intestines of Argas persicus. Parasitology, 1, 302-310.

Neumann, W. and E. Habermann. 1954. Beitrage zur charakterisierung der wirkstoffe des bienengiftes. Arch. Exp. Pathol. Pharmakol. 222, 367-387.

Ornstein, L. 1964. Disc electrophoresis. I. Background and theory. Ann. N. Y. Acad. Sci. 121, 321-349.

Oxer, D. T. 1948. The preparation of canine anti-tick serum. J. Aust. Vet. Ass. 24, 95-96.

Pavlosky, E. N. and N. I. Chodukin. 1929. Uber die antikoaguline undere wirksame Bestandteile der Zecke Ornithodorus papillipes. Z. Parasitenk. 2, 90-96.

Purnell, R. E. and L. P. Joyner. 1968. The development of Theileria parva in the salivary glands of the tick, Rhiphecephalus appendiculatus. Parasitology. 58, 725-732.

Radola, B. J. 1968a. Thin-layer gel filtration of proteins. I. Method. J. Chromatog. 38, 61-77.

Radola, B. J. 1968b. Thin-layer gel filtration of proteins. II. Applications J. Chromatog. 38, 78-90.

Ramsay, J. A. 1971. Insect rectum. Phil. Trans. Roy. Soc. Lond. B. 262, 251-260.

Reichenow, E. 1940. The piroplasmasida. In Protozoan parasites of domestic animals and of man. Levine, N. D., Burgess Pub. Co., Minneapolis. pp. 305-308.

Riek, R. F. 1957a. Studies on the reactions of animals to infestation with ticks. I. Tick anemia. Aust. J. Agri. Res. 8, 209-214.

Riek, R. F. 1957b. Studies on the reactions of animals to infestation with ticks. II. Tick toxins. Aust. J. Agri. Res. 8, 215-223.

Riek, R. F. 1959. Studies on the reactions of animals to infestation with ticks. V. Laboratory animals as hosts for the cattle tick, Boophilus microplus (Canestrini). Aust. J. Agri. Res. 10, 614-619.

Riek, R. F. 1962. Studies on the reaction of animals to infestation with ticks. VI. Resistance of cattle to infestation with the tick Boophilus microplus (Canestrini). Aust. J. Agri. Res. 13, 532-550.

Riek, R. F. 1964. The life cycle of Babesia bigemina (Smith and Kilborne, 1893) in the tick vector Boophilus microplus (Canestrini). Aust. J. Agri. Res. 15, 802-821.

Riek, R. F. 1966. The development of Babesia spp. and Theileria spp. in ticks with special reference to those occurring in cattle. In Biology of Parasites. Soulsby, E. J. L. (ed.) Acad. Press, New York. pp. 15-30.

Ristic, M. 1960. Anaplasmosis. Advan. Vet. Sci. 6, 111-192.

Ristic, M. 1965. Anaplasma marginale a non-protozoan blood parasite. Abstr. Pap. 2. Int. Conf. Protozol. (London, 29 July - 5 August). pp. 32-33.

Ristic, M. 1966. The vertebrate developmental cycle of Babesia and Theileria. In Biology of Parasites. Soulsby, E. J. L. (ed.) Acad. Press, New York. pp. 128-139.

Roberts, J. A. 1968. Acquisition by the host of resistance to the cattle tick, Boophilus microplus (Canestrini). J. Parasitol.

Roshdy, M. A. 1972. The subgenus Persicargas (Ixodoidea, Argasidae, Argas) 15. Histology and histochemistry of the salivary glands of A. (P.) persicus (Oken). J. Med. Entomol. 9, 143-148.

Shapiro, A. L., E. Vinuela and J. V. Maizel Jr. 1967. Molecular weight estimation of polypeptide chains by electrophoresis in SDS-polyacrylamide gel. Biochem. Biophys. Res. Commun. 28, 815-820.

Shih, Chi-yen, J. R. Sauer, P. Eikenbary, J. A. Hair and J. H. Frick. 1973. The effects of desiccation and rehydration on the lone star tick. J. Insect. Physiol. 19, 505-514.

Tatchell, R. J. 1967. A modified method for obtaining tick oral secretion. J. Parasitol. 53, 1106-1107.

Tatchell, R. J. 1967b. Salivary secretion in the cattle tick as a means of water elimination. Nature (Lond.). 23, 940-941.

Tatchell, R. J. 1968. Host-parasite interactions and the feeding of blood sucking arthropods. Parasitology. 59, 431-456.

Tatchell, R. J. 1969. The ionic regulatory role of the salivary secretion of the cattle tick, Boophilus microplus. J. Insect. Physiol. 15, 1421-1430.

Till, W. M. 1959. New cell types in the salivary gland of the brown ear tick, Rhipicephalus appendiculatus Neumann. Nature (Lond.). 184, 1078-1079.

Till, W. M. 1961. A contribution to the anatomy and histology of the brown ear tick, Rhipicephalus appendiculatus Neumann. Mem. Entomol. Soc. S. Afr. 6, 124.

Tolksdorf, S., M. H. McCready, D. R. McCullagh, and E. Schwenk. 1949. The turbidimetric assay of hyaluronidase. J. Lab. Clin. Med. 34, 74-89.

Trager, W. 1939. Acquired immunity to ticks. J. Parasitol. 25, 57-81.

Williamson, J. 1956. The composition of tsetse-fly saliva. II. Analysis of amino acids and sugars by paper partition chromatography. Ann. Trop. Med. Parasitol. 50, 334-344.

Williams, R. J. and E. M. Lansford. 1967. The encyclopedia of biochemistry. Reinhold Pub. Co., New York. p. 876.

Worthington Enzyme Manual, 1972. Worthington Biochemical Corporation, Freehold, New Jersey.

VITA

Robert Wells Barker

Candidate for the Degree of

Doctor of Philosophy

Thesis: STUDIES ON THE CHARACTERIZATION OF ORAL SECRETION AND SALIVARY GLAND HOMOGENATE EXTRACTS OF AMBLYOMMA AMERICANUM (ACARINA: IXODIDAE)

Major Field: Entomology

Biographical:

Personal Data: Born in Oklahoma City, Oklahoma, February 7, 1944, the son of Mr. and Mrs. W. R. Barker.

Education: Attended school in Oklahoma City until grade six; moved to Braggs, Oklahoma and graduated from Braggs High School in 1962; received the Associate of Science degree from Connors College in May, 1964; received the Bachelor of Science degree in Education Biology from Northeastern State College in May, 1967.

Professional Experience: Biology instructor in secondary public school for 3 years and research assistant at Oklahoma State University.

Societies: Omega Tau Sigma (Honorary), Entomological Society of America, American Mosquito Control Association, Wildlife Society and Sigma Xi.

Honors: Received Sigma Xi Research Grant, National Science Foundation Research Award, Graduate Excellence Award and Entomological Society of America Entomology Research Institute Graduate Student Award.