CHANGES IN DOPAMINE-SENSITIVE ADENYLATE CYCLASE ACTIVITY IN SALIVARY GLANDS OF FEEDING FEMALE LONE STAR TICKS *AMBLYOMMA AMERICANUM* (L.)

By

MARY LOUISE SCHRAMKE

Bachelor of Arts

Kalamazoo College

Kalamazoo, Michigan

1977

Submitted to the Faculty of the Graduate College
of the Oklahoma State University
in partial fulfillment of the requirements
for the Degree of
MASTER OF SCIENCE
May, 1982

CHANGES IN DOPAMINE-SENSITIVE ADENYLATE CYCLASE ACTIVITY IN SALIVARY GLANDS OF FEEDING FEMALE LONE STAR TICKS AMBLYOMMA AMERICANUM (L.)

Thesis Approved:

John R. Sauer
Thesis Adviser

Richard C. Essenberg

L. D. Eckenberg

Norman N. Durham
Dean of the Graduate College

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PREFACE

This study was designed to determine the importance of the enzyme adenylate cyclase in the salivary glands of female lone star ticks Amblyomma americanum (L.), as this enzyme catalyses the production of intracellular cyclic AMP, a molecule known to play a key role in the fluid secretory process of feeding female ixodid ticks.

The author would like to express sincere gratitude to her major adviser, Dr. John Sauer, for the encouragement to complete this study and the assistance in the preparation of this thesis.

The support, lab space and ideas of Dr. Richard Essenburg and Dr. Raymond Eikenbary, who served as members of the author's committee, are gratefully acknowledged. The invaluable assistance and instruction provided by Dr. Stephen Schmidt is also sincerely appreciated.

The graduate students and staff of the laboratories in which I worked in both the Biochemistry and Entomology departments were always supportive and will always be appreciated. I would also like to thank Dr. Jackie Hair and his staff for their integral role in providing and maintaining the experimental animals and facilities used in the course of this study.

I would also like to thank my husband Dean Hector and his parents Pat and Neil for their encouragement to continue my work here at Oklahoma State University.

To my friends, Rhonda and Melanie, Elaine and Tim, and Becky, thank

you for listening to all the complaints and comments as my work drew to a close.

This thesis is dedicated to my parents, Frank and Lorraine, and my family for their love and friendship which helped me grow as a person and fostered my desire to pursue a scientific career.

CHAPTER I

INTRODUCTION

Ixodid tick salivary glands function in excreting excess fluid while the tick is attached and feeding on the host (Gregson, 1967; Tatchell, 1967; Kaufman and Phillips, 1973a). Salivary glands also participate in the uptake of water in unfed ticks (Rudolph and Knülle, 1974; McMullen et al., 1976). Salivary glands play an important role in maintaining homeostatic conditions within ixodid female ticks as large bloodmeals are consumed for egg nourishment and subsequent oviposition (Balashov, 1972). Ingested host blood is concentrated within the tick gut as excess ions and water from the bloodmeal are transported across the gut epithelium into the hemolymph and are collected and secreted back into the host via the salivary glands (Tatchell, 1967, 1969; Kirkland, 1971; Kaufman and Phillips, 1973 a, b, c; Meredith and Kaufman, 1973). The various functions and components of acarine salivary glands, including fluid secretion in ixodid ticks have been reviewed by Sauer (1977).

Control of salivary gland secretion is thought to be mediated via nerves rather than hormones (Kaufman and Phillips, 1973b). Much evidence has been gathered in support of this hypothesis, particularly concerning control of fluid secretion involving catecholamines. Chloride uptake and fluid secretion in isolated whole tick salivary glands are stimulated when glands are incubated in media containing catecholamines (Sauer et al., 1974; Kaufman and Phillips, 1973b, Kaufman, 1976; Needham

and Sauer, 1975; Sauer et al., 1979). In addition, the salivary glands of several tick species are innervated (Coons and Roshdy, 1973; Megaw, 1976; Binnington, 1981), and catecholamines have been found to exist in nervous tissue and salivary glands of ticks (Megaw and Robertson, 1974; Binnington and Stone, 1977).

Cyclic AMP is now recognized as an intracellular "second messenger" mediating many of the effects of catecholamines and other hormones in peripheral tissues (Sutherland et al., 1968). The level of cyclic AMP within a cell depends upon the activities of at least two enzymes or enzyme systems. Adenylate cyclase catalyses the formation of cyclic AMP from ATP, whereas a phosphodiesterase catalyses the hydrolysis of cyclic AMP to 5'-AMP. Most of the adenylate cyclase activity is associated with the cell surface membranes of different tissues (Davoren and Sutherland, 1963). The adenylate cyclase enzyme system is composed of three components: The receptor component (R) containing a specific binding site for hormones or neurotransmitters, the catalytic unit (C) and the guanine nucleotide regulatory unit (G) which binds GTP (Rodbell, 1980).

Hormones and neurotransmitters appear to play an important role in tissue development (Greengard, 1971; McMahon, 1974). Hormones or neurotransmitters bind to specific receptors on the cell surface of their target tissue which leads to the activation of adenylate cyclase to produce intracellular cyclic AMP which affects numerous metabolic processes within target cells (Robinson et al., 1971). Cyclic AMP has been shown to enhance the synthesis of enzymes and other cell components which are essential for tissue growth and development (Greengard, 1971). Phasic alternations in tissue cyclic AMP and adenylate cyclase have been reported in the development of mammalian liver (Bar and Hahn, 1971), brain

(Schmidt et al., 1970), heart (Novak et al., 1972), lung (Nijjar, 1979) and other tissues (Braun and Hechter, 1970; Palmer, 1972). Rasenick et al. (1976) described a six-fold increase in brain cyclic AMP that occurred within 24 hours after transferring pupae of the silkmoth, Hyalophora cecropia from four months of cold storage to room temperature. Levels of cyclic AMP fell to half the maximum value after 48 hours and continued to decline slowly during the silkmoth pharate adult development. Rasenick et al. (1976) have hypothesized that the sudden increase in the intracellular level of cyclic AMP in the insect brain may be an essential step in the initiation of adult insect development. Bodnaryk (1978) reported a six-fold increase in cyclic AMP and a two-fold increase in cyclic GMP in the brain of Mamestra configurata Wlk., after the initiation of neurosecretion, which suggested that cyclic nucleotides are important in establishing brain processes during adult insect brain differentiation and perhaps in the overall differentiation process itself.

The application of exogenous cyclic AMP stimulates fluid secretion in whole salivary gland preparations of ixodid ticks and the addition of theophylline, and inhibitor of phosphodiesterase, enhances the fluid secretory process in tick salivary glands in the presence of dopamine (Sauer et al., 1979). The secretory competence of isolated tick salivary glands depends on the weight of the tick from which the glands were excised and thus on the stage of feeding of the developing tick (Kaufman, 1976; Megaw, 1976). Whole gland homogenates of tick salivary glands have been shown to contain a cyclic nucleotide phosphodiesterase whose activity changes with stage of tick feeding (McMullen and Sauer, 1978). Kaufman et al. (1976) have confirmed the existence of a Na,K-ATPase in the salivary glands of Amblyomma hebraeum (Koch) and the activity of this

enzyme increased with time spent by the tick on the host and correlates to the increased secretory rates seen at the same time. Recently, Schmidt et al. (1982a) have demonstrated the presence of a dopamine-sensitive adenylate cyclase in cell-free preparations of ixodid tick salivary glands, and that factor(s) exist in the cytoplasm which enhance adenylate cyclase in the particulate fraction. This study was initiated to determine if the dopamine-sensitive adenylate cyclase activity in tick salivary glands changed in response to female tick feeding and development in the adult stage.

CHAPTER II

MATERIALS AND METHODS

Experimental Animals

Adult female lone star ticks Amblyomma americanum (L.) were the source of salivary glands in assays for adenylate cyclase. Ticks were reared by a modification of the method of Gladney and Drummond (1970) as reported by Patrick and Hair (1976). The ticks were allowed to attach and feed on restrained sheep. Ticks were contained on the sheep by orthopedic stockinette cells which were affixed with formica cement to the sheep wool. Twenty to thirty pairs of adult ticks were placed into each cell and five cells were placed on each sheep. In experiments where the ticks were used which had been attached to the host for a specified number of days, all unattached ticks were removed from the cells within 12 hours after placement. Ticks of various weights, representative of the entire adult feeding process (ca 4 to 900 mg) were sampled in this study.

Tissue Preparation

Paired salivary glands were dissected from female ticks in cold 50 mM Tris-HCl buffer, pH 7.4, using the procedure described by Needham and Sauer (1975). Tissue homogenization was of two types based on the number of pairs of glands homogenized and the method of homogenization. Preliminary investigations utilized multiple pairs of salivary glands

(usually 3-4 pairs) which were homogenized with a loose-fitting ground glass homogenizer. The second type of homogenization involved single pairs of salivary glands which were homogenized with a 1cc disposable tuberculin syringe (21g needle) in a 0.5 ml Pierce® reacti-ware vessel. A ratio of 100 µl buffer per pair of salivary glands was maintained in both types of tissue homogenization.

Adenylate Cyclase Assay

Adenylate cyclase assay was performed as described by Schmidt et al. (1982a) using [α-^{32}P] ATP as substrate. The assay medium contained in 50 µl: 50 mM Tris-HCl buffer, pH 7.4, 3.84 mM $MgCl_2$, 0.1 mM EDTA, 0.5 mM cyclic AMP, 20 mM creatine phosphate, 50 U/ml creatine phosphokinase and enzyme.

The reactants were preincubated for three minutes at 30°C. Assays were initiated by the addition of 11-15 µl of salivary gland homogenate which contained 20-70 µg of protein depending on the weight of the tick(s) from which the glands were excised. The reaction was terminated after 15 minutes by the addition of 1 ml of 1N HCl and the samples were immediately heated in boiling water for 10 mintues. Schmidt et al. (1982a) have reported that the enzyme is stable and the reaction linear with respect to time for 20 minutes. [α-^{32}P] Cyclic AMP was isolated from the reaction mixture by a $ZnSO_4$ and $Ba(OH)_2$ precipitation procedure as described by Nakai and Brooker (1975). In the second precipitation, aliquots of 70 µl each were taken in triplicate from the clear supernatant fraction of each purified reaction mixture which contained the [α-^{32}P] cyclic AMP. Each aliquot was placed in 6 ml of cocktail and counted on a Beckman LS-3150T liquid scintillation counter. Machine

counting efficiencies were monitored using an external standard. The scintillation cocktail contained (per liter): 750 ml toluene, 250 ml 2-ethoxy-ethanol, 7g of 2,5-Diphenyl-Oxazole (PPO), and 0.2g of 1,4-bis [2(5-Phenyl-oxazolyl) benzene (POPOP)]. A seventy-six percent recovery of the reaction product was assumed in this study based on the reported 70-80% recovery of [$^{14}_{-C}$] Cyclic AMP from the purified reaction mixture obtained by Schmidt, et al. (1982a).

Adenylate cyclase activity measured in the absence of dopamine is referred to as "basal activity" and in its presence (10 µM final concentration) as "dopamine-stimulated activity." Total enzyme activity is expressed as p moles cyclic AMP produced per minute per pair of salivary glands and specific activity is p moles cyclic AMP produced per minute per milligram of tissue protein. Schmidt et al. (1982a) reported maximal stimulation of adenylate cyclase activity in ixodid tick salivary gland homogenates using 10 µM dopamine, so this concentration was used in assessing dopamine-stimulated enzyme activity throughout this study. Protein concentration was determined by the method of Lowry et al. (1951) with bovine serum albumin as standard.

Data Analysis

Statistical analyses were carried out using the 1979 edition of the Statistical Analysis System, SAS (Barr et al., 1979). Analysis of variance (General Linear Models Proceedure) was used to examine the relationships of enzyme activity to tick weight and host utilized (sheep number) and the dependence of protein content of tick salivary glands upon tick weight during the adult tick feeding process.

Chemicals

[α-^{32}P] Adenosine 5'-triphosphate, tetra-(triethylamonium) salt (10-30 Ci/mmol) was obtained from New England Nuclear, Boston, Ma., U.S.A. Creatine phosphokinase (EC 2.7.3.2) the essentially salt-free powder from rabbit muscle, cyclic AMP as the crystalized free acid, the sodium salt of ATP and phosphocreatine were obtained from Sigma Chemical Co., St. Louis, Mo. U.S.A. All other chemicals used were reagent grade or greater purity.

Abbreviations

ATP	Adenosine 5'-triphosphate
Cyclic AMP	Adenosine 3':5'-cyclic monophosphate acid
DA	Dopamine
EDTA	Ethylene diamine tetracetic acid
GDP	guanosine diphosphate
GMP	guanosine 5'-phosphate
GTP	guanosine triphosphate
MIX	3-isobutyl-1-methylxanthine

CHAPTER III

RESULTS

Total Protein Content of Tick Salivary Glands

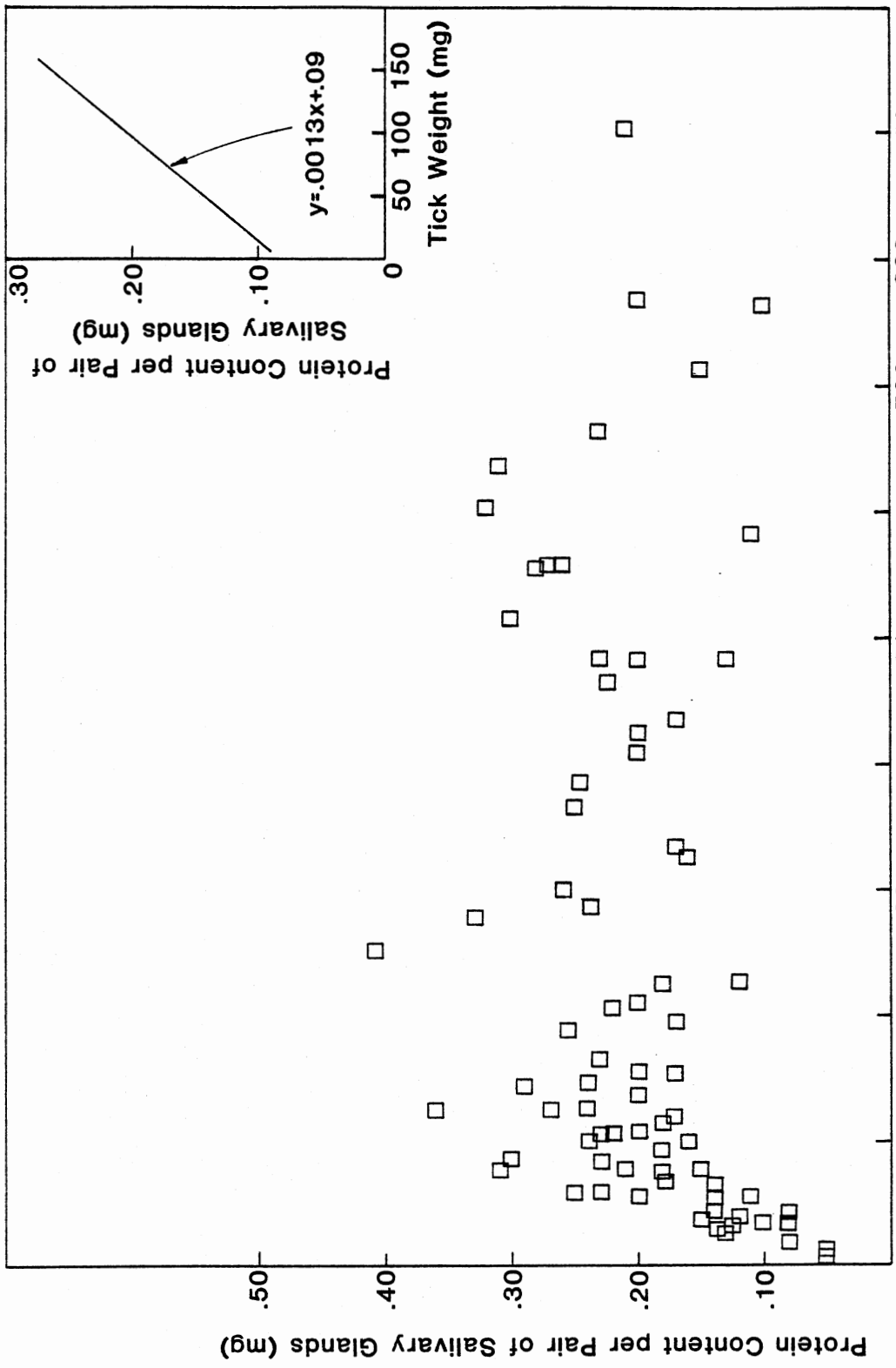
Pooled Gland Assays

Because salivary glands are known to undergo substantial growth and development during tick feeding (McSwain et al., 1982), it was important to estimate the protein content of salivary glands as a function of tick feeding to see if enzyme activity changed at rates different from changes in tissue protein. Preliminary adenylate cyclase assays included homogenates of three to four pairs of glands from low (≤150 mg) and high (>150 mg) weight ticks. Of the twenty-five pooled gland experiments, seven were of a mean weight of less than 150 mg. The mean total protein content of gland pairs from low weight ticks was 0.394±0.095 mg, and for high weight ticks it was 0.362±0.089 mg.

Single Gland Pair Assays

The protein content of a pair of salivary assayed individually, rather than as pooled glands from ticks of similar weight, showed an increase in total protein content from 0.006±0.001 mg/gland pair in unfed female ticks (n = 9) to 0.196±.06 mg/gland pair in ticks weighing less than 150 mg, the approximate tick weight at which maximum gland protein was first observed (Figure 1). Glandular protein remained relatively

Figure 1. Changes in Salivary Gland Pair Total Protein Content (mg) from Feeding Female Adult Ticks of Various Weights (mg). Insert shows the line and equation that describes the best estimate of the change in gland pair total protein content with change in tick weight to 150 mg.


Protein Content per Pair of Salivary Glands (mg)
.50
.40
.30
.20
.10
0
100
200
300
400
500
600
700
800
900
Tick Weight (mg)
Protein Content per Pair of Salivary Glands (mg)
.30
.20
.10
0
50 100 150
Tick Weight (mg)
y=.0013x+.09

constant at this level in heavier ticks (ca 150-800 mg). The positive slope of the line estimating the protein content of glands from low weight ticks differed significantly from the line estimating protein in glands of heavier ticks ($p < 0.001$). These results agree well with the earlier findings of McSwain et al. (1982) on the changes in protein content in salivary glands of feeding A. americanum. Glandular protein in replete ticks (detached from the host 12-24 hours earlier) did not vary significantly from that in glands of high weight feeding ticks.

Adenylate Cyclase Activity

Pooled Gland Assays

Schmidt et al. (1982a, b) have characterized adenylate cyclase in detail in salivary glands of feeding female A. americanum weighing 50-150 mg. Adenylate cyclase in glands from ticks at other stages of tick feeding (weight) has not been characterized. Therefore, "basal" and dopamine-stimulated adenylate cyclase was measured in salivary glands from ticks of all weights representative of the complete cycle of female feeding on sheep. Glands from at least three or four ticks were used to provide enzyme to test both "basal" and dopamine-stimulated activity in a pooled homogenate (Tables I and II). In all experiments dopamine-simulated activity was higher than "basal" activity. Because of the considerable variation in enzyme activities it was not possible to demonstrate statistically significant differences when comparing "basal" and dopamine-stimulated activities in low weight (Table I) to "basal" and dopamine-stimulated activities in high weight ticks (Table II) in these assays.

TABLE I

ADENYLATE CYCLASE ACTIVITY IN TICK SALIVARY GLANDS OF POOLED HOMOGENATES
LOW WEIGHT TICKS

Observation Number	Mean Tick Weight* ± S.D. (mg)	Basal Specific Activity (pmoles cAMP formed/min/mg protein)	Dopamine Specific Acyivity (pmoles cAMP formed/min/mg protein)	Mean Protein Content per Gland Pair (mg)
		Low Weight Ticks		
1	33.9 ± 7.3 (4)	90.7	230.8	0.218
2	77.4 ± 17.2 (3)	28.1	67.3	0.347
3	78.4 ± 3.2 (4)	71.7	145.2	0.407
4	113.5 ± 15.0 (4)	134.7	205.2	0.470
5	127.2 ± 8.2 (4)	53.7	100.1	0.427
6	133.1 ± 15.7 (3)	65.3	94.5	0.540
7	146.2 ± 27.6 (4)	39.3	148.3	0.347
8	156.3 ± 9.2 (3)	63.8	119.9	0.340
9	171.1 ± 38.9 (3)	47.7	143.6	0.180
$\bar{X}$ Low Weight Ticks (± S.D.)		66.1 ± 29.8	139.4 ± 49.4	0.364 ± 0.110

* Number of Gland pairs used in experiment indicated in parentheses.

TABLE II

ADENYLATE CYCLASE ACTIVITY IN TICK SALIVARY GLANDS
OF POOLED HOMOGENATES-HIGH WEIGHT TICKS

Observation Number	Mean Tick* Weight ±S.D. (mg)	Basal Specific Activity (pmoles cAMP fromed/min/ mg protein)	Dopamine Specific Activity	Mean Protein Content per Gland Pair (mg)
		High Weight Ticks		
10	225.05 ± 38.8 (4)	82.5	194.3	0.420
11	298.3 ± 48.13 (3)	64.5	135.0	0.215
12	358.3 ± 19.6 (4)	95.6	144.4	0.413
13	364.5 ± 41.3 (4)	127.9	218.7	0.300
14	377.4 ± 13.9 (3)	77.5	121.5	0.473
15	393.7 ± 16.3 (4)	84.1	140.9	0.346
16	525.1 ± 62.9 (3)	161.1	205.8	0.393
17	552.8 ± 26.0 (3)	97.8	192.5	0.370
18	581.2 ± 139.6 (3)	51.1	109.3	0.313
19	589.5 ± 45.5 (3)	87.4	127.4	0.260
20	592.2 ± 59.9 (4)	20.3	27.5	0.530
21	656.9 ± 88.1 (4)	47.7	132.1	0.387
22	695.2 ± 116.9 (4)	190.6	317.6	0.286
23	699.4 ± 62.6 (4)	29.5	32.8	0.361
24	774.8 ± 99.7 (3)	68.3	115.8	0.140
25	911.9 ± 130.2 (4)	115.6	120.4	0.327
$\bar{X}$ High Weight Ticks (± S.D.)		87.5 ± 43.6	146.0 ± 67.9	0.345 ± 0.090

*Number of gland pairs used in experiment indicated in parenthesis.

Single Gland Pair Assays

To obtain a better indication of enzyme activity in glands from a single tick, adenylate cyclase was measured in homogenates of salivary gland pairs dissected from individual ticks of various weights representative of the entire female feeding process. In addition, host effect on enzyme activities in salivary glands removed from ticks feeding on different ovine hosts were compared. "Basal" and dopamine-stimulated adenylate cyclase were negligible in salivary glands of unfed females (Table III). Subsequent to attachment to the host, the female tick begins to feed and a gradual weight gain occurs from ca 5 mg in the unfed tick to ca 20 mg on day seven post-attachment (Table III). Low levels of "basal" specific activity were detectable at this stage of feeding and interestingly, the dopamine-stimulated specific activity was of similar magnitude (Table III). The ability of dopamine to stimulate adenylate cyclase specific activity above "basal" activity is salivary gland homogenates increased markedly as tick feeding continued and as further increases in tick weight occurred (Figures 2, 3, 4, and 5).

When the specific activity of adenylate cyclase in salivary glands was analysed for a linear relationship over the tick weights tested, the estimated slopes of dopamine-stimulated and basal activity showed considerable variation within each treatment (Figures 2, 3, and 4). Figure 5 is a compilation of all results illustrated in Figures 2, 3, and 4. Adenylate cyclase activities in salivary glands were grouped according to two classes of tick weight from which glands were excised: low weight ($\leq$200 mg) and high weight (>200 mg) and according to the sheep on which the ticks fed. The tick weight at which maximum enzyme activity occurred

TABLE III

ADENYLATE CYCLASE ACTIVITY IN HOMOGENATES OF SALIVARY GLANDS FROM UNFED FEMALE TICKS AND FEMALE TICKS WHICH HAD FED ON AN OVINE HOST FOR SEVEN DAYS

Unfed			Attached Seven Days		
Basal Specific Activity (pmoles cAMP formed/min/mg protein)	Dopamine Specific* Activity	Mean Tick Weight (mg)	Basal Specific Activity (pmoles cAMP formed/min/mg protein)	Dopamine Specific* Activity	Mean Tick Weight (mg)
—	—	4.7 ± 0.5 (3)**	25.8 ± 3.2	28.4 ± 2.9	18.9 ± 1.5 (3)**

*Dopamine-Stimulated activity measured at 10^{-5}M dopamine.

**Number of experiments indicated in parenthesis. Error expressed as ±S.D.

Figure 2. Adenylate Cyclase Specific Activity in Salivary Gland Pair Homogenates from Feeding Female Adult Ticks of Various Weights Reared on Two Different Ovine Hosts, 06(A) and 16(B). Basal specific activity (●); dopamine-stimulated specific activity (▲). Predicted lines for change in specific activity with change in tick weight are shown; basal (———) and dopamine-stimulated specific activity (- - - -). Changes in salivary gland pair specific activity are illustrated for glands from low weight (≤200 mg) and high weight (>200 mg) ticks. Equations for lines of linear regression are listed in Table IV.

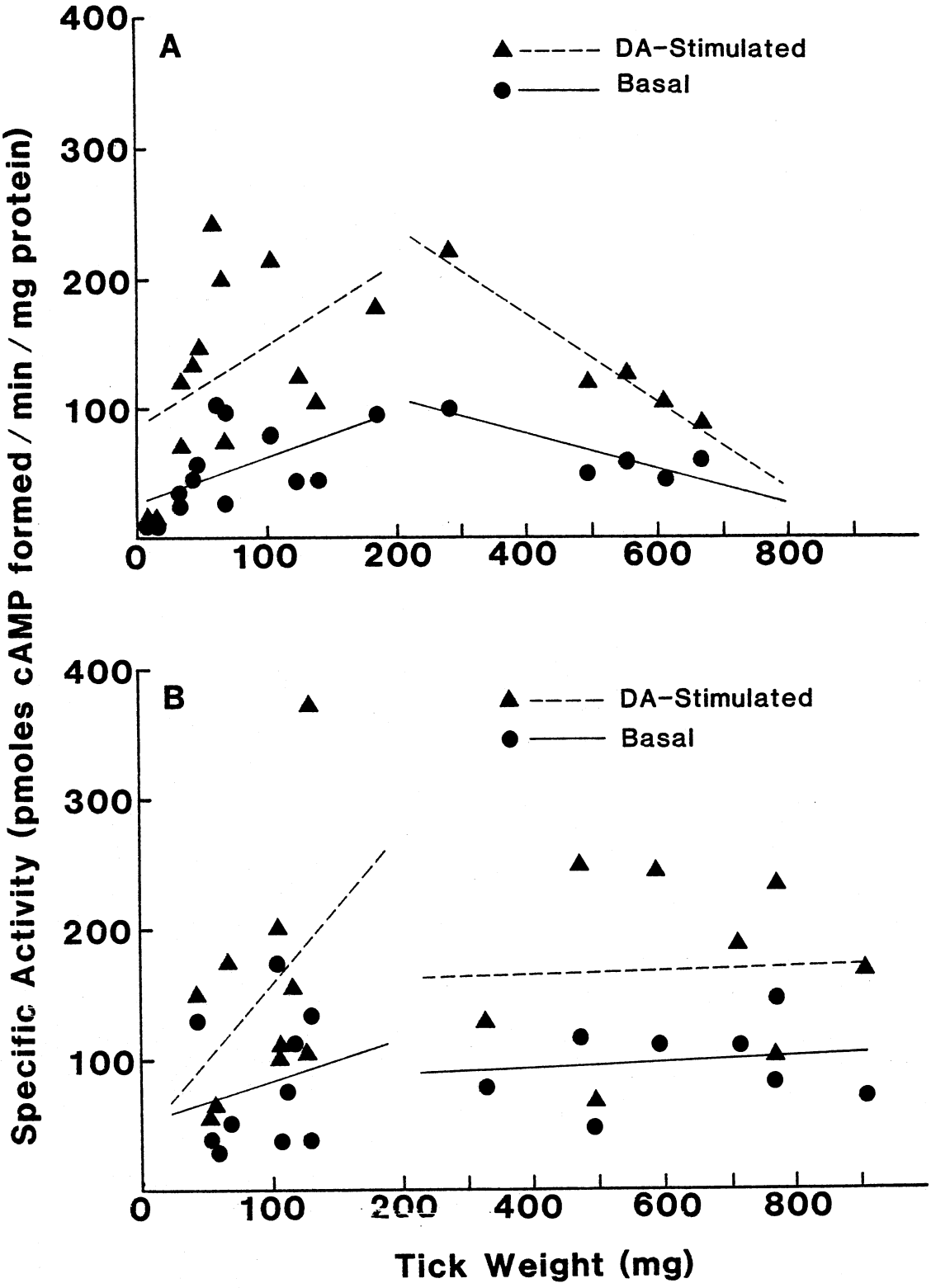

A
DA-Stimulated
Basal
400
300
200
100
0
100
200
400
600
800
B
DA-Stimulated
Basal
400
300
200
100
0
100
200
400
600
800
Specific Activity (pmoles cAMP formed / min / mg protein)
Tick Weight (mg)

Figure 3. Adenylate Cyclase Specific Activity in Salivary Gland Pair Homogenates from Feeding Female Adult Ticks of Various Weights Reared on Two Different Ovine Hosts, 74(A) and 58(B). Basal specific activity (●); dopamine-stimulated specific activity (▲). Changes in salivary gland pair specific activity are illustrated in glands from low weight (≤200 mg) and high weight (>200 mg) ticks. Predicted lines for change in specific activity with change in tick weight are shown; basal (——) and dopamine-stimulated (-----) specific activity. Equations for lines of linear regression are listed in Table IV. No salivary glands were analysed for enzyme activity from low weight ticks reared on ovine host 74(A).

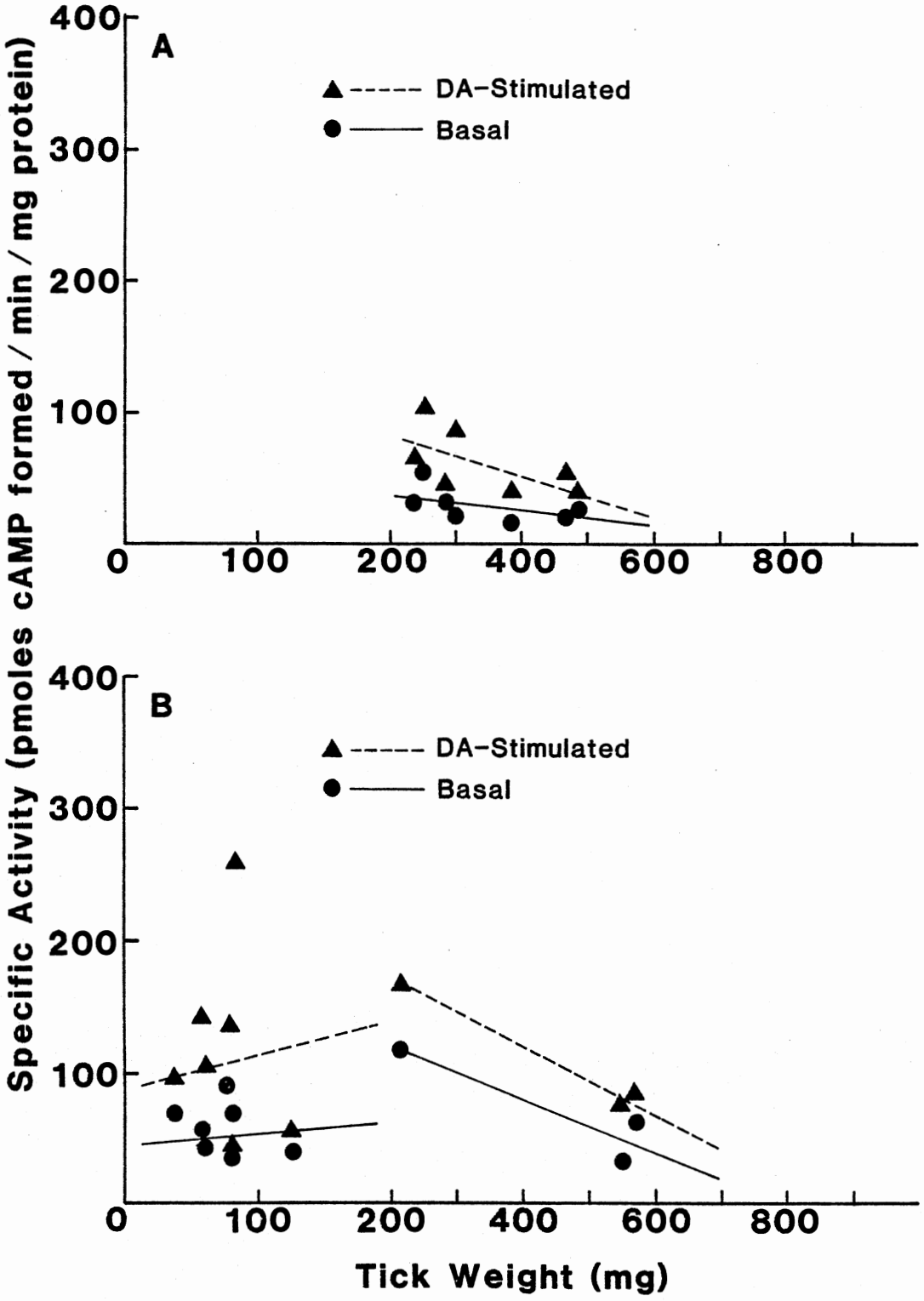

A
DA-Stimulated
Basal
400
300
200
100
0
100
200
400
600
800
B
DA-Stimulated
Basal
400
300
200
100
0
100
200
400
600
800
Specific Activity (pmoles cAMP formed / min / mg protein)
Tick Weight (mg)

Figure 4. Adenylate Cyclase Specific Activity in Salivary Gland Pair Homogenates from Feeding Female Adult Ticks of Various Weights Reared on Two Different Ovine Hosts, 73(A) and 95 (B). Basal specific activity (●); dopamine-stimulated specific activity (▲). Changes in salivary gland pair specific activity are illustrated in glands from low weight (≤200 mg) and high weight (>200 mg) ticks. Predicted lines for change in specific activity with change in tick weight are shown; basal (———) and dopamine-stimulated (-----) specific activity. Equations for lines of linear regression are listed in Table IV. Predicted lines for basal and dopamine-stimulated specific activity are not illustrated for high weight ticks reared on 73(A) due to a lack of representative tick gland pairs assayed.

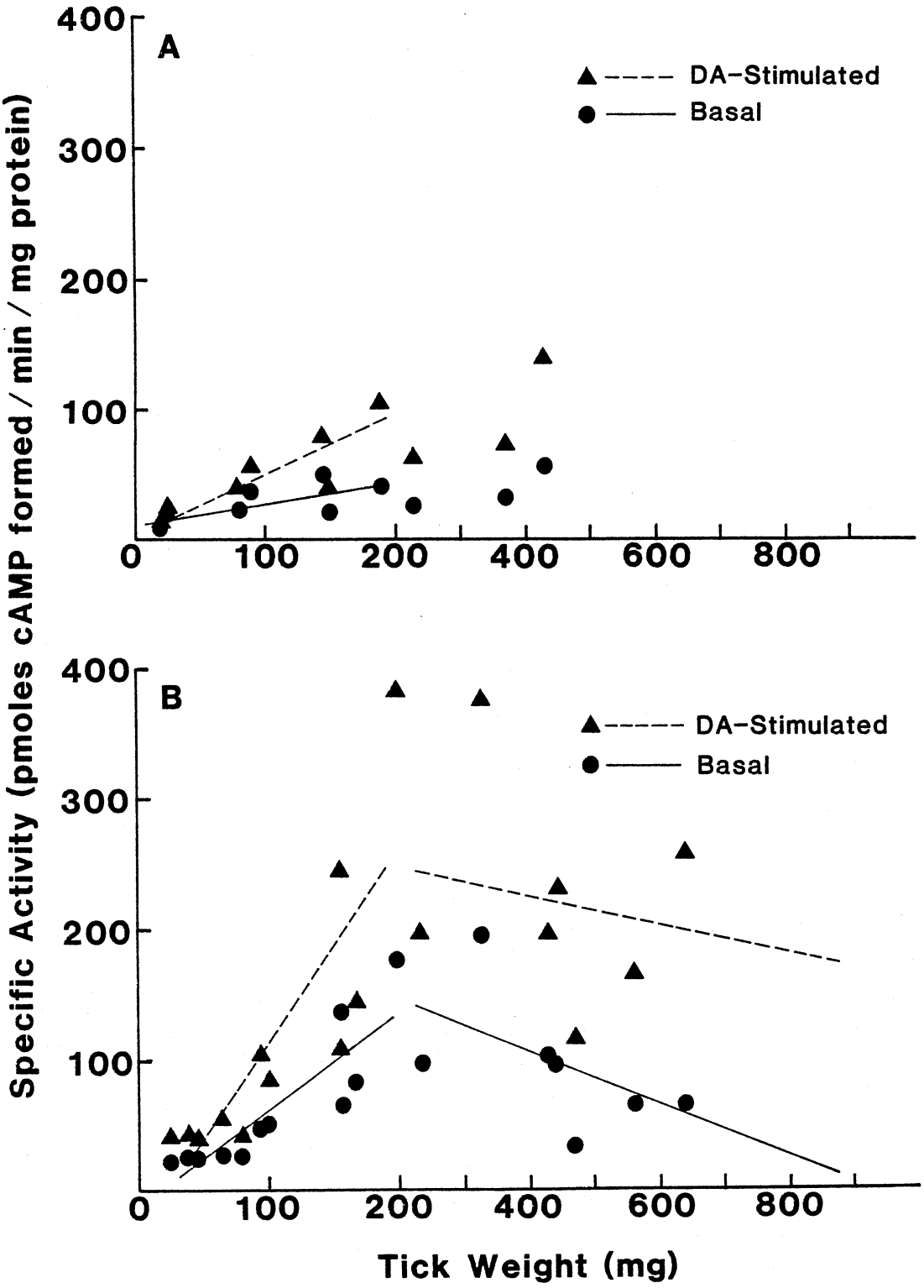
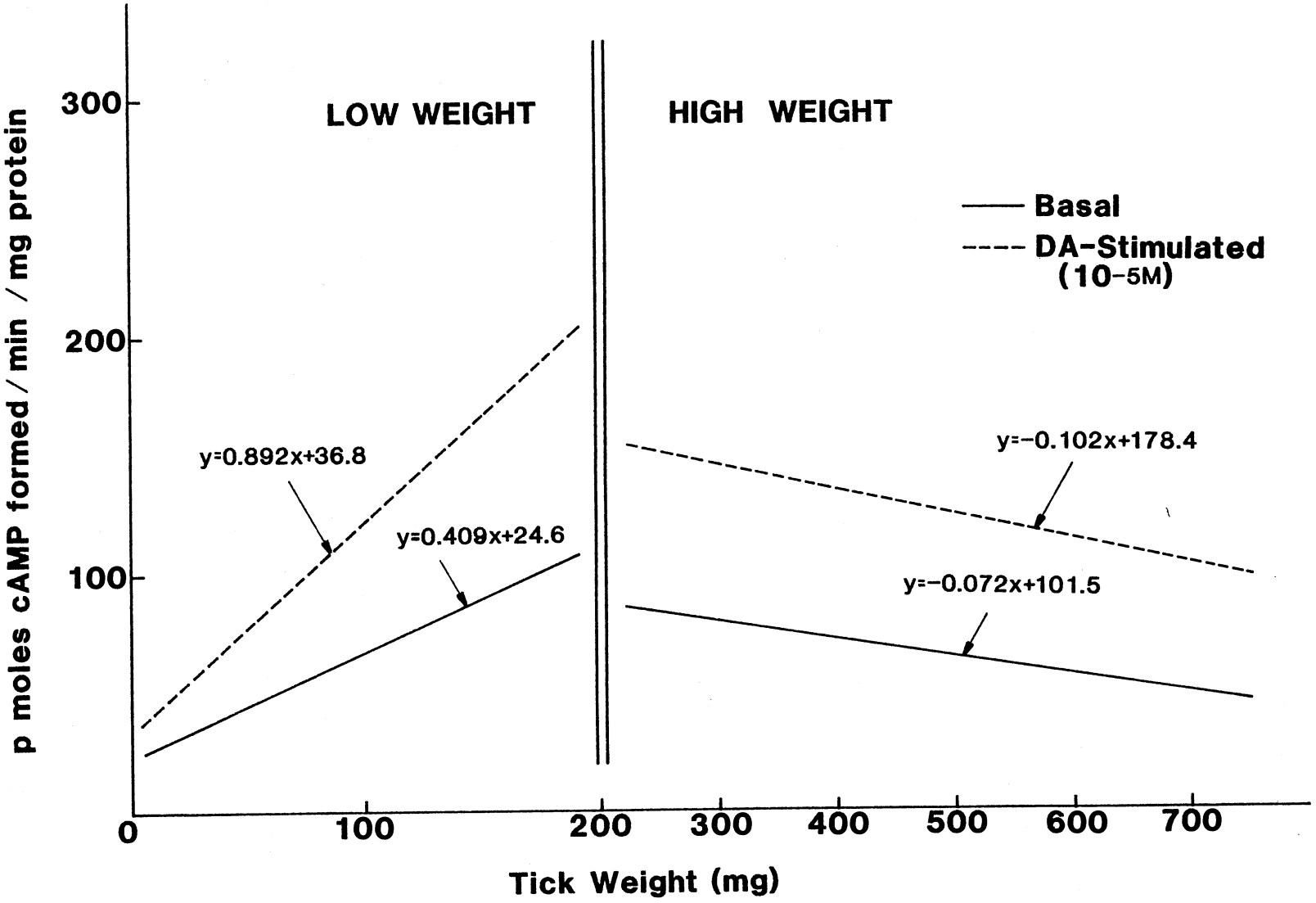

A
DA-Stimulated
Basal
400
300
200
100
0
100
200
400
600
800
B
DA-Stimulated
Basal
400
300
200
100
0
100
200
400
600
800
Specific Activity (pmoles cAMP formed / min / mg protein)
Tick Weight (mg)

Figure 5. Composite of Adenylate Cyclase Specific Activity in Salivary Gland Homogenates from Feeding Female Adult Ticks Reared on Six Different Ovine Hosts. Changes in salivary gland pair specific activity are illustrated for glands from low weight (≤200 mg) and high weight (>200 mg) ticks. Predicted lines and respective equations for change in specific activity with change in tick weight are shown, basal (——) and dopamine-stimulated (10^{-5}M) specific activity (-----).


LOW WEIGHT
HIGH WEIGHT
Basal
DA-Stimulated (10-5M)
y=0.892x+36.8
y=0.409x+24.6
y=-0.102x+178.4
y=-0.072x+101.5
p moles cAMP formed / min / mg protein
300
200
100
0
100
200
300
400
500
600
700
Tick Weight (mg)

appeared somewhat higher (200 mg) than the tick weight at which maximum gland protein was first observed (150 mg) although the considerable variation in results precluded an unambiguous statistical comparison of this apparent difference. Salivary glands from female ticks reared on six separate ovine hosts were tested for adenylate cyclase activity. Predicted lines which estimated the change in both "basal" and dopamine-stimulated specific activity with change in weight of the feeding tick from which the glands were removed were compared between the different hosts used. Slopes of the lines estimating the changes in enzyme activity, either "basal" or dopamine-stimulated, in salivary glands from low weight ticks reared on different hosts were not significantly different from one another. The same host-comparative relationship held between changes in enzyme activity in glands removed from high weight ticks (Table IV). In low weight ticks, both "basal" and dopamine-stimulated adenylate cyclase activity increased with increasing tick weight to 200 mg (Figure 5). Dopamine-stimulated activity increased more rapidly than "basal" activity ($p < 0.001$). Adenylate cyclase activity decreased in salivary glands taken from high weight ticks (Figure 5). Dopamine-stimulated activity was significantly greater than "basal" enzyme activity for ticks weighing more than 20 mg for each gland pair assayed ($p > 0.001$, paired t-test). Enzyme activity from ticks ($n = 15$) which had fed to repletion and detached from the host also showed that the dopamine-stimulated activity was significantly greater than the "basal" response ($p < 0.005$, paired t-test).

TABLE IV

COMPOSITE ANALYSIS OF ADENYLATE CYCLASE ACTIVITY IN SALIVARY GLAND HOMOGENATES FROM TICKS REARED ON VARIOUS SHEEP-SINGLE GLAND ASSAYS

		Linear Regression Analysis of Specific Activity vs. Tick Weight†			
		Low Weight Ticks (≤200 mg)		High Weight Ticks (>200 mg)	
Sheep No.	Treatment[Δ]	Slope*	Intercept**	Slope*	Intercept**
06	B	0.326 ± 0.180	28.2 ± 25.1 (13)	-0.133 ± 0.110	130.5 ± 95.4 (5)
	D	0.608 ± 0.370	88.5	-0.343 ± 0.270	308.0
16	B	.304 ± 0.350	52.7 ± 53.1 (10)	0.029 ± 0.070	77.7 ± 68.2 (8)
	D	1.178 ± .700	38.8	0.016 ± 0.130	155.4
58	B	0.093 ± 0.440	43.5 ± 51.0 (8)	-0.200 ± 0.120	159.5 ± 87.2 (3)
	D	0.242 ± 0.880	88.8	-0.260 ± 0.230	223.3
73	B	0.159 ± 0.210	10.9 ± 39.3 (7)	0.102 ± 0.230	4.6 ± 122.4 (3)
	D	0.430 ± 0.430	6.8	0.286 ± 0.460	-3.9
74	B	—	—	-0.064 ± 0.140	48.6 ± 76.6 (7)
	D	—	—	-0.162 ± 0.270	117.2
95	B	0.747 ± 0.180	-12.8 ± 33.0 (11)	-0.208 ± 0.100	188.2 ± 74.4 (7)
	D	1.528 ± 0.360	-36.8	-0.102 ± 0.080	178.4
All Sheep	B	0.409 ± 0.100	24.6 40.2 (49)	-0.072 ± 0.040	101.5 ± 87.4 (3)
	D	0.892 ± 0.200	36.8	-0.102 ± 0.080	178.4

[Δ]Treatments are: B = Basal; D = Dopamine-stimulated activity (10^{-5}M).

†Regression of specific activity (pmoles cAMP formed/min/mg protein) on tick weight (mg). The number of pairs of tick salivary glands used in regression analysis are indicated in parentheses. Error expressed as ±S.D.

*pmoles cAMP formed/min/mg protein/mg tick weight.

**pmoles cAMP formed/min/mg protein.

Host Effect on Salivary Gland Adenylate Cyclase Activity

There is a well documented effect of previous host exposure to feeding ticks affecting the growth and development of subsequent tick feeding and final engorgement weight (McGowan et al., 1981). McGowan et al. (1981) have shown that cattle immunized with extracts of Amblyomma americanum (L.) significantly reduced the adult female tick final engorgement weight of ticks which fed on these cattle. A marked effect on average "basal" and dopamine-stimulated adenylate cyclase activity was seen when comparing activity in salivary glands of ticks which were reared on hosts with no prior exposure to ticks in the laboratory to that of adenylate cyclase activity of ticks reared on hosts with previous exposure to ticks in the Laboratory (Table V). However, percent stimulation of adenylate cyclase by dopamine over "basal" activity remained consistent (Table V).

TABLE V

EFFECT ON PREVIOUS EXPOSURE OF HOST TO TICKS ON SALIVARY GLAND ADENYLATE CYCLASE ACTIVITY

Sheep No.	Mean Enzyme Activity* Basal (pmoles cAMP formed/min/mg protein)	Mean Enzyme Activity* DA-Stimulated (pmoles cAMP formed/min/mg protein)	Percent DA-Stimulation Above Basal	Previous Exposure of Host to Ticks in the Laboratory	Mean Replete Tick Weight ±S.D.** (mg)
6	53.9 ± 29.9	131.1 ± 65.9 (18)	143	0	692 ± 113 (33)
16	80.6 ± 45.0	154 ± 76.9 (18)	92	0	698 ± 125 (61)
95	75.7 ± 52.0	156.2 ± 107.2 (18)	106	0	620 ± 153 (20)
58	55.6 ± 28.2	106.2 ± 65.3 (11)	91	1	598 ± 56 (3)
73	30.1 ± 14.0	61.8 ± 35.8 (10)	105	2	331 ± 107 (5)
74	26.6 ± 12.4	61.5 ± 25.2 (7)	131	2	548 ± 47 (4)

*Number of pairs of glands assayed to obtain average enzyme activity indicated in parentheses. DA-Stimulated is activity assayed with dopamine (10^{-5}M). Error expressed ± S.D.

**Number of ticks used to obtain average replete tick weight indicated in parentheses.

CHAPTER IV

DISCUSSION AND CONCLUSIONS

Results of the present study of adenylate cyclase in ixodid female salivary glands indicate: 1) Little adenylate cyclase activity is present in salivary glands of unfed females. 2) Adenylate cyclase activity increases in salivary glands following tick attachment to a host but the ability of dopamine to stimulate the enzyme significantly does not occur until after a considerable period of slow tick feeding and possibly mating. The latter hypothesis remains to be tested. 3) Enzyme activity increases in glands as the tick continues to feed reaching a peak at a tick weight of ca 200 mg; activity then declines as the tick continues to feed. 4) Dopamine-stimulated adenylate cyclase activity changes at rates different from changes in "basal" activity. 5) Enzyme activity changes at rates different from changes in total protein content of the glands. 6) There is a host effect on enzyme activity, much of which can be attributed to prior exposure of the host to ticks in the present experiments.

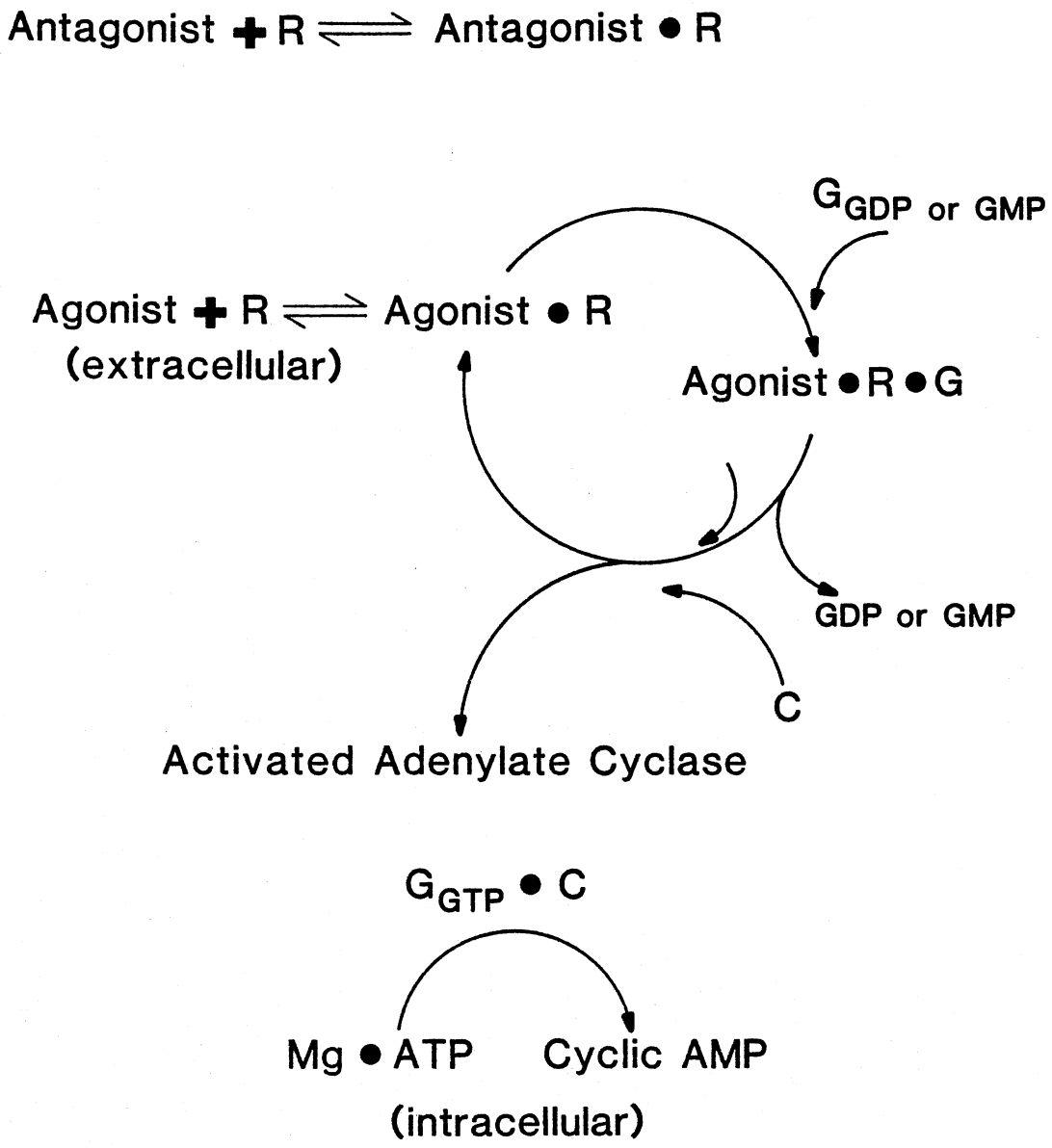
Hormonally responsive adenylate cyclase is a multi-component system consisting of three physically distinct proteins which "float" freely within the phospholipid bilayer of the plasma membrane (Ross and Gilman, 1980). Oriented toward the outer membrane is the receptor subunit (R) which has a specific binding site for hormones or neurotransmitters. Other subunits include the guanine nucleotide-binding regulatory protein

(G) and the catalytic unit (C) at the inner face of the membrane, which converts substrate ATP to cyclic AMP (Rodbell, 1980). Hormone binding to the receptor promotes dissociation of GMP or GDP and the subsequent binding of GTP to the G-protein. Binding of the GTP to the G-protein promotes its association to the catalytic moiety and the activation of adenylate cyclase (Pfeuffer, 1979). Both agonists (hormones or neurotransmitters) and antagonists bind to receptors, but only agonists promote association of receptors to the G-protein (Limbird et al., 1980). A GTP-ase associated with the G-protein controls the "off" rate of the activated state of the enzyme by the hydrolysis of the bound GTP to GDP and the subsequent dissociation of G_{GDP} and C (Figure 6) (Pfueffer, 1979). Guanine nucleotides decrease the affinity of receptor subunit for agonists (but not antagonists) by accelerating the dissociation of the receptor-agonist complex (Limbird et al., 1980).

Adenylate cyclase activity in tick salivary glands was found in feeding ticks weighing greater than 20 mg. Low, but similar levels of "basal" and dopamine-stimulated adenylate cyclase activity was detected in salivary glands from ticks which had attached to the host and fed for seven days. Enzyme activity in unfed females was negligible. McSwain et al. (1982) reported that in the absence of mating, ticks will not feed to repletion and increase in weight greater than 27±4mg. Mating appears to occur within five to seven days after the female tick has attached to a host (McSwain et al., 1982).

There is no detailed description of copulation in ixodid ticks but males can attach to the host ventrally adjacent to the female, copulate and detach from the host and mate with as many as 37 different females (Gladney and Drummond, 1970). Behavioral and anatomical evidence suggest

Figure 6. Components and Activation Process of the Adenylate Cyclase System as Described by Limbird et al. (1980). Both agonists and antagonists bind to receptor (R) with high affinity and specificity but only agonists are capable of promoting association of the GTP-binding protein (G) with the receptor. When GTP occupies the G-protein the affinity of the agonist for the receptor is reduced and the catalytically active form of adenylate cyclase (CG_{GTP}) is created. Intracellular cyclic AMP formation continues until the bound GTP is hydrolized to GDP or GMP.


Antagonist + R ⇌ Antagonist • R
G GDP or GMP
Agonist + R ⇌ Agonist • R
(extracellular)
Agonist • R • G
GDP or GMP
C
Activated Adenylate Cyclase
G GTP • C
Mg • ATP
Cyclic AMP
(intracellular)

that a period of sexual maturation is induced by feeding and is necessary before male A. americanum become sexually active (Haggart and Davis, 1981). Male ticks begin to respond to a sex pheromone, 2-6-dichorophenol only after about seven days of feeding (Berger et al., 1971). Electrophysiological data implicated changes in the tick central nervous system occurred which controled the probable physiological mechanisms related to tick attraction and mating (Haggart and Davis, 1981). A nerve branching from the lateral plexus, part of the peripheral nervous system in ixodid ticks, innervates the posterior region of the salivary gland in the ixodid tick, Boophilus microplus (Binnington, 1978). As the nerve reaches the gland, it divides further innervating lobular ducts and gland acini ending in varicose nerve endings in the gland (Binnington, 1981). The anterior region of the gland is innervated by a branch of the palpal nerve (Binnington and Tatchell, 1973). There are lateral segmental organs associated with the lateral plexus which innervates the salivary glands and it was postulated that they function as neurohemal organs (Obenchain and Oliver, 1975; Panfilova, 1978). Recent ultrastructural studies have shown that these are not neurohemal terminals and that the organs probably function as true endocrine glands (Binnington, 1981). Sexual behavior and mating appear to be, in part, controlled by nerves after initiation via pheromone-mediated attraction of males to females. Copulation appears to initiate a multitude of physiological mechanisms in the female during engorgement. Copulation may provide the stimulus leading to and ultimately contribute to the expression of adenylate cyclase activity in the salivary glands of feeding female lone star ticks. Alternately, the increased feeding induced by copulation may provide the primary signal for increased enzyme activity.

Dopamine-stimulated adenylate cyclase specific activity changed at rates different from "basal" activity (Figure 1). The increase in "basal" activity may be due to an increase in endogenous activators such as tissue dopamine, or an increased ability of GTP to activate adenylate cyclase system in the protein components of the adenylate cyclase system in the salivary gland membranes as the tick feeds and develops as an adult. Cytoplasmic factors modulate hormonal stimulation of adenylate cyclase in many different systems (Beaumont et al., 1979; MacNeil et al., 1980). MacNeil et al. (1980) tested a variety of species for cytosolic activating factors of adenylate cyclase and found no evidence of species specificity as cytoplasm stimulated adenylate cyclase activity from the same and unrelated tissue to the same degree. The nature of these variously described protein activators present in the cytosol and whether they act by binding GTP or act at the same site as GTP is unknown (MacNeil et al., 1980). It will be of interest to determine the nature of the activator(s) present in the cytosol from salivary glands of ticks weighing 50-150 mg (Schmidt et al., 1982a) and test for their presence or absence throughout the feeding process. These activator(s) may contribute to the increased ability of dopamine to stimulate the tick salivary gland adenylate cyclase. Phospholipids are known to play an important role in the integrity of cell membranes and undoubtedly contribute to a functional adenylate cyclase system (Ross and Gilman, 1980). The involvement of phospholipids in hormone-responsive adenylate cyclases in various tissues has been studied in many laboratories (see Anand-Srivastava and Johnson, 1981) and may provide the link between the receptor and transducing subunits (G-protein) of the adenylate cyclase system (Anand-Srivastava and Johnson, 1981).

Maximum protein content of the salivary glands was attained at a tick weight (150 mg) lower than the tick weight at which the apparent maximum enzyme specific activity was attained (200 mg), but maximum fluid secretory capability in whole *in vitro* salivary gland preparations occurred (ca 400 mg) (Sauer et al., 1979). Salivary glands from high weight ticks show a decrease in adenylate cyclase specific activity as ticks undergo the rapid stage of feeding and maximally secrete fluid back into the host. Cellular fractionation studies have shown the existence of a stage-specific, heat stable inhibitor of adenylate cyclase activity in the slime mold *Dictyostelium discoideum* (Cripps and Rutherford, 1981). The cellular slime mold aggregates in response to the chemotactic stimulus of cyclic AMP when food is exhausted or removed (Konijn et al., 1967; Barkley, 1969). The cells then form migrating psuedoplasmodia which subsequently differentiate into fruiting bodies. Cyclic AMP concentrations have been shown to oscillate coincidental to adenylate cyclase activity (Roos et al., 1977). These changes were not due to changes in available ATP levels; rather, they seemed related to regulation of the catalytic function of the enzyme (Cripps and Rutherford, 1981). The inhibitor appeared in cells which exhibited high cyclic AMP concentration, a time when high adenylate cyclase activity would be expected. This inhibitor appeared only in stages where adenylate cyclase activity was low and when cyclic AMP is thought to play a key role in slime mold aggregation (Cripps and Rutherford, 1981). In tick salivary glands, fluid secretory ability is at a maximum in the rapidly feeding, high weight tick and cyclic AMP is also thought to play a key role in the fluid transport process during this tick developmental phase (Sauer et al., 1979). The presence of a probable cytosolic inhibitor in salivary glands of high weight ticks

(S. Schmidt, personal communication) may account for the decrease in adenylate cyclase activity in ticks of this weight and may be a needed regulatory factor on overall concentration of cyclic AMP as the phosphodiestrase activity of salivary glands in this weight of ticks is at its lowest level (McMullen and Sauer, 1978). It should be interesting to further characterize the physical characteristics of this inhibitor of salivary glands throughout the feeding process and determine if it is a general or a specific inhibitor of enzyme activity.

The decrease in the average adenylate cyclase activity seen in the salivary glands of ticks reared on hosts which had been previously exposed to tick infestation in the laboratory coincides with the reduced engorgement weight of ticks which had fed on these "pre-exposed" hosts. The reduced enzyme rate may demonstrate the importance of adenylate cyclase as an indicator of the physiological growth and development of ixodid ticks as they feed as adults.

REFERENCES CITED

Anand-Srivastava, M. B. and R. A. Johnson. 1981. Role of phospholipids in coupling of adenosine and dopamine receptors to striatal adenylate cyclase. J. Neurochem. 36(5): 1819-1828.

Balashov, Y. S. 1972. A translation of blood sucking ticks (Ixodoidea) Vectors of diseases of man and animals. Misc. Publ. Entomol. Soc. Am. 8: 161-376.

Bar, H-P, and P. Hahn. 1971. Development of rat liver adenyl cyclase. Canadian J. Biochem. 49(1): 85-89.

Barr, A. J., J. H. Goodnight, J. P. Sall, W. H. Blair, and D. M. Chilko. 1979. SAS user's guide--1979 edition Raleigh, North Carolina: SAS Institute, Inc.

Barkley, D. S. 1969. Adenosine-3', 5'- phosphate: Identification as acrasin in a species of cellular slime mold. Science 165: 1133-1134.

Beaumont, C., J-P. Piau, S. Fischer, J. Delaunay, and G. Schapira. 1979. Stimulation of erythroid cells adenylate cyclase by soluble factors. Biochem. Biophys. Res. Comm. 91(4): 1250-1257.

Berger, R. S., J. C. Dukes, and Y. S. Chow. 1971. Demonstration of a sex pheromone in three species of hard ticks. J. Med. Entomol. 8: 84-864.

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

Binnington, K. C. 1981. Innervation of coxal muscles, heart and other organs in the cattle tick, Boophilus microplus Canestrini (Acarina: Ixodidae). Int. J. Insect Morphol. and Embryol. 10(2): 109-119.

Binnington, K. C. and B. F. Stone. 1977. Distribution of catecholamines in the cattle tick Boophilus microplus. Comp. Biochem. Physiol. 58C: 21-28.

Binnington, K. C. and R. J. Tatchell. 1973. The nervous system and neurosecretory cells of Boophilus microplus (Acarina: Ixodidae). Z. Wiss. Zool. Abt. A. 185: 193-206.

Bodnaryk, R. P. 1978. Levels of brain cyclic AMP and cyclic GMP during the initiation of adult development in the bertha army worm Mamestra configurata (WLK). Insect Biochem. 8: 383-387.

Braun T. and O. Hechter. 1970. Glucocorticoid regulation of ACTH sensitivity of adenyl cyclase in rat fetal membranes. Proc. Natl. Acad. Sci. U.S. 66: 995-1001.

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

Cripps, M. M. and C. L. Rutherford. 1981. A stage-specific inhibitor of adenylate cyclase in Dictyostelium discoideum. Exp. Cell Res. 133: 309-316.

Davoren, P. R. and E. W. Suterhland. 1963. The cellular location of adenylate cyclase in the pigion erythrocyte. J. Biol. Chem. 238: 3016-3023.

Gladney, W. J. and R. O. Drummond. 1970. Mating behavior and reproduction of the lone star tick, Amblyomma americanum (L.). Ann. Entomol. Soc. Amer. 63(4): 1036-1039.

Gregson, J. D. 1967. Observations on the movement of fluids in the vicinity of the mouthparts of naturally feeding Dermacentor andersoni: Stiles. Parasitol. 57: 1-8.

Greengard, O. 1971. Enzymic differentiation in mammalian tissues. Essay Biochem. 7: 159-205.

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

Kaufman, W. R. 1976. The influence of various factors on fluid secretion by in vitro salivary glands of ixodid ticks. J. Exp. Biol. 64: 727-42.

Kaufman, W. R., P. A. Diehl, and A. A. Aeschlimann. 1976. Na, K-ATPase in the salivary gland to the ixodid tick Amblyomma hebraeum (Koch) its relation to the process of fluid secretion. Experientia 32: 986-87.

Kaufman, W. R. and J. E. Phillips. 1973a. Ion and water balance in the ixodid tick Dermacentor andersoni. I. Routes of ion and water secretion. J. Exper. Biol. 58: 523-536.

Kaufman, W. R. and J. E. Phillips. 1973b. Ion and water balance in the Ixodid tick Dermacentor andersoni. II. Mechanism and Control of salivary secretion. J. Exper. Biol. 58: 537-547.

Kaufman, W. R. and J. E. Phillips. 1973c. Ion and water balance in the ixodid tick Dermacentor andersoni. III. Influence of monovalent ions and osmotic pressure on salivary secretion. J. Exper. Biol. 58: 549-564.

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

Knoijn, T. M., T. G. C. Van de Meene, J. T. Bonner, and D. S. Barkley. 1967. The acrasin activity of adenosine-3', 5' cyclic phosphate. Proc. Natl. Acad. Sci. U.S. 58(1): 1152-1154.

Limbird, L. E., D. M. Gill, and R. J. Lefkowitz. 1980. Agonist-promoted coupling of the β-adrenergic receptor with the guanine nucleoticle regulatory protein of the adenylate cyclase system. Proc. Natl. Acad. Sci. U.S. 77(2): 775-779.

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

McGowan, M. J., R. W. Barker, J. T. Homer, R. W. McNew, and K. H. Holscher. 1981. J. Med. Entomol. 18(4): 328-332.

McGowan, M. J., R. W. Barker, J. T. Homer, R. W. McNew, and K. H. Holscher. 1981. Success of tick feeding on calves immunized with Amblyomma americanum (Acari: Ixodidae) extract. J. Med. Entomol. 18(4): 328-332.

McMahon, D. 1974. Chemical messengers in development: a hypothesis. Science. 185: 1012-1021.

MacNeil, S., A. Crawford, H. Amirrasooli, S. Johnson, A. Pollock, C. Ollis, and S. Tomlinson. 1980. Stimulation of hormone-responsive adenylate cyclase activity by a factor present in the cytosol. Biochem. J. 188: 393-400.

McMullen, H. and J. R. Sauer. 1978. The relationship of phosphodiesterase and cyclic AMP to the process of fluid secretion in the salivary glands of the ixodid tick Amblyomma americanum. Experientia. 34: 1030-1031.

McMullen, H., J. R. Sauer, and R. L. Burton. 1976. Possible role in uptake of water vapor by ixodid tick salivary glands. J. Insect. Physiol. 22: 1281-1285.

McSwain, J. L., R. C. Essenburg, and J. R. Sauer. 1982. Protein changes in the salivary glands of the feeding female lone star tick, Amblyomma americanum, during feeding. J. Parasitol. (in press).

Megaw, M. J. W. 1976. Structure and function of the salivary glands of the tick, Boophilus microplus Canestrini (Acarina: Ixodidae). Int. J. Insect Morph. and Empryol. 8(2): 67-83.

Megaw, M. J. W. and H. A. Robertson. 1974. Dopamine and noradrenaline in the salivary glands and brain of the tick Boophilus microplus. Effect of reserpine. Experientia. 30: 1261-1262.

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

Nakai, C. and G. Brooker. 1975. Assay for adenylate cyclase and cyclic nucleoticle phosphodiesterases and the preparation of high specific activity ^{32}-P labeled substrates. Biochem. Biophys. Acta. 391: 222-239.

Needham, G. R. and J. R. Sauer. 1975. Control of fluid secretion by isolated salivary glands of the lone star tick. J. Insect. Physiol. 21: 1893-1898.

Nijjar, M. S. 1979. Regulation of rat lung adenylate cyclase by cytoplasmic factor(s) during development. Biochim. Biophys. Acta. 548: 43-50.

Novak, E., G. I. Drummond, J. Skada, and P. Hahn. 1972. Developmental changes in cyclic AMP, protein kinase, phosphorylase kinase and phosphorylase in liver, heart and skeletal muscle of the rat. Arch. Biochem. Biophys. 150: 511-518.

Obenchain, F. D. and J. H. Oliver. 1975. Neurosecretory system of the American dog tick Dermacentor variabilis (Acari: Ixodidae). II. Distribution of secretory cell types. axonal pathways and putative neurohemal-neuroendocrine associations; comparative histological and anatomical implications. J. Morph. 145: 269-294.

Palmer, G. C., M. J. Schmidt, and G. A. Robison. 1972. Development and characteristics of the histamine-induced accumulation of cyclic AMP in the rat cerebral cortex. J. Neurochem. 19: 2251-2256.

Panfilova, I. M. 1978. Lateral organs in Ixodes persulcatus (Parasitiformes, Ixodidae) Zool. Zh. 57: 190-196.

Patrick, C. D. and J. A. Hair. 1976. Laboratory rearing proceedures and equipment for multi host ticks (Acarina:Ixodidae). I. Med. Entomol. 12: 389-390.

Pfeuffer, T. 1979. Guanine nucleoticle-controlled interactions between components of adenylate cyclase. FEBS Lett. 101: 85-89.

Rasenick, M. M., J. Neuburg, and S. J. Berry. 1976. Brain cyclic AMP levels in the initiation of adult development in the cecropia silkmoth. J. Insect Physiol. 22: 1453-1456.

Robison, G. A., R. W. Butcher, and E. W. Sutherland. 1971. Cyclic AMP. Academic Press, New York.

Rodbell, M. 1980. The role of hormone receptors and GTP-regulatory proteins in membrane transduction. Nature. 284: 17-22.

Roos, W., C. Scheidegger, and G. Gerisch. 1977. Adenylate cyclase activity oscillations as signals for cell aggregation in Dictyostelium discoideum. Nature (Lond.) 266: 259-261.

Ross, E. M. and A. G. Gilman. 1980. Biochemical properties of hormone sensitive adenylate cyclase. Ann. Rev. Biochem. 49: 533-564.

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

Sauer, J. R. 1977. Acarine salivary glands--Physiological relationships. J. Med. Entomol. 14: 1-9.

Sauer, J. R., J. H. Frick, and J. Hair. 1974. Control of ^{36}Cl uptake by isolated salivary glands of the lone star tick. J. Insect Physiol. 20: 1771-1778.

Sauer, J. R., G. R. Needham, H. L. McMullen, and R. D. Morrison. 1979. Cyclic nucleotides, calcium and salivary secretion in ixodid ticks. Recent Adv. in Acarology. 1: 365-373.

Schmidt, M. J., E. C. Palmer, W. Dettbarn, and G. A. Robison. 1970. Cylcic AMP and adenyl cyclase in the developing rat brain. Develop. Psychobiol. 3(1): 53-67.

Schmidt, S. P., R. C. Essenburg, and J. R. Sauer. 1982a. A dopamine-sensitive adenylate cyclase in the salivary glands of Amblyomma americanum (L.) Comp. Biochem. Physiol. (in press).

Schmidt, S. P., R. C. Essenburg, and J. R. Sauer. 1982b. Evidence for a D_1 dopamine receptor in the salivary glands of Amblyomma americanum (L.) (in preparation).

Sutherland, E. W., G. A. Robison, and R. W. Butcher. 1968. Some aspects of the biological role of adenosine 3',5'-monophosphate (cyclic AMP) Circulation. 37: 279-306.

Tatchell, R. J. 1967. Salivary secretion in the cattle tick as a means of water elimination. Nature (Lond.). 213: 940-941.

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

VITA

Mary Louise Schramke

Candidate for the Degree of

Master of Science

Thesis: CHANGES IN DOPAMINE-SENSITIVE ADENYLATE CYCLASE ACTIVITY IN SALIVARY GLANDS OF FEEDING FEMALE LONE STAR TICKS, AMBLYOMMA AMERICANUM (L.)

Major Field: Entomology

Biographical:

Personal Data: Born in Saginaw County, Saginaw, Michigan, January 11, 1955, the daughter of Frank and Lorraine Schramke.

Education: Graduated from Stevenson High School, Sterling Heights, Michigan, June, 1973; received the Bachelor of Arts degree from Kalamazoo College, Kalamazoo, Michigan, with a major in Biology June, 1977; completed the requirements for the Master of Science degree at Oklahoma State University in May, 1982.

Professional Experiences: Assistant to Director of Laboratory Operations, International Research and Development Corporation, Mattawan, Michigan, October, 1977, to March, 1978; Research Assistant, Peregrine Falcon Survey Team, Gila National Forest, New Mexico, U.S. Forest Service-Chihuahuan Desert Research Institute Cooperative Study, April to July, 1978; Laboratory Technician II, Animal Science Department, Oklahoma State University, Stillwater, Oklahoma, September, 1978, to June, 1979; Laboratory Technician II, Department of Entomology, Oklahoma State University, Stillwater, Oklahoma, July, 1979, to December, 1980; Research assistant, Department of Entomology, Oklahoma State University, Stillwater, Oklahoma, January, 1981, to January, 1982.

Societies: Entomological Society of America.