PHOSPHOLIPASE A_2 ACTIVITY IN THE SALIVARY GLANDS OF THE LONE STAR TICK *Amblyomma americanum* (L.) (ACARINA: IXODIDAE)

BY

MARK R. SURDICK

Bachelor of Science

in Arts and Science

Oklahoma State University

Stillwater, Oklahoma

1984

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
July, 1991

PHOSPHOLIPASE A_2 ACTIVITY IN THE SALIVARY GLANDS OF THE LONE STAR TICK *Amblyomma americanum* (L.) (ACARINA: IXODIDAE)

Thesis Approved:

Jack W. Dillwith
Thesis Adviser

John R. Fann

Richard Berberet

Richard C Essenberg

Norman N Durham
Dean of the Graduate College

ACKNOWLEDGEMENTS

I would like at this time to recognize those people who have actually been of assistance to me in carrying out this study: Dr. Jack Dillwith, Dr. John Sauer, Dr. Richard Essenberg, Dr. Janet McSwain, Dr. Douglas Bergman, Mr. Mike Shipley, and Mr. Jim Tucker.

Also , I would like to thank my parents for their patient and very generous support, both financial and moral. Were it not for them, I would have had to give up on this graduate program a long time ago.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Figure Page

CHAPTER I

INTRODUCTION

Amblyomma americanum (L.), the lone star tick, is a serious pest of man and livestock. This species of tick is very widespread geographically and has been known to transmit Lyme disease (Schulze *et al.*, 1984).

The lone star tick, like other ixodid ticks, possesses certain adaptations to allow it to parasitize its mammalian hosts without interference from the host's defenses. It has been speculated that eicosanoids such as prostaglandin E_2 and prostacyclin in tick saliva suppress the immune and inflammatory responses of the host (Ribeiro *et al.*, 1985). Prostaglandin E_2 has already been found in the saliva of *Ixodes dammini* Spielman (Ribeiro *et al.*, 1988), *Boophilus microplus* (Canestrini) (Dickinson *et al.*, 1976), *Hyalomma anatolicum excavatum* (Koch) (Shemesh *et al.*, 1979), and *Amblyomma americanum* (Ribeiro *et al.*, personal communication).

Eicosanoids are derivatives of arachidonic acid and other 20-carbon polyunsaturated fatty acids, which are usually released from cellular phospholipids by the action of phospholipase A_2 (PLA_2) enzymes. Phospholipase A_2 activity regulates the overall production of eicosanoids, and it is

the rate-limiting step in eicosanoid biosynthesis (Flower and Blackwell, 1976). The purpose of this study was to determine if phospholipase A_2 activity exists in A. americanum salivary glands and to study the properties of this activity if found. This study was the first one to examine the activity of these eicosanoid precursor-releasing enzymes in ticks. Objectives of this study were:

1. Determine if PLA_2 activity is present in tick salivary glands

2. Characterize tick salivary gland PLA_2 activity, if found, in terms of:
   A. Optimal incubation time.
   B. Effect of salivary gland protein concentration.
   C. Calcium requirement.
   D. Optimum pH.
   E. Optimum incubation temperature.
   F. Metal ion effects.
   G. Substrate specificity.
   H. Subcellular location of the enzyme

3. Compare activity of PLA_2 in fresh and frozen salivary glands and gland homogenates.

CHAPTER II

LITERATURE REVIEW

The ability of ixodid or hard ticks to parasitize their hosts is mediated by the well-developed salivary glands possessed by these arachnids. These organs secrete cement to fix the tick to the host, analgesics to prevent pain to the host during biting, and anticoagulants to prevent blood clotting. Salivary glands also function in allowing the tick to take in water from the atmosphere as well as in secreting excess fluid out of the tick's body during feeding (Kaufman, 1989). The process of feeding by ticks follows a consistent pattern. The tick initially inserts its mouthparts into the skin of the host animal, where it anchors itself by secreting a cement from its salivary glands. Various agents in tick saliva create a feeding lesion under the skin around the mouthparts. The parasite derives its nourishment from this pool of blood. During feeding, the tick alternates between blood sucking for nutrition and salivation for injection of feeding aids and excess fluid elimination (Kaufman, 1989).

There are two stages of feeding in ixodid ticks. The first stage is the slow-feeding phase, where the tick will take in enough blood to grow to 200 mg, ten times its

original weight, within 10 days. The fast phase of feeding is characterized by a further fivefold weight increase beyond 200 mg that occurs within 24 hours (Kaufman, 1989).

There are certain obstacles to blood feeding by arthropods present in vertebrate hosts that must be overcome if the parasite is to be successful in obtaining food. These obstacles are the normal vertebrate responses to blood vessel damage, which include vasoconstriction, platelet aggregation, blood coagulation, and immune reactions (Ribeiro, 1986).

It has been proposed that the mechanism by which hard ticks evade many of these host defenses is by secreting prostaglandin E_2 (PGE_2) and prostacyclin in their saliva (Ribeiro et al., 1985). These eicosanoids can cause dilation of blood vessels to allow increased blood flow to the feeding site and are potent inhibitors of platelet aggregation, vasoconstriction, macrophage activation, and neutrophil activity. The suppression of the immune system by PGE_2 may not only prevent host immune response to the tick, but may also protect any tick- borne pathogen introduced with the saliva, allowing the pathogen to gain an early foothold in the host animal.

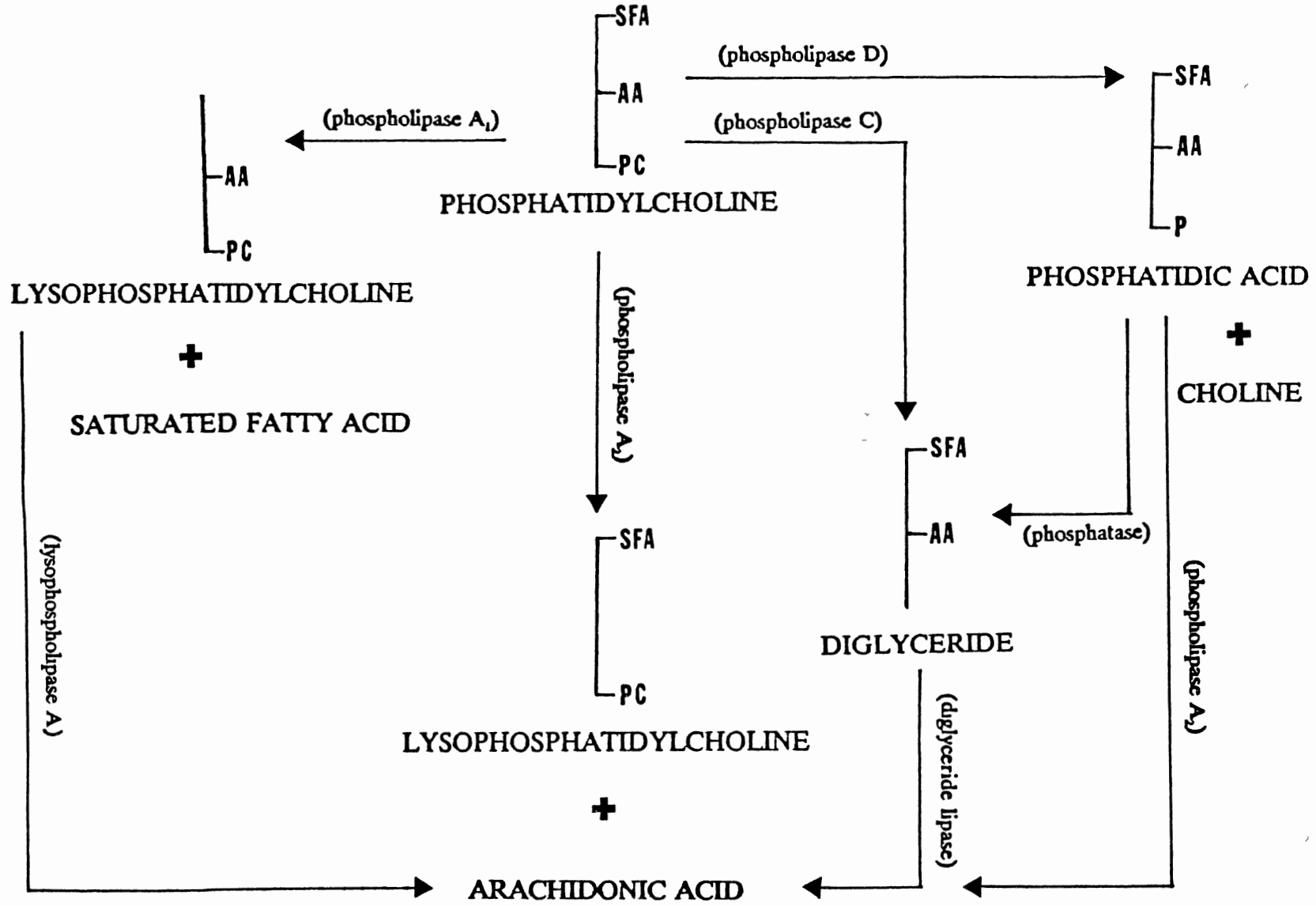
Prostaglandin E_2, confusingly enough, is also an inflammatory agent. It promotes the effects of bradykinin, which acts to increase pain in the host at the feeding site. However, in I. dammini saliva, a kininase activity has been found that destroys bradykinin (Ribeiro et al., 1985). In

this way, the tick is able to exploit the advantageous effects to blood feeding of these eicosanoids while avoiding their inflammatory effects.

Prostaglandin E_2 has been found in the saliva of I. dammini (Ribeiro et al., 1985), B. microplus (Dickinson et al.,1976), and H. anatolicum excavatum (Shemesh et al., 1979). Preliminary studies by Ribeiro et al.(personal communication) have shown the presence of PGE_2 and $PGF_{2\alpha}$ in the saliva of the lone star tick, A. americanum . Salivary PGE_2 in this species, which has been implicated as a vector of Lyme disease (Schulze et al., 1984), could perhaps facilitate the establishment of the Lyme disease pathogen in the host as well as allowing the tick to parasitize its host.

The precursor of prostaglandin E_2 is arachidonic acid. The rate-limiting step in eicosanoid synthesis is the hydrolysis of arachidonic acid from the sn-2 position of cellular phospholipids by phospholipase A_2 (PLA_2) enzymes (Flower and Blackwell, 1976). Arachidonic acid can also be released by a phospholipase C-diglyceride lipase pathway, a phospholipase D-phosphatase-diglyceride lipase pathway, a phospholipase D-PLA_2 pathway, or a phospholipase A_1-lysophospholipase pathway (Holtzmann, 1991; Dennis et al., 1991). However, the major source of free arachidonic acid for eicosanoid synthesis is believed to be phospholipase A_2 (Dennis et al., 1991). The reactions and products of phospholipases are shown in Figure 1.

Figure 1. Reactions of Phospholipases with Phosphatidylcholine.SFA: saturated fatty acid, AA: arachidonic acid, P: phosphate, C: choline.


SFA
AA
PC
PHOSPHATIDYLCHOLINE
(phospholipase D)
(phospholipase A1)
(phospholipase C)
AA
PC
LYSOPHOSPHATIDYLCHOLINE
SFA
AA
P
PHOSPHATIDIC ACID
CHOLINE
SATURATED FATTY ACID
(phospholipase A2)
SFA
AA
DIGLYCERIDE
(phosphatase)
SFA
PC
LYSOPHOSPHATIDYLCHOLINE
(lysophospholipase A1)
(diglyceride lipase)
(phospholipase A2)
ARACHIDONIC ACID

Phospholipase A_2 activity is ubiquitous and has been found in bacteria, plants, protozoa, insects, and many vertebrates (Van den Bosch, 1980). The most studied PLA_2 activities are the secreted extracellular phospholipases A_2 that are found in snake and bee venoms as well as in pancreatic secretions. Less is known about intracellular PLA_2 activities, as they are less stable and present in lower quantities (Jain and Berg, 1989).

The phospholipases A_2 that have received the most attention in arthropods up to this time are extracellularly secreted PLA_2 in the venoms of stinging insects. The majority of these enzymes are very similar to each other and to mammalian digestive and reptilian venom PLA_2 as well. They have an approximate molecular weight of 14,000, a peptide chain of about 123 amino acids, high heat-stability, and dependence on calcium at millimolar levels for activation (Dijkstra et al., 1978). The amino acid sequences from two hymenopteran species have recently been determined. The structure of the PLA_2 from the honeybee, Apis mellifera L., shows a close homology to mammalian enzymes such as bovine pancreatic PLA_2 (Kuchler et al., 1989). This is in contrast with the amino acid sequence of phospholipase A_2 in the venom of the hornet Vespa orientalis Adono. This extremely toxic secreted PLA_2 has a very different structure from other known enzymes of this type (Korneev et al., 1989).

Evidence for non-secreted PLA_2 in insects has also been

found. Phospholipase A_1 and A_2 activity as determined by the release of lysophospholipid from phospholipid has been found in such diptera species as Musca domestica L., Phormia regina (Meigen), Glossina morsitans Westwood, and Culex pipiens fatigans (L.) (Bridges, 1983; Rao and Subrahmanyam, 1969). The PLA activity in the Culex pipiens larva is the most extensively characterized non-secreted arthropod PLA at this time. It has an optimum pH of 9 and an optimum temperature of 45° C. This PLA activity is unique in that it is inhibited by calcium, which activates most PLA_2 enzymes (Rao and Subrahmanyam, 1969). No reports of phospholipase A_2 activity from ticks have been published.

Because phospholipase A_2 is a soluble enzyme acting upon an insoluble substrate, normal kinetic parameters fail to accurately describe the enzyme's activity. In the presence of substrate dispersed to the monomeric level, almost no PLA_2 activity is detected. Concentrations of substrate above the solubility limit of the solution greatly increase phospholipase A_2 activity. A PLA_2 enzyme does not bind to individual lipid molecules, but rather to the lipid-water interface (Verger and de Hass, 1976). Thus, PLA_2 activity is at least as dependent on the surface area of lipid exposed to aqueous media as it is on absolute substrate concentration.

Phospholipase A_2 has two modes of action in its activity in interfaces: the hopping mode and the scooting mode. In the hopping mode, the enzyme in the aqueous phase

binds to a lipid-water interface, acts upon a substrate molecule, and then returns to the aqueous phase. In other words, the enzyme "hops" from interface to interface. In the scooting mode, after the enzyme has acted upon a substrate molecule in an interface, it binds to an adjacent substrate molecule in the same interface (Jain and Berg, 1989).

Among the many PLA_2 , there are a number that act on specific fatty acids in the 2-position of phospholipids. Phospholipase A_2 enzymes specific for arachidonic acid in the sn-2 position include those found in human placental blood vessels (Karnachouw and Chan, 1985), human platelet cytoplasm (Kim et al., 1988), human platelet membranes (Jesse and Cohen, 1976), and hamster heart (Tam et al., 1984). Hamster heart phospholipase A_2 was also found to be specific for not only the fatty acid in the sn-2 position, but was also affected by that in the 1-position as well (Cao et al., 1987). Other PLA_2 are less specific. The phospholipase A_2 activities found in rat platelets and human polymorphonuclear cells act equally on arachidonic or oleic acid (Schalkwijk et al., 1990). Human osteoarthritic synovial fluid PLA_2 shows a similar lack of specificity for the fatty acid substrates linoleic or arachidonic acid (Parks et al.,1990).

Phospholipase A_2 may also show specificity for the phospholipid head group. Human platelets contain two forms of PLA_2, one that is specific for phosphatidylcholine and one that is specific for phosphatidylethanolamine (Cheung

and Ballou, 1989). A PLA_2 purified from human rheumatiod arthritic synovial fluid was able to hydrolyse phosphatidylethanolamine more completely than phosphatidylcholine or phosphatidylserine (Hara et al., 1989). Peritoneal exudates from rats injected with caseinate contained a phospholipase A_2 that preferred phosphatidylethanolamine and phosphatidylserine to phosphatidylcholine (Chang et al., 1987a). The PLA_2 in hamster heart, however, was specific for phosphatidylcholine (Cao et al., 1987). There are PLA_2 activities, however, that lack phospholipid substrate specificity. Horse platelet phospholipase A_2 acts equally on phosphatidylcholine, phosphatidylethanolamine, or phosphatidylinositol (Van den Bosch, 1980). The PLA_2 from human monocytic leukemic U937 cells released arachidonic acid in equal amounts from phosphatidylcholine, phosphatidylethanolamine, and phosphatidylinositol (Diez and Mong, 1990).

Another type of substrate specificity observed in PLA_2 enzymes involves the linkage of the fatty acid in the 1-position. Most phospholipids have their fatty acids esterified to the glycerol backbone, but there are others in which the fatty acids are attached by an ether linkage. The PLA_2 in dog myocardial cytosol has a high specificity for ether-linked phospholipids (Hazen et al., 1990). Sheep platelet PLA_2 is extremely specific for these types of substrates, as it is 100x more active on ether-linked phospholipids than it is on esterified phospholipids (Loeb

and Gross, 1986). Rat platelet PLA_2, by comparison, is highly specific for the ester-linked phospholipids (Colard et al., 1987). The phospholipase A_2 in human platelets and human leukemic monocytes does not distinguish between these substrates (Kramer et al., 1988; Diez and Mong, 1990).

The pH at which an enzyme shows greatest activity is an important parameter in its characterization. The pH optima for PLA_2 activities vary greatly between species and tissues. Most are active between pH 7-9.5 (Van den Bosch, 1980; Kramer et al., 1989; Stefanski et al., 1986). However, phospholipase A_2 activities with acid pH optima have been observed. Rabbit polymorphonuclear leucocytes contain two PLA_2 activities, one of which has a 5.5 pH optimum (Franson et al., 1974). Another phospholipase A_2 active at acid pH (6.4) is found in dog myocardial cytosol (Hazen et al., 1990).

The activity of many PLA_2 enzymes is dependent on calcium. The majority of phospholipases A_2 are active only in the presence of calcium (Van den Bosch, 1980; Jain and Berg, 1989). The phospholipase A_2 in human osteoarthritic synovial fluid specifically requires calcium. It has no activity without this element, and other metal ions such as magnesium, copper, barium, or manganese, cannot be substituted (Parks et al., 1990). A similar enzyme activity has been observed in rat platelets (Horigome et al., 1987).

Calcium-dependent phospholipase A_2 enzymes also vary in the concentration of calcium that they require for

activation. Intracellular PLA_2 enzymes are activated by submicromolar concentrations of calcium (Loeb and Gross, 1986; Diez and Mong, 1990). The secreted PLA_2, on the other hand, require calcium at the millimolar level before they are activated (Alonzo et al., 1986; Kramer et al., 1990; Parks et al.,1990).

This is not to say that calcium-independent PLA_2 activities do not exist. The PLA_2 activity in hamster heart cytosol does not show any requirement for calcium or any other divalent metal ion (Cao et al., 1987). Another calcium-independent phospholipase A_2 is found in canine myocardial cytosol (Hazen et al., 1990). The phospholipase A activity in mosquito larval homogenates is not only active without calcium, but actually appears to be inhibited by it (Rao and Subrahmanyam, 1969).

There are many substances that can inhibit PLA_2 activity. Some, like mepacrine, block the enzyme-substrate interface (Jain and Jahardigar, 1985). Others, such as triflouroperazine, bind calcium (Chang et al., 1987b). Para-bromophenacylbromide is an inhibitor that forms a covalent bond with histidine near the Ca^{++}-binding site of the PLA_2 molecule. This inhibitor is more effective at lower calcium concentrations (Chang et al., 1987a). Manoalide, a sesquiterpenoid isolated from the sponge Luffanella variabilis Cannaget, inhibits PLA_2 by interfering with the phospholipid-binding site of the enzyme. The hydrolysis of phosphatidylcholine by phospholipase A_2 is inhibited by

manoalide, while that of phosphatidylethanolamine is not, indicating that manoalide has no effect on the active site of the enzyme (Bennet et al., 1987). Manoalide is also much more effective in inhibiting extracellular PLA_2 than it is in suppressing the activity of the intracellular enzymes. Vitamin E has been observed to inhibit platelet PLA_2, but it is unknown whether this inhibition is due to interaction with the enzyme itself or with the substrate (Douglas et al., 1986).

There is no specific inhibitor for PLA_2 at this time as the inhibitors discussed previously can act upon other phospholipases as well (Chang et al., 1987b). One development in PLA_2 inhibitors that is showing some promise is the use of substrate analogs. Two recently tested analogs are Wyeth-Ayerst PLA_2 inhibitors. One of these, WY-49422, was shown to inhibit mammalian, snake venom, and bee venom phospholipase A_2 in the same relative concentration range. The other, WY-48489, was specific for mammalian PLA_2 (Marshall and Chang, 1990).

CHAPTER III

MATERIALS AND METHODS

The ticks used in this study were adult females of the lone star tick, A. americanum. Unfed ticks were maintained at 20°C and 98% humidity before being placed on sheep. Removal of the partially-fed ticks was performed prior to their engorgement on the blood of their hosts (Patrick and Hair, 1975). Feeding stages of ticks were determined on the basis of weight, as only ticks in the fast-feeding stage exceeded 200 mg in weight. Unless otherwise indicated, slow-feeding ticks were used in all enzyme characterization studies.

The salivary glands of partially-fed ticks were dissected out of their bodies in 0.1 M morpholinopropane sulfonic acid (MOPS) buffer at pH 6.8. The glandular tissue was stored frozen at -15°C in a 0.5 M piperazine N, N-bis-(2-ethane sulfonic acid) (PIPES) buffer (pH 6.8) containing 20mM ethylene glycol tetraacetic acid (EGTA) and 40% glycerol.

The majority of chemical reagents used in this study were purchased from Sigma Chemical Company (St. Louis, Missouri), while Fisher Scientific (Houston, Texas) was the source of most of the organic solvents utilized. Butanol and

diethyl ether, however, were purchased from EM Science (Cherry Hill, New Jersey) and Mallinckrodt (Paris, Kentucky), respectively. The radiolabeled phospholipid substrates and 2,5-diphenyloxazole (PPO) for scintillation cocktails came from New England Nuclear (Boston, Massachusetts). These substrates included 1-palmitoyl, 2-arachidonyl phosphatidylethanolamine, 1-stearoyl, 2-arachidonyl phosphatidylinositol, and 1-palmitoyl phosphatidylcholine with the following fatty acids in the 2-position: arachidonic, linoleic, oleic, and palmitic. The exception was 1-stearoyl, 2-arachidonyl phosphatidylcholine, which was purchased from Amersham (Arlington Heights, Illinois). All substrates used were labeled with ^{14}C as the 1-carbon of the fatty acid in the 2-position.

Enzyme assays for PLA_2 activity used as a substrate 1 nmole of 2-^{14}C arachidonyl phosphatidylcholine (53-56 mCi/mMol) dissolved in toluene-ethanol (1:1,v/v) which was added in a 12x75 mm disposable culture tube. The solvent was evaporated under nitrogen and the substrate was dispersed by vortexing in 80 ul of a 0.1 M Tris buffer (pH 9) containing 5 mM $CaCl_2$ in the same tube. Reactions were started by adding 20 ul of a homogenate of six salivary glands in 1 ml of 0.1 M Tris buffer (pH 9). Incubations were performed at 37°C (Parks et al., 1990).

In preliminary experiments to test for release of arachidonic acid, reactions were stopped with 2N HCl. The reaction mixture was then applied to a 3 x 0.5 cm silicic

acid column (Bio-Sil A: Bio-Rad Laboratories, Richmond, California) equilibrated with hexane-dioxane-acetic acid (70:30:1,v/v). Arachidonic acid was eluted from the column with 6 ml of the equilibrating solvent (Cosentino and Ellis, 1981).

In all subsequent experiments, enzyme reactions were stopped by addition of 100 *u*l of butanol and 10 *u*l of 0.18 mg/ml carrier arachidonic acid (Hazen et al., 1990). After vortexing and centrifugation in a Model HNS centrifuge (International Equipment Company, Needham Heights, Massachusetts) at 2000 rpm, 60 *u*l of the butanol phase was removed and placed on channeled 250*u* silica gel GF TLC plates with preadsorbent zone (Analtech, Newark, Delaware), which were developed in a solvent of hexane-diethyl ether-acetic acid (70:30:1,v/v). Lanes containing lipid and arachidonic acid standards on each plate were sprayed with Rhodamine 6G dye for visualization under long-wave ultraviolet light. The regions of the unsprayed sample lanes corresponding to the locations of the standards were scraped directly into 20 ml scintillation vials (Franson et al., 1974). After addition of 10 ml of a scintillation cocktail containing 6 g PPO/liter toluene, radioactivity in samples was determined using a Beckman LS 5000CE liquid scintillation counter (Beckman Instruments, Palo Alto, California). Each sample was counted for 5 minutes using an open window for ^{14}C measurement.

The concentration of tick salivary gland protein was

determined using a dye-binding assay, which allowed for calculation of enzyme activity as umoles/min/mg protein (Bradford, 1976). Bovine serum albumin was used as the protein standard. Samples were assayed in 96-well Falcon (Beckton Dickinson, Lincoln Park, New Jersey) microtiter plates (Simpson and Sonne, 1982) and read on an automatic plate reader (Model 310, Bio-Tek Instruments Inc., Winooski, Vermont).

Homogenates of gland tissue were incubated in Tris buffers of varying pH to determine the relationship between pH and activity of PLA_2. The effect of gland protein concentration on enzyme activity was tested using dilutions of the homogenates in Tris buffer. Incubations in temperatures ranging from 25-75°C were performed in a shaking water bath.

In order to determine if any observed activity was due to phospholipase A_2 activity and not the activity of phospholipase C acting together with diglyceride lipase, 100 uM neomycin was added to the assay mixture to test for inhibition of activity (Axelrod et al., 1988). In a related experiment, the regions of the TLC plates where diglyceride would be located were visualized by iodine vapors, scraped off, and counted to determine if this PLC pathway or a phospholipase D-phosphatase-diglyceride lipase pathway was responsible for the observed liberation of free arachidonic acid (Rubin et al., 1990). Thin-layer chromatography was performed as described above.

Phosphatidic acid and lysophosphatidylcholine fractions were also studied to test for the presence of phospholipase D or PLA_1 activity. These were separated from the neutral lipid products on a silicic acid column by the method of Lynch and Thompson (1986), and then loaded onto TLC plates to be developed in chloroform-methanol-hexane-acetic acid-boric acid 40:20:30:10:1.8 v/v/v/v/w (Gilfillan et al., 1983). Standards were visualized with iodine vapors and corresponding areas were scraped for determination of radioactivity as described previously.

Additional substrates for specificity studies included 2-^{14}C arachidonyl phosphatidylethanolamine, 2-^{14}C arachidonyl phosphatidylinositol, and phosphatidylcholine with linoleic, oleic, and palmitic acid labeled at the sn-2 position .

In order to determine if PLA_2 activity in tick salivary glands is calcium-dependent, assays using a calcium-free 0.1 M Tris buffer (pH 9) containing 5 mM EGTA were performed. Buffers containing 5mM nitrilotriacetic acid (NTA) at pH 9 with calcium concentrations between 1.5-4.5 mM, which yeilded free concentrations of calcium of 0.25-5.0 uM, were used to study the effect of intracellular calcium levels on activity (Perrin and Sayce, 1967). The calmodulin inhibitor, calmidazolium, was dissolved in dimethylsulfoxide (DMSO) and added to the reaction mixture at 20 uM concentration to determine if any calcium effects were mediated by calmodulin (Gietzen et al., 1981). Experiments testing for the metal ion requirements of the enzyme activity utilized buffers

with magnesium, copper, zinc, and manganese substituted for the calcium in the standard reaction buffer.

In order to determine the subcellular location of the PLA_2, homogenates were centrifuged at 15,000xg and aliquots of resulting supernatants further centrifuged at 100,000xg. Pellet fractions were resuspended in the same volume of buffer as the final volume of their corresponding supernatants.

Data was analyzed by determining means and standard deviations (SD). Additional analysis used the Student's t-test at the 0.05 significance level.

CHAPTER IV

RESULTS

In preliminary experiments using the procedure of Cosentino and Ellis (1981) to separate the lipid products, radiolabeled free arachidonic acid was released from ^{14}C-arachidonyl phosphatidylcholine when incubated with tick salivary gland homogenates (Figure 2). Almost no labeled arachidonic acid was found in reaction mixtures that did not contain tick salivary gland protein or in those that were stopped by adding 2N HCl with tick gland homogenate.

The possibility existed during the course of this study that the phospholipase activity observed was not necessarily due to PLA_2. Arachidonic acid can also be released by a phospholipase C-diglyceride lipase pathway, a phospholipase D-phosphatase-diglyceride lipase pathway, or a phospholipase A_1-lysophospholipase A pathway (Holtzman, 1991; Dennis *et al.*, 1991). Neomycin, which inhibits phospholipase C, did not inhibit the release of arachidonic acid by tick salivary gland homogenates (Figure 3). Furthermore, no radiolabeled diglyceride was found in the products of incubation, eliminating the possibility of any pathway involving diglyceride lipase as the source of radioactive arachidonic acid (Table I). The absence of labeled phosphatidic acid in

Figure 2. Release of Arachidonic Acid From the 2-Position of Phosphatidylcholine by A. americanum Salivary Gland Homogenate. A: no homogenate, B: reaction with homogenate stopped immediately, C: incubation for 15 minutes with tick gland protein. Results expressed as radioactivity of arachidonic acid fraction mean ± SD, n= 3.

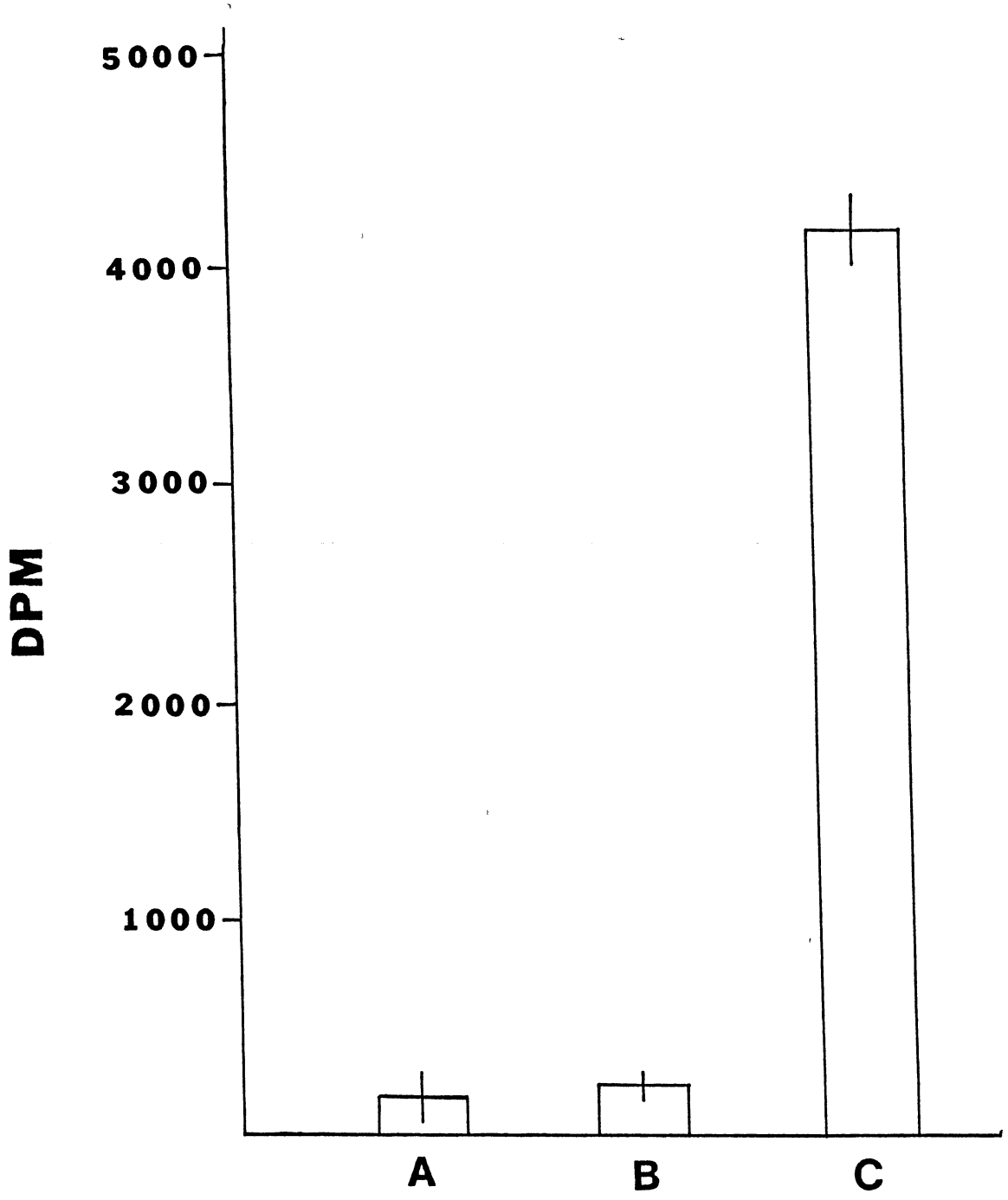

5000
4000
3000
2000
1000
DPM
A
B
C

Figure 3. Effect of 100uM Neomycin on A. americanum Salivary Gland PLA_2 Activity. Specific activity expressed as nanomoles product released/minute/mg protein. Bars represent mean $\pm$ SD, n= 3. Results determined not to be significantly different by Student's t-test at 0.05 level.

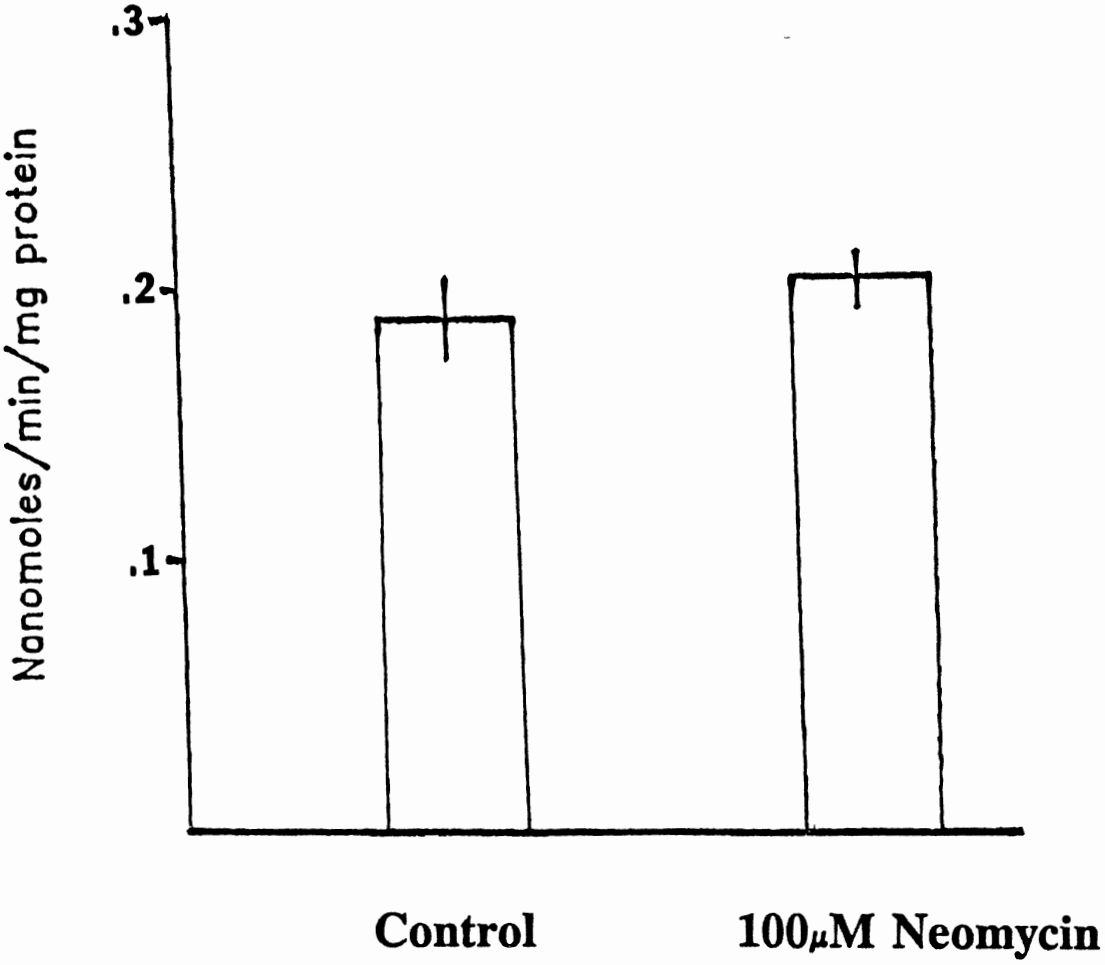

.3
.2
.1
Nanomoles/min/mg protein
Control
100μM Neomycin

TABLE I

DISTRIBUTION OF RADIOLABELED ARACHIDONIC ACID IN ASSAY PRODUCT LIPID FRACTIONS

Phospholipid degradation product	DPM*
Diglyceride	82.11 ± 12.2
Arachidonic acid	2418.88 ± 114.39
Phosphatidic acid	19.16 ± 2.63
Lysophosphatidylcholine	79.86 ± 16.73

* Background radiation = 100 DPM or less, Mean ± SD, n= 3

the reaction products rules out the presence of phospholipase D activity altogether, while the lack of radioactivity in the lysophosphatidylcholine fraction indicates that a PLA_1 pathway is not responsible for the observed release of free arachidonic acid.

Tick salivary gland PLA_2 activity was observed to be directly proportional to incubation time (Figure 4). The amount of activity increased rapidly in the first 8 minutes,then there was a leveling off beyond that period.

Phospholipase A_2 activity in A. americanum was found to be directly proportional to salivary gland protein concentration (Figure 5). Some activity seemed to be lost at lower concentrations (<4ug/assay) of gland protein diluted in 100 mM Tris buffer, indicating some instability at low total protein concentrations. When total protein was kept constant by diluting tissue homogenates in buffer containing 0.25 mg/ml bovine serum albumin, the PLA_2 showed a more linear relationship to gland protein.

Despite the instability of tick salivary gland PLA_2 in low total protein levels, this enzyme activity was found to be stable with respect to freezing. Phospholipase A_2 from A. americanum showed little or no loss of activity when whole glands (Figure 6) or three separate gland tissue homogenates were frozen at -20° C for 24-36 hours (Figure 7). Stability of frozen homogenates was not affected by the length of time the glands had been frozen. This stability of the PLA_2 with freezing and thawing made working with this tissue much

Figure 4. Phospholipase A_2 Activity in A. americanum Salivary Glands as a Function of Incubation Time. Points represent means $\pm$ SD, n= 3.

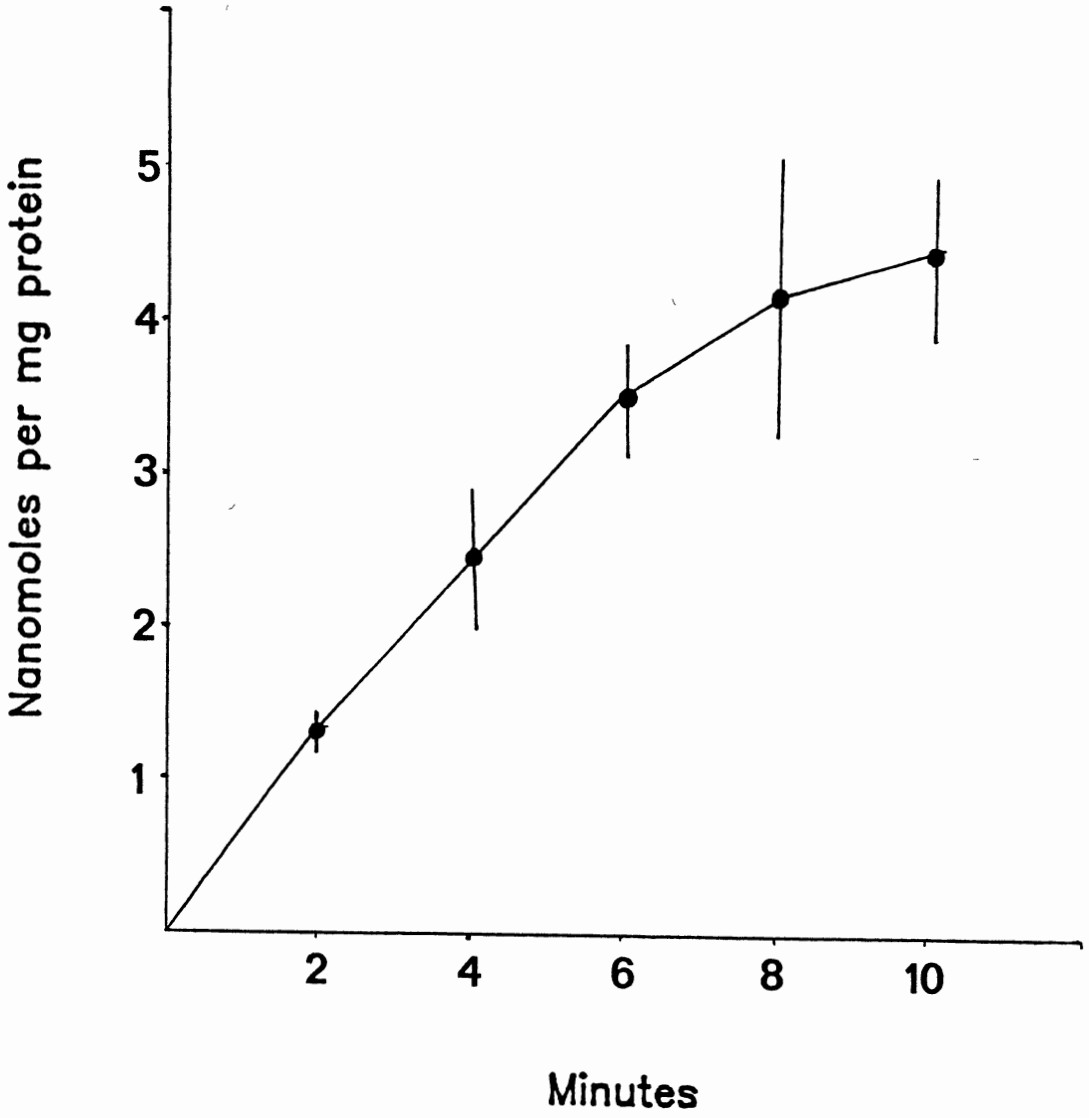

Nanomoles per mg protein
5
4
3
2
1
2
4
6
8
10
Minutes

Figure 5. PLA_2 Activity in A. americanum Salivary Glands as a Function of Gland Tissue Protein Concentration. Homogenates Diluted in Tris Buffer ± 0.25 mg/ml Bovine Serum Albumin. Points represent means ± SD, n= 3.

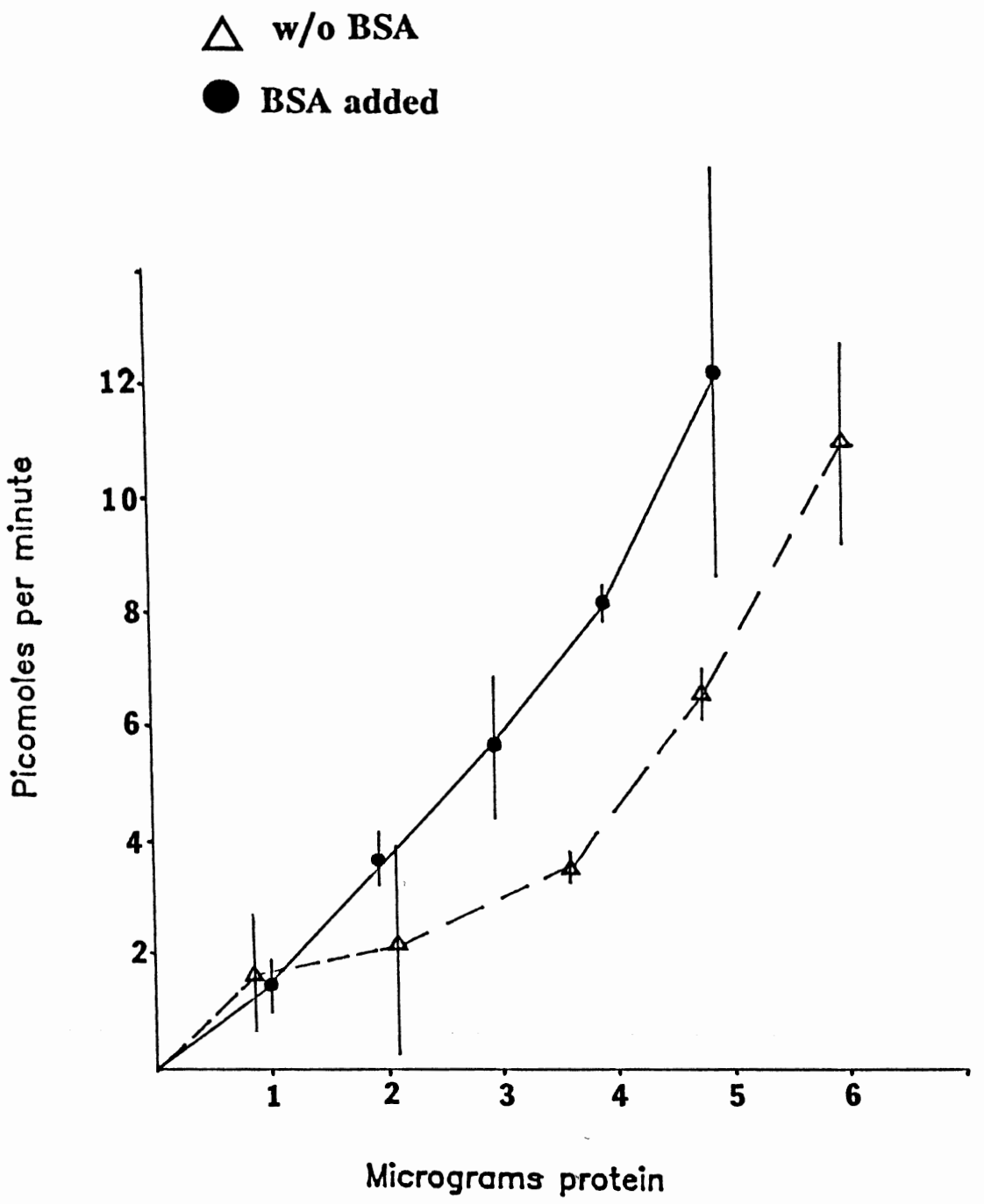

△ w/o BSA
● BSA added
Picomoles per minute
12
10
8
6
4
2
1
2
3
4
5
6
Micrograms protein

Figure 6. PLA_2 Activity in Fresh and Frozen A. americanum Salivary Glands. Frozen glands stored at -20°C for 24-36 hours. Bars represent means ± SD, n= 3. Results determined not to be significantly different by Student's t-test at 0.05 level.

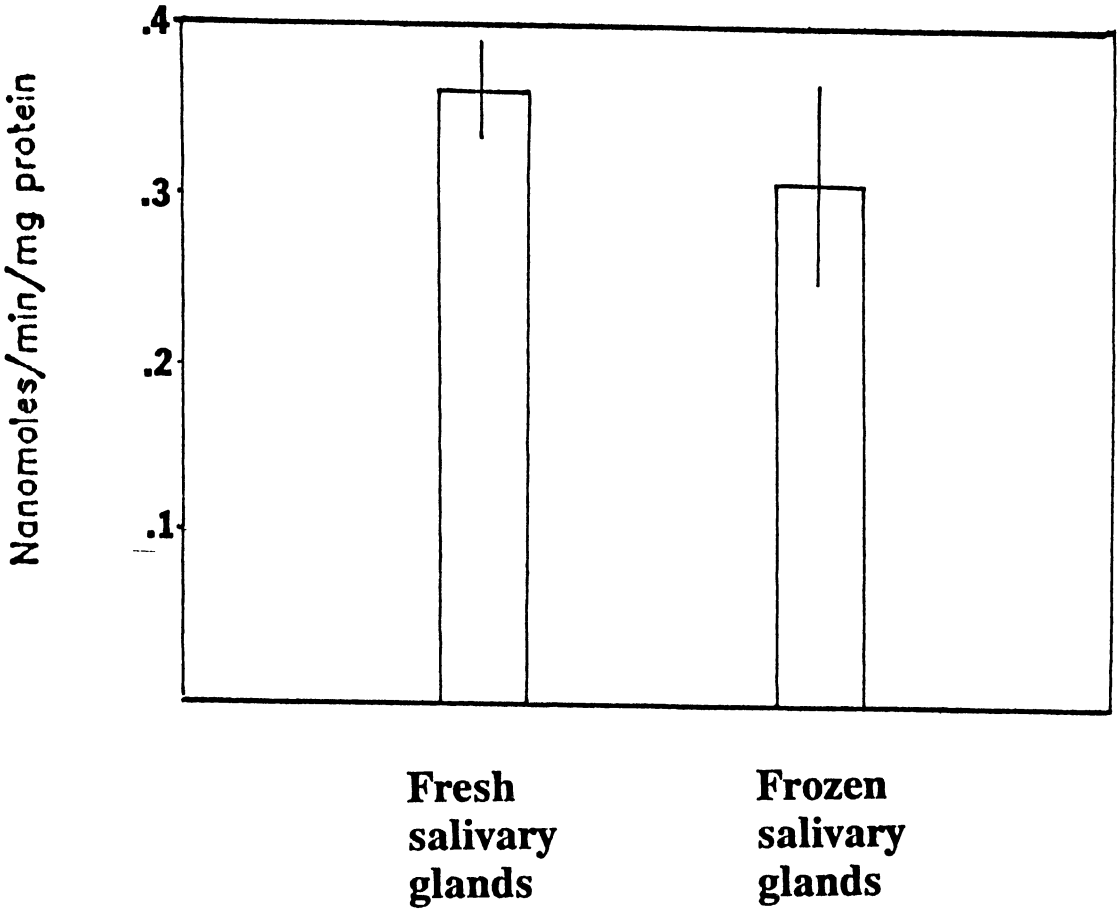
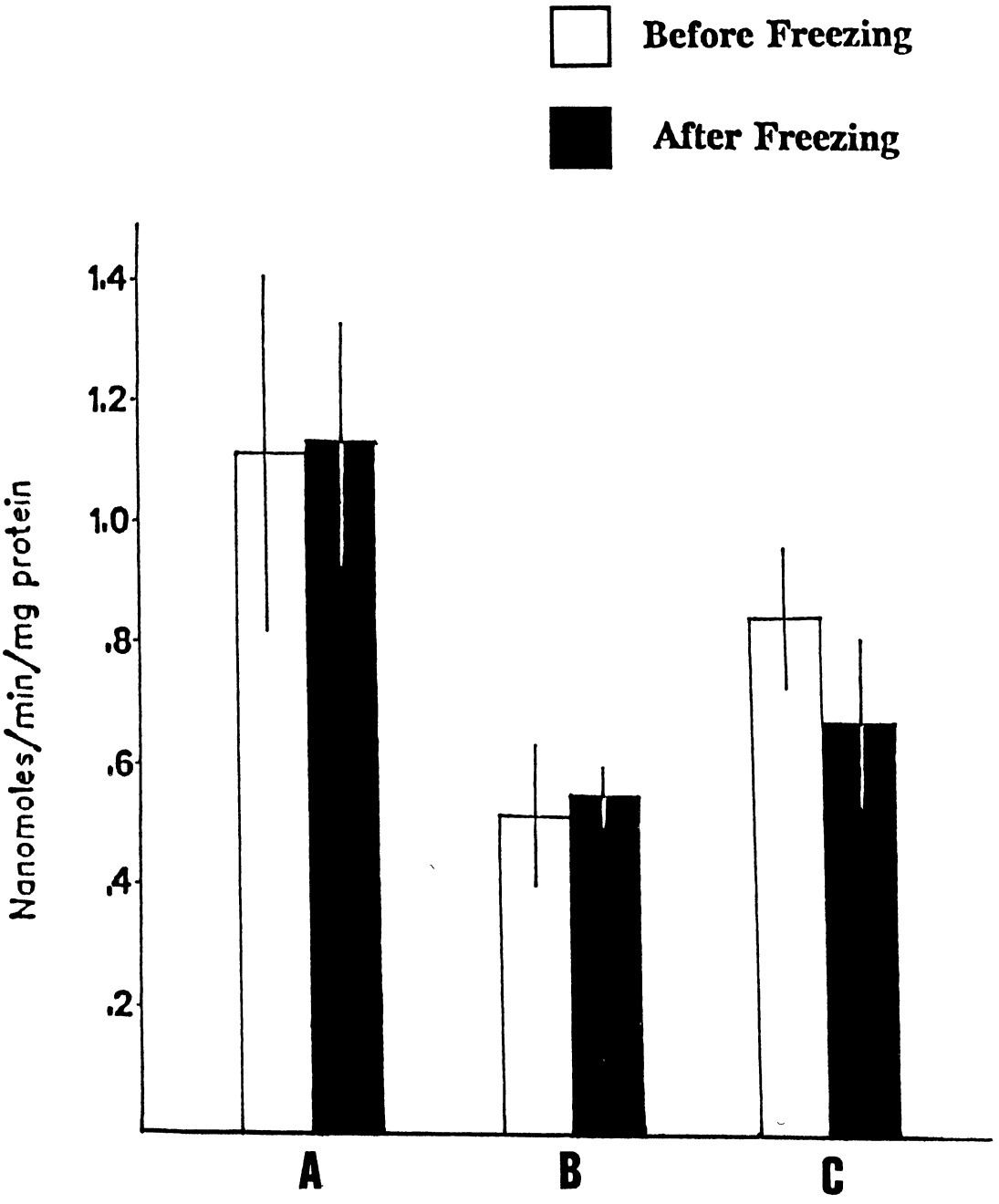

Nanomoles/min/mg protein
.4
.3
.2
.1
Fresh salivary glands
Frozen salivary glands

Figure 7. Effect on PLA_2 Activity of Freezing 3 Different Homogenates of A. americanum Salivary Glands at -20°C for 24-36 Hours. A= fresh glands, B= glands frozen for 6 months, C= glands frozen 1 day. Bars represent means ± SD, n= 3. Results not significantly different according to Student's t-test at 0.05 level.


Before Freezing
After Freezing
Nanomoles/min/mg protein
1.4
1.2
1.0
.8
.6
.4
.2
A
B
C

easier, as it eliminated the need to obtain freshly dissected glands for each assay.

Optimum temperature range for the lone star tick salivary gland PLA_2 was between 37-47° C (Figure 8).

The pH at which the greatest PLA_2 activity took place was about 9 (Figure 9). The data from these initial experiments outlined the optimum conditions for which a standard assay (5 minutes, 37°C, pH 9) for all subseqent experiments was developed.

Tick salivary gland phospholipase A_2 was found to be calcium-dependent, as little or no arachidonic acid was released when phosphatidylcholine was incubated with gland homogenates in the absence of calcium (Figure 10). Activity was not inhibited, but was increased significantly, by the calmodulin inhibitor R24571 (calmidazolium), suggesting that activation of PLA_2 by calcium is not mediated by calmodulin.

The minimum concentration of calcium necessary for activation was found to be in the submicromolar range, with half-maximal activation at 0.5uM calcium (Figure 11).

The phospholipase A_2 activity at millimolar calcium concentration was approximately equal to the maximum activity already reached at micromolar calcium levels, eliminating the possibility of higher activities at millimolar calcium concentrations (Figure 12).

As seen in Table II, none of the metal ions tested could fully substitute for calcium in activating tick salivary gland PLA_2. Manganese gave partial enzyme

Figure 8. Effect of Assay Incubation Temperature on PLA_2 Activity in A. americanum Salivary Glands. Points represent means ± SD, n= 3.

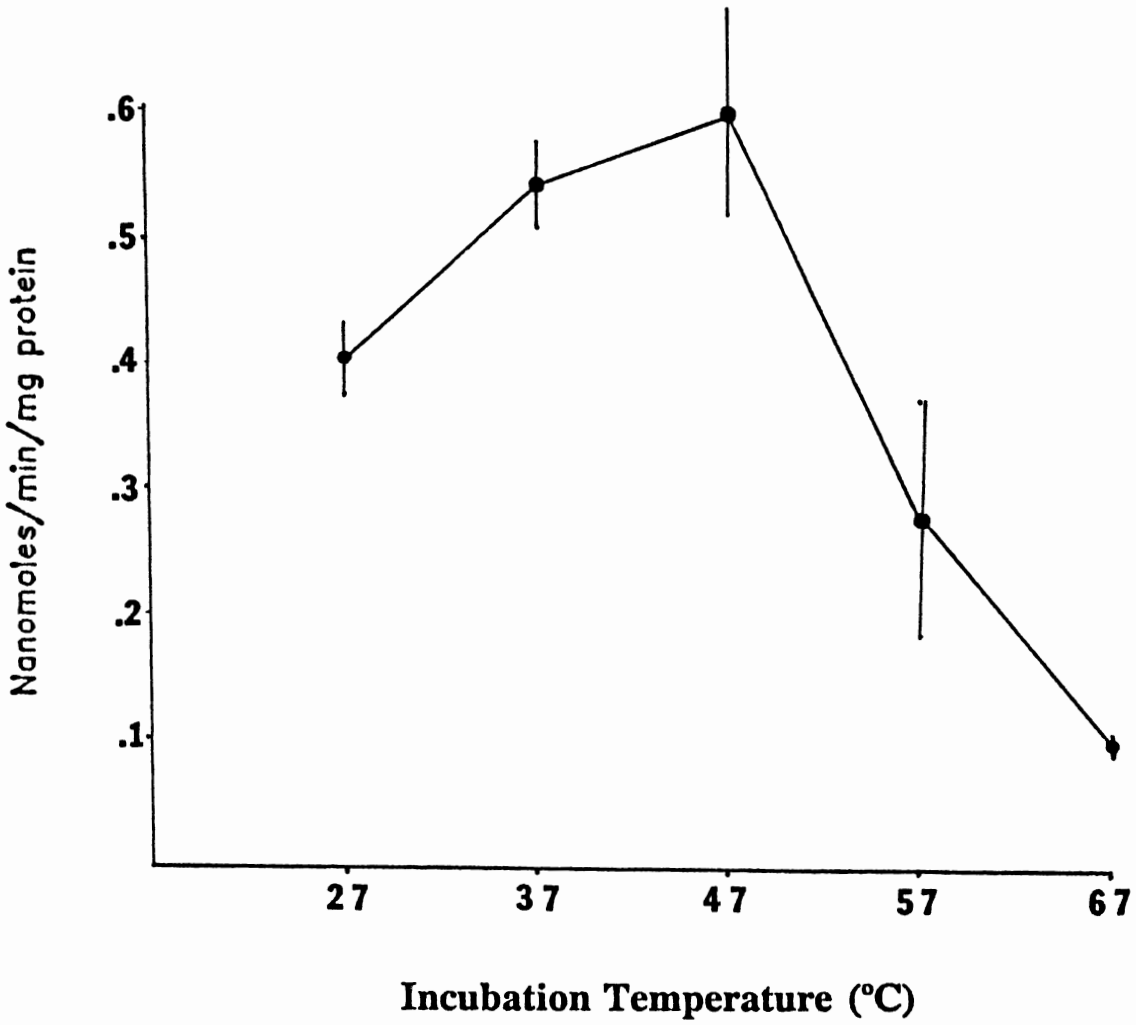

.6
.5
.4
.3
.2
.1
Nanomoles/min/mg protein
27
37
47
57
67
Incubation Temperature (°C)

Figure 9. Effect of Assay Buffer pH on PLA_2 Activity in A. americanum Salivary Glands. Points represent means ± SD, n= 3.

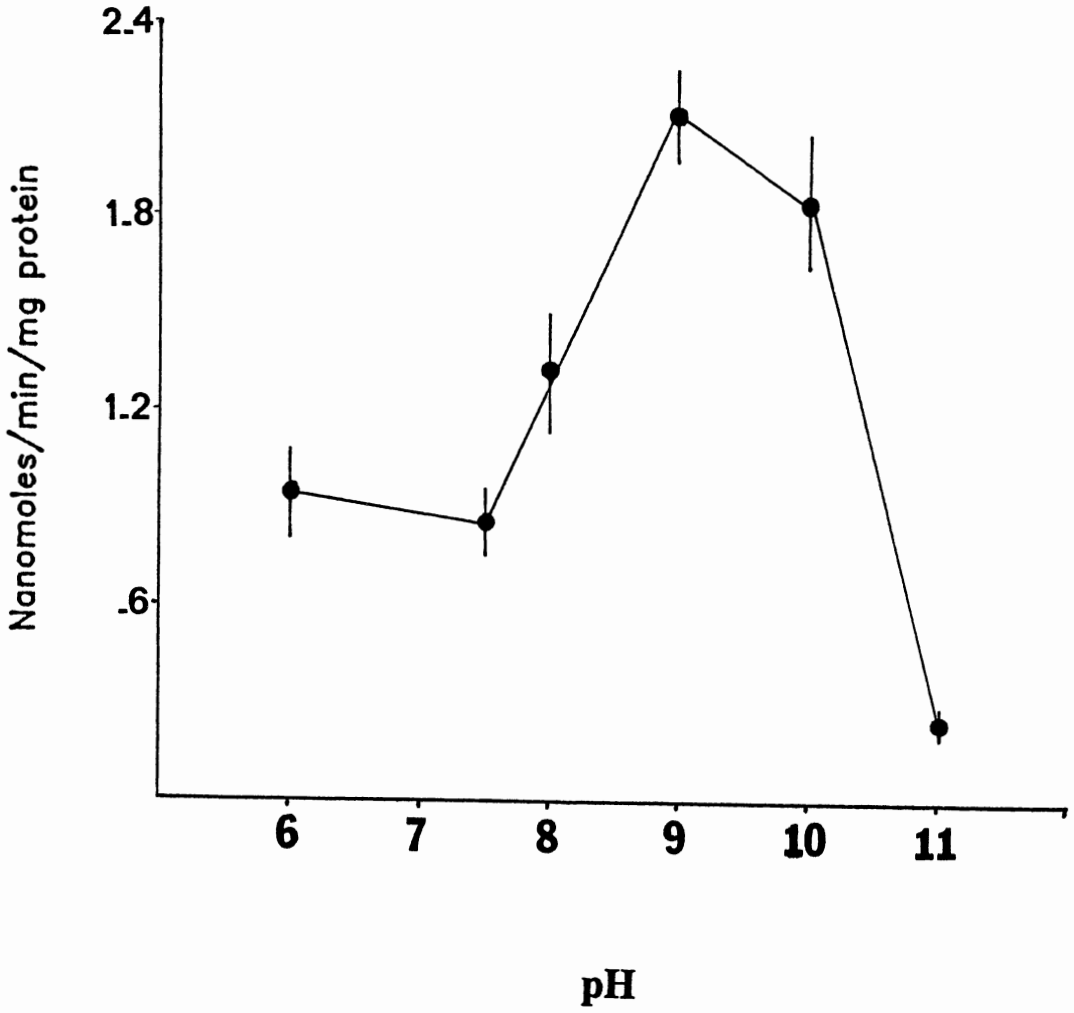

2.4
1.8
1.2
.6
Nanomoles/min/mg protein
6
7
8
9
10
11
pH

Figure 10. Calcium Dependence of,and Effect of Calmodulin Inhibitor R24571 on, A. americanum Salivary Gland PLA_2 Activity. Bars represent means ± SD, n= 3. Activity with calcium + R24571 significantly greater than that with calcium alone according to Students t- test at 0.05 level.

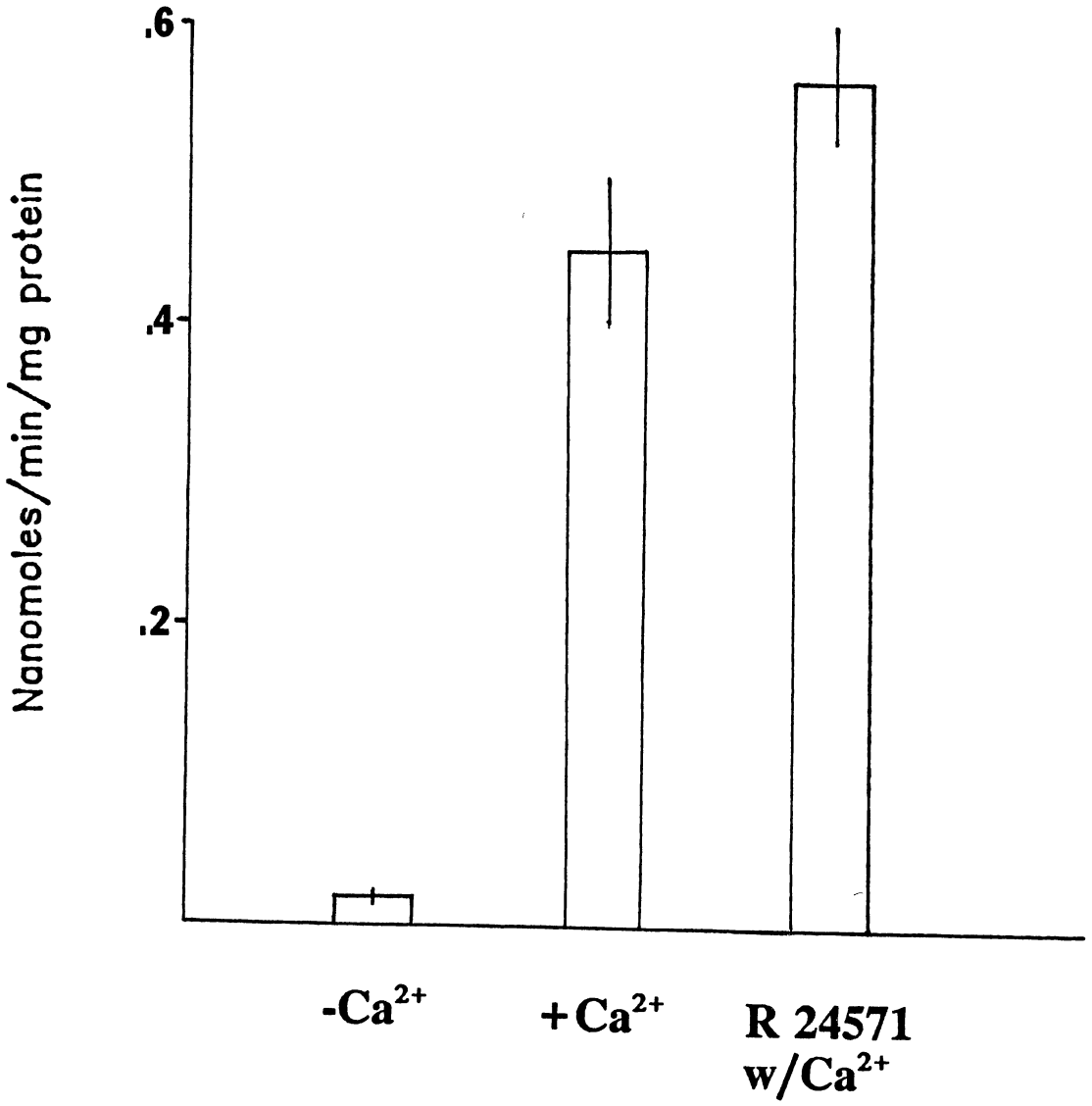

.6
.4
.2
Nanomoles/min/mg protein
-Ca2+
+Ca2+
R 24571
w/Ca2+

Figure 11. Effect of Calcium Concentration on PLA_2 Activity in A. americanum Salivary Glands. Points represent means ± SD, n= 3.

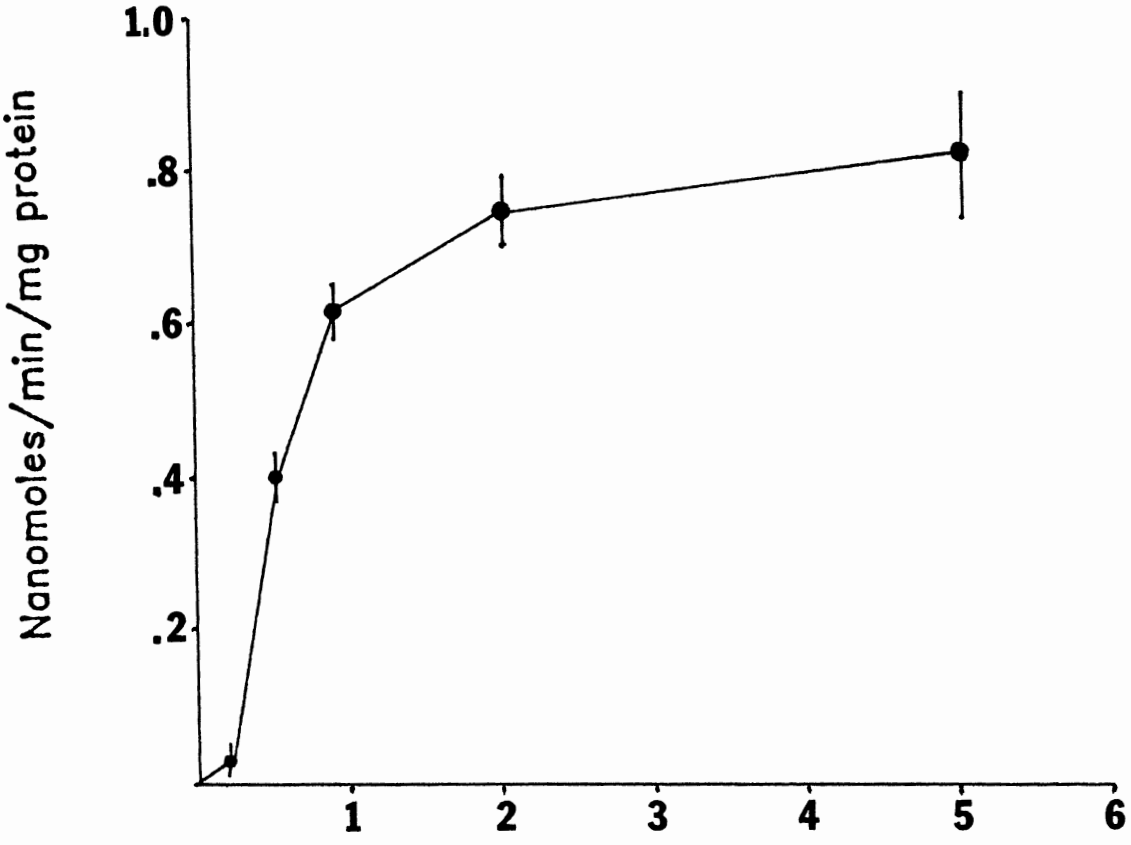

1.0
.8
.6
.4
.2
Nanomoles/min/mg protein
1
2
3
4
5
6
μM Calcium concentration.

Figure 12. Comparison of A. americanum Salivary Gland PLA_2 Activity at Micromolar and Millimolar Calcium Concentrations. Bars represent means $\pm$ SD, n= 3. Results not significantly different according to Student's t-test at 0.05 level.

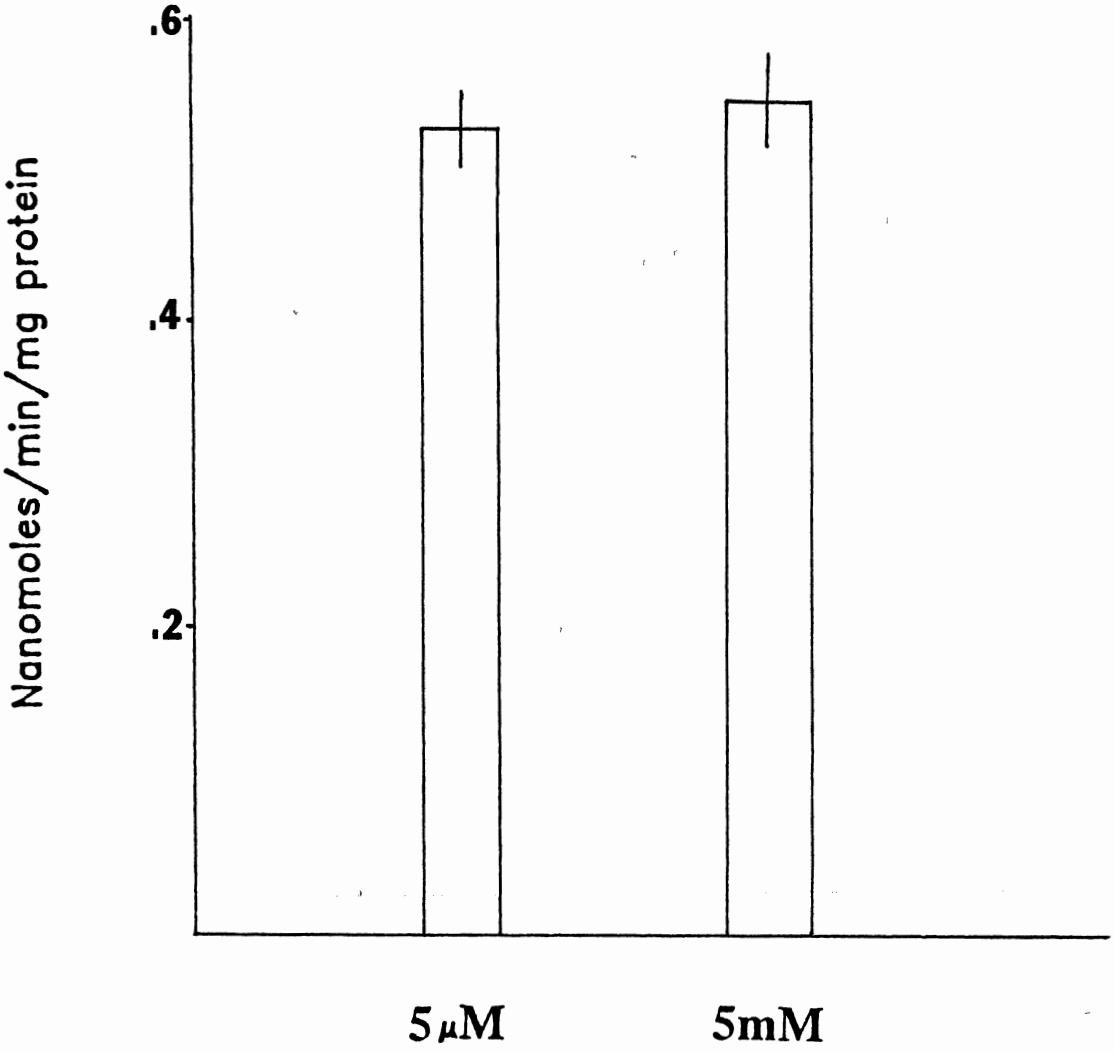

.6
.4
.2
Nanomoles/min/mg protein
5 μM
5mM
Calcium concentration.

TABLE II

EFFECT OF VARIOUS METAL IONS AS SUBSTITUTES FOR, OR ADDITIVES TO, CALCIUM ON PLA_2 ACTIVITY*

Cation	Activity	+ 5mM $CaCl_2$
5mM $CaCl_2$ (control)	100%	-
5mM $MgCl_2$	1%	84%
5mM $CuCl_2$	2%	100%
5mM $MnCl_2$	26%	19%
5mM $ZnCl_2$	5%	27%

* expressed as percent of control

activation when substituted for calcium and appeared to be inhibitory in the presence of calcium. Magnesium and zinc gave little or no activation when substituted for calcium. In the presence of 5mM calcium, magnesium was slightly inhibitory and zinc was greatly inhibitory. Copper had no effect on activity, neither activating PLA_2 in the absence of calcium or inhibiting PLA_2 in the presence of calcium.

After a 15,000xg centrifugation of tick salivary glands homogenized in calcium-free buffer, it was observed that the majority of PLA_2 activity was found in the supernatant fraction (Figure 13). Further centrifugation of this fraction at 100,000xg yielded a supernatant that again contained most of the salivary gland phospholipase A_2 activity. These results indicate that the PLA_2 activity in question is cytosolic and not membrane-bound. Glands homogenized with calcium and centrifuged at 100,000xg also yielded most of their PLA_2 activity in the soluble fraction.

The activity of tick salivary gland PLA_2 on various substrates is shown in Table III. Arachidonic acid was cleaved off in greater amounts from phosphatidylcholine than from phosphatidylethanolamine or phosphatidylinositol, indicating specificity for the phospholipid head group. The phospholipase A_2 activity in A. americanum salivary glands appeared to be specific for arachidonic acid in the 2-position, as arachidonic acid was cleaved off in apparently greater quantities than were linoleic, oleic, or palmitic acid (Table IV).

Figure 13. Tick Salivary Gland PLA_2 Activity in Different Subcellular Fractions. P: pellet, S: supernatant. Results expressed as % of total activity of each centrifugation.

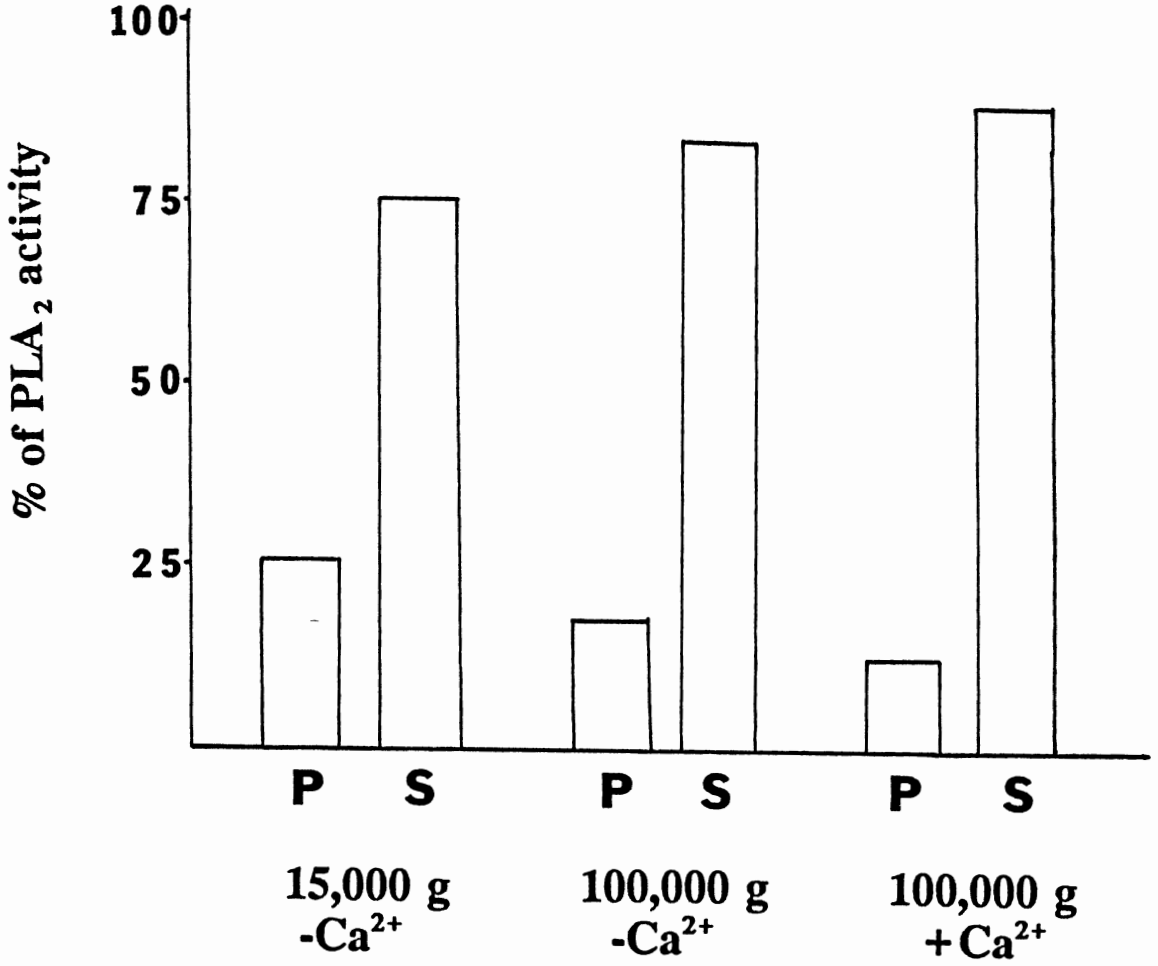

100
75
50
25
% of PLA2 activity
P
S
P
S
P
S
15,000 g
-Ca2+
100,000 g
-Ca2+
100,000 g
+Ca2+

TABLE III

ACTIVITY OF SALIVARY GLAND PLA_2 ON VARIOUS PHOSPHOLIPID SUBSTRATES

Substrate	PLA_2 activity (nMol/min/mg protein)*
2-arachidonyl phosphatidylcholine	1.67 ± 0.27
2-arachidonyl phosphatidylethanolamine	0.95 ± 0.19
2-arachidonyl phosphatidylinositol	0.62 ± 0.05

* Mean ± SD, n= 3.

TABLE IV

ACTIVITY OF SALIVARY GLAND PLA_2 ON VARIOUS FATTY ACID SUBSTRATES

Substrate	PLA_2 activity (nMol/min/mg protein)*
2-arachidonyl phosphatidylcholine	1.10 ± 0.04
2-linoleoyl phosphatidylcholine	0.60 ± 0.04
2-oleoyl phosphatidylcholine	0.56 ± 0.01
2-palmitoyl phosphatidylcholine	0.58 ± 0.11

* Mean ± SD, n= 3

CHAPTER V

DISCUSSION

Radioactive free arachidonic acid was released when tick salivary gland homogenate was incubated with phospholipid labeled in the 2-position, indicating the presence of a phospholipase A_2. The possibility of phospholipase C and diglyceride lipase activity being responsible for this release was eliminated by the fact that neomycin, which is inhibitory to phospholipase C, did not inhibit the reaction. A possible phospholipase D-phosphatase-diglyceride lipase pathway was ruled out along with the PLC pathway by the absence of radiolabeled diglyceride in the assay products. In fact, phospholipase D could be ruled out entirely, as no labeled phosphatidic acid was recovered from the reaction products. The lack of labeled lysophosphatidylcholine as a product makes a phospholipase A_1-lysophospholipase A pathway equally unlikely. The observed specificity for the fatty acid in the 2-position and for phosphatidylcholine is also consistent with PLA_2 activity. Thus, it is reasonable to conclude that the measured activity in tick salivary glands is phospholipase A_2.

The plot of PLA_2 activity against time reached a

plateau at approximately 10 minutes, much earlier than that obtained for the activity in homogenates of human placental blood vessel tissue, which continued to increase in a linear fashion after 30 minutes (Karnauchow and Chan, 1985).

Tick salivary gland PLA_2 showed lower activity when diluted to low total protein concentrations and had to be diluted in a buffer containing bovine serum albumin before a linear relationship between salivary gland protein concentration and activity could be established. A similar phenomenon was observed during attempts to purify the phospholipase A_2 in human monocytic leukemic cells, in which that enzyme activity was likewise stabilized by addition of BSA (Diez and Mong, 1990).

Many phospholipase A_2 enzymes show optimum activity at or near pH 9, including those found in human platelet membranes, hamster heart, and human rheumatoid arthritic synovial fluid (Jesse and Cohen, 1976; Tam et al., 1984; Hara et al., 1989). Thus, the pH optimum for tick salivary gland PLA_2 is not unusual.

The optimum temperature range seen for tick salivary gland PLA_2 is similar to that in mosquito larvae PLA, which showed greatest activity at temperatures between 37-47°C (Rao and Subrahmanyam, 1969).

The activation of enzyme activity at submicromolar calcium levels would suggest that PLA_2 observed in A. americanum salivary glands is intracellular, as secreted PLA_2 require calcium concentrations in the millimolar range

for activation (Kramer et al., 1990; Diez and Mong, 1990).

A biphasic calcium dependence has been observed in human platelet PLA_2, which showed peak activities at 10uM and 2mM calcium concentrations (Kramer et al., 1988). Tick salivary gland phospholipase A_2 did not display this kind of behavior, as activity was no greater at millimolar calcium concentrations than the maximum activity observed at micromolar levels.

The results of the metal ion study are different from those obtained with other PLA_2 activities. In human osteoarthritic synovial fluid PLA_2 activity, other metal ions were completely unable to substitute for calcium in activating enzyme activity. Copper had an inhibitory effect on this PLA_2 activity in the presence of calcium, while magnesium did not (Parks et al., 1990). Hamster heart PLA_2 could be fully activated by either calcium or magnesium (Tam et al., 1984). Rat liver macrophage phospholipase A_2 activity was activated to a greater degree by magnesium and calcium together than by calcium alone (Krause et al., 1991). This contrasts sharply with the calcium-mediated activation of tick salivary gland PLA_2 activity, which was inhibited in the presence of magnesium. Mosquito larva PLA_2 was inhibited by calcium as well as by zinc (Rao and Subrahmanyam, 1969).

The intracellular PLA_2 found in human leukemic monocytes was found to be cytosolic in the absence of calcium, and became membrane-bound in its presence (Diez and

Mong, 1990). This phenomenon was not observed with tick salivary gland phospholipase A_2, which was similar to the cytosolic enzyme found in human platelets (Kramer et al., 1986) in that most activity was found in the supernatant of all centrifugations.

The phospholipase A_2 in A. americanum salivary glands was more specific for phosphatidylcholine than for phosphatidylethanolamine or phosphatidylinositol. Phospholipases A_2 with similar specificity for this phospholipid substrate include those found in human platelets (Cheung and Ballou, 1989) and in hamster heart (Cao et al., 1987).

Tick salivary gland PLA_2 appeared to show specificity in regard to fatty acid substrates, seemingly preferring arachidonic acid to linoleic, oleic, or palmitic acid in the 2-position. Similar results were seen in experiments on PLA_2 from human placental blood vessels (Karnauchow and Chan, 1985), hamster heart (Tam et al., 1984), and human platelets (Jesse and Cohen, 1976; Kim et al., 1988).

CHAPTER VI

SUMMARY AND CONCLUSIONS

The hard ticks have the ability to evade the immune responses of their vertebrate hosts. This ability may be due to eicosanoids in the saliva of these parasites. Eicosanoids are derived from arachidonic acid, which is usually released from cellular phospholipids by the action of phospholipase A_2 enzymes.

A phospholipase A_2 activity was found in the lone star tick, Amblyomma americanum, that was seen to be directly proportional to incubation time and salivary gland protein concentration. This PLA_2 was unstable when diluted to low total protein concentration, but was very stable when frozen. Tick salivary gland phospholipase A_2 was further characterized by a pH optimum of 9 and an incubation temperature optimum range of 37-47°C.

Activity was dependent on calcium, which could activate the PLA_2 at intracellular concentrations without mediation by calmodulin. Other metal ions could not fully substitute for calcium in activating the enzyme.

A. americanum salivary gland phospholipase A_2 appeared to be a cytosolic enzyme, with most observed activity occurring in the supernatant of centrifuged homogenates.

The substrate specificity of tick salivary gland PLA_2 was fairly high for the fatty acid in the 2-position as well as for the head group of phospholipid substrates, preferentially hydrolyzing arachidonic acid from the 2-position of phosphatidylcholine.

This study is not only the first reported finding of phospholipase A_2 in ticks, but is also the most extensive characterization of an arthropod non-secreted PLA_2 ever attempted. The information gained in this investigation will prove valuable as the basis for further studies on the characterization and regulation of this PLA_2 activity as it affects tick feeding. Hopefully, the knowledge gained from this work, especially in regard to enzyme stability, can lead to the eventual purification of the PLA_2 from the salivary glands of this tick species. Preliminary data on the saliva of A. americanum suggests that a secreted PLA_2 activity is present in this substance. Further work is needed to characterize tick salivary phospholipase A_2 if found. The techniques developed in this study can also be used to perhaps characterize the PLA_2 in the salivary glands of other tick species. Comparisons of the amount and properties of this enzyme activity between different tick species may give important clues as to why some ticks are more effective disease vectors than others.

REFERENCES CITED

Alonzo, F., P. M. Henson, and C. A. Leslie. 1986. A cytosolic phospholipase in human neutrophils that hydrolyses arachidonyl- containing phosphatidylcholine. Biochim. Biophys. Acta, 878: 273-280.

Axelrod, J., R. M. Burch, and C. Jelsema. 1988. Receptor-mediated activation of phospholipase A_2 via GTP-binding proteins: arachidonic acid and its metabolites as second messengers. Trends In Neurosciences, 11: 117-123.

Bennet, C.F., S. Mong, M. A. Clarke, L. I. Kruse, and S. T. Crooke. 1987. Differential effects of manoalide on secreted and intracellular phospholipases. Biochem. Pharmacol., 36: 733-740.

Bradford, M. M. 1976. A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein dye binding. Anal. Biochem., 72: 246-254.

Bridges, R. G. 1983. Insect phospholipids. In Metabolic Aspects of Lipid Nutrition in Insects, edited by T. E. Mittler and R. H. Dadd. Westview Press, Boulder, Colo. p.170.

Cao, Y., S. Tan, G. Arthur, H. Chen, and P. Clay. 1987. The purification and characterization of a phospholipase A in hamster heart cytosol for the hydrolysis of phosphatidyl- choline. J. Biol. Chem., 262: 16927-16935.

Chang, H. W., I. Kudo, M. Tomita, and K. Inoue. 1987a. Purification and characterization of extracellular phospholipase A_2 from peritoneal cavity of caseinate-treated rat. J. Biochem., 102: 147-154.

Chang, J., J. H. Musser, H. McGregor. 1987b. Phospholipase A_2: function and pharmacological regulation. Biochem. Pharmacol., 36:2429-2436.

Cheung, W. Y. and L. R. Ballou. 1989. Substrate- specific forms of phospholpase A_2 in human platelets. In Advances in Prostaglandin, Thromboxane, and Leukotriene Research vol. 19. Raven Press Ltd., New York. pp. 584-589.

Colard, O., M. Breton, and G. Bereziat. 1987. Hydrolysis of endogenous phospholipids by rat platelet phospholipase A_2: ether or acyl bond and polar head group selectivity. Biochim. Biophys. Acta, 921: 333-340.

Cosentino, M. J. and L. C. Ellis. 1981. A rapid simple radiometric assay for phospholipase A_2 activity. Prostaglandins, 22: 309-322.

Dennis, E. A., S. G. Rhee, M. M. Billah, and Y. A. Hannun. 1991. Role of phospholipases in generating lipid second messengers in signal transduction. FASEB J. 5: 2068-2077

Dickinson, R. G., J. E. O'Hagan, M. Schotz, K. C. Binnington, and M. P. Hegarty. 1976. Prostaglandin in the saliva of the cattle tick Boophilus microplus. Australian J. Exp. Biol. Med. Sci., 54:475-486.

Dijkstra, B. W., J. Drenth, K. Kalk, and P. Vandermaelen. 1978. Three-dimensional structure and disulfide bond connections in bovine pancreatic phospholipase A_2. J. Mol. Biol., 124: 53-60.

Diez, E. and S. Mong. 1990. Purification of a phospholipase A_2 from human monocytic leukemic U937 cells. J. Biol. Chem., 265: 14654-14661.

Douglas, C. E., A. C. Chan, and P. Choy. 1986. Vitamin E inhibits platelet phospholipase A_2. Biochim. Biophys. Acta, 876: 639-645.

Flower, R. J. and G.J. Blackwell. 1976. The importance of phospholipase A_2 in prostaglandin biosynthesis. Biochem. Pharmacol., 25: 285-291.

Franson, R., P. Patriarca, and P. Elsbach. 1974. Phospholipid metabolism by phagocytic cells. phospholipases A_2 associated with rabbit polymorphonuclear leukocyte granules. J. Lipid Res., 15: 380-388.

Gietzen, K., A. Wuthrich, and H. Bader. 1981. R24571: A new powerful inhibitor of red blood cell Ca^{++}- transport ATPase and of calmodulin- regulated functions. Biochem. Biophys. Res. Comm., 101: 418-425.

Gilfillan, A. M., A. J. Chu, D. A. Smart, and S. A. Rooney. 1983. Single plate separation of lung phospholipids including disaturated phosphatidylcholine. J. Lipid Res., 24: 1651-1656.

Hara, S., I. Kudo, H. W. Chang, K. Matsuda, T. Miyamoto, and K. Inoue. 1989. Purification and characterization of extracellular phospholipase A_2 from human synovial fluid in rheumatoid arthritis., J. Biochem., 105: 395-399.

Hazen, S. L., R. J. Stuppy, and R. W. Gross. 1990. Purification and characterization of canine myocardial cytosolic phospholipase A_2 . J. Biol. Chem., 265: 10622-10630.

Holtzman, M.J. 1991. Arachidonic acid metabolism. Am. Rev. Respir. Dis., 143: 188-203.

Horigome, K., M. Hayakawa, K. Inoue, and S. Nojima. 1987. Purification and characterization of phospholipase A_2 released from rat platelets. J. Biochem., 101: 625-631.

Jain, K. J. and O. G. Berg. 1989. The kinetics of interfacial catalysis by phospholipase A_2 and regulation of interfacial activation: hopping versus scooting. Biochim. Biophys. Acta, 1002: 127-156.

Jain, M. K. and D. V. Jahagirdar. 1985. Action of phospholipase A_2 on bilayers.Effect of inhibitors. Biochim. Biophys. Acta, 814: 319-326.

Jesse, R. L. and P. Cohen. 1976. Arachidonic acid release from diacyl phosphatidylethanolamine by human platelet membranes. Biochem. J., 158: 283-287.

Karnauchow, T. M. and A. C. Chan. 1985. Characterization of human placental blood vessel phospholipase A_2 , demonstration of substrate selectivity for arachidonyl-phosphatidylcholine. Int. J. Biochem. 17: 1317-1319.

Kaufman, W. R. 1989. Tick- host interaction: a synthesis of current concepts. Parasitology Today, 5: 47-56.

Kim, D. K., I. Kudo, and K. Inoue. 1988. Detection in human platelets of phospholipase A_2 activity which preferentially hydrolyses an arachidonyl residue. J. Biochem. 104: 492-494.

Kramer, R. M., G. C. Checani, A. Deykin, C. R. Pritzker, and D. Deykin. 1986. Solubilization and properties of Ca^{2+}-dependent human platelet phospholipase A_2. Biochim. Biophys. Acta, 878: 394-403.

Kramer, R. M., J. A. Jakubowski, and D, Deykin. 1988. Hydrolysis of 1-alkyl-2-arachidonyl-sn-glycero-3-phosphocholine, a common precursor of platelet-activating factor and eicosanoids by human platelet phospholipase A_2. Biochim. Biophys. Acta, 959: 269-279.

Kramer, R. M., C. Hession, B. Johansen, G. Hayes, P. McGray, E. P. Chow, R. Tizard, and R. D. Pepinsky. 1989. Structure and properties of a human non- pancreatic phospholipase A_2. J. Biol. Chem., 264: 5768-5775.

Kramer, R. M., B. Johansen, C. Hession, and R. B. Pepinsky. 1990. Structure and properties of a secretable phospholipase A_2 from human platelets. In Phospholipases A_2, edited by P. Y. K. Wong and E. A. Dennis. Plenum Press, New York. pp. 55-81.

Krause, H., P. Dieter, A. Specking, A. Ballhorn, E. Ferber, and K. Decker. 1991. Synergistic effect of magnesium and calcium ions in the activation of phospholipase A_2 of liver macrophages. Biochem. Biophys. Res. Comm., 175: 532-536.

Korneev, A. S., S. I. Salikhov, and M. U. Tuichibaev. 1989. Amino acid sequence of orientotoxin I and orientotoxin II from the hornet Vespa orientalis venom. Bioorg. Khim., 15: 127-129.

Kuchler, K., M. Gmachl, M. J. Sippl, and G. Kreil. 1989. Analysis of the cDNA for phospholipase A_2 from honeybee venom glands. The deduced amino acid sequence reveals homology to the corresponding vertebrate enzymes. Eur. J. Biochem., 184: 249-254.

Loeb, L. A. and R. W. Gross. 1986. Identification and purification of sheep platelet phospholipase A_2 isoforms. J. Biol. Chem., 261: 10467-10470.

Lynch, D. V. and G. A. Thompson. 1986. Analysis of phospholipid molecular species by gas chromatography and coupled gas chromatography- mass spectrometry. In Modern Methods of Plant Analysis, edited by H. F. Linskens and J. F. Jackson. Springer- Verlag, Berlin. pp. 100-120.

Marshall, L. A. and J. Y. Chang. 1990. Pharmacological control of phospholipase A_2 activity in vitro and in vivo. In Phospholipase A_2, edited by P. Y. K. Wong and E. A. Dennis. Plenum Press , New York. pp. 169-182.

Parks, T. F., S. Lukas, and A. F. Hoffman. 1990. Purification and characterization of a phospholipase A_2 from human osteoarthritic synovial fluid. In Phospholipase A_2, edited by P. Y. K. Wong and E. A. Dennis. Plenum Press, New York. pp. 55-81

Patrick, C. D. and J. A. Hair. 1975. Laboratory rearing procedures and equipment for multi- host ticks (Acarina: Ixodidae). J. Med. Entomol., 12: 389-390.

Perrin, D. O., and I. G. Sayce. 1967. Computer calculation of equilibrium concentrations in mixtures of metal ions and complexing species. Talanta, 14: 833-842.

Rao, R. H. and D. Subrahmanyam. 1969. Studies on the phospholipase A in larvae of Culex pipiens fatigans. J. Insect Physiol., 15: 149-159.

Ribeiro, J. M. C. 1986. Role of saliva in feeding by a hard tick. In Host- Regulated Developmental Mechanisms in Vector Arthropods . edited by Dov Borodovsky and Andrew Spielman. pp. 121-124.

Ribeiro, J. M. C., G. T. Makoul, J. Levine, D. R. Robinson, and A. Spielman. 1985. Antihemostatic, antiinflammatory, and immunosuppressive properties of the saliva of a tick, Ixodes dammini. J. Exp. Med., 161: 332-344.

Ribeiro, J. M. C., G. T. Makoul, and D. R. Robinson. 1988. Ixodes dammini: evidence for salivary prostacyclin secretion. J. Parasitol., 74: 1068-1069.

Rubin, R. P., R. H. Thompson, and S. G. Laychock. 1990. Characterization of phospholipase A_2 in purified zymogen granule membranes. Biochim. Biophys. Acta.1045: 245-251.

Schalkwijk, C. G., F. Marki, and H. Van den Bosch. 1990. Studies on the acyl- chain specificity of cellular phospholipases A_2. Biochim. Biophys. Acta. 1044: 139-146.

Schulze, T. L., G. S. Bowen, E. M. Bosler, M. F. Lakat, W. E. Parkin, R. Altman, B. G. Ormiston, and J. K. Shisler. 1984. Amblyomma americanum: a potential vector for Lyme disease in New Jersey. Science, 224: 601-603.

Shemesh, M., A. Hadani, A. Shklar, L. S. Shore, and F. Meleguir. 1979. Prostaglandins in the salivary glands and reproductive organs of Hyalomma anatolicum excavatum Koch (Acari: Ixodidae). Bulletin of Entomological Research, 69: 381-385.

Simpson, I. and O. Sonne. 1982. A simple, rapid, and sensitive method for measuring protein concentration in subcellular membrane fractions prepared by sucrose density ultracentrifugation. Anal. Biochem., 119: 424-427.

Stefanski, E., W. Pruzanski, B. Sternby, and P. Vadas. 1986. Purification of a soluble phospholipase A_2 from synovial fluid in rheumatoid arthritis. J. Biochem., 100: 1297-1303.

Tam, S. W., R. Y. K. Man, and P. C. Choy. 1984. Hydrolysis of phosphatidylcholine by phospholipase A_2 in hamster heart. Can. J. Biochem. Cell Biol., 62: 1269-1274.

Van den Bosch, H. 1980. Intracellular phospholipases A_2. Biochim. Biophys. Acta, 604: 191-246.

Verger, R. and G. H. de Hass. 1976. Interfacial enzyme kinetics of lipolysis. Ann. Rev. Biophys. Bioeng., 5: 77-117.

VITA

Mark R. Surdick

Candidate for the Degree of

Master of Science

Thesis: PHOSPHOLIPASE A_2 ACTIVITY IN THE SALIVARY GLANDS OF THE LONE STAR TICK Amblyomma americanum (L.) (ACARINA: IXODIDAE)

Major Field: Entomology

Biographical:

Personal Data: Born in Fort Worth, Texas, August 17, 1962, the son of Thomas A. and Shirley Surdick.

Education: Graduated Northeast High School, Oklahoma City, Oklahoma, in May 1980; Received Bachelor of Science Degree in Biology from Oklahoma State University in December 1984; completed requirements for the Master of Science degree at Oklahoma State University in July, 1991.