AGING OF UNFED ADULT FIELD REARED LONE STAR TICKS, AMBLYOMMA AMERICANUM (L.)

By

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LONE STAR TICKS, AMBLYOMMA

AMERICANUM (L.)

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PREFACE

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CHAPTER I

INTRODUCTION

Aging in animals is an interesting process. For example, the age distribution of a population of insects may influence the effectiveness of a control program. Adult ixodid ticks have been observed to live for up to three years in a free-living, unfed stage, close to the ground, preferring areas with low dense brush (Hoch et al., 1971). Nymphal stages also overwinter in the same type of habitat. Replete ticks of both stages enter diapause and overwinter until the following spring at which time they molt to the next successive stage. Because of this overlapping of generations, "old" and "young" ticks may coexist in the same population. To date, the best method to control Lone Star ticks includes vegetative management in conjunction with the broadcast use of acaricides. Although these methods are effective, differential susceptibility of ticks of different ages has been observed (Mount, personal comm. 1981). The reported life stage susceptibility of Lone Star ticks to acaricides, is: larvae> nymphs> adult males> adult females. The ability to determine the age distribution

for a given population would allow for optimization of spraying times and rates, and improve control while reducing the likelihood of developing a resistant strain of tick.

The study of aging is also of gerontological interest. Rockstein (1973), and Miquel et al. (1981), state that insects grow old and manifest aging processes (in postmitotic tissue such as heart and nervous tissue) identically to those of higher vertebrates. This and their small size, short lifespan, availability and manageability are reasons why arthropods are used increasingly by biologists, biochemists, biophysicists, and entomologists as experimental organisms. Insects have cells, which under the microscope, are indistinguishable from those of higher animals (Smith 1968).

Curtis (1963) reviewed several theories of aging including the "wear and tear" theory, the somatic mutation theory, and a theory which states that somatic cells accumulate a certain number of mutations culminating in senescence and death. Rockstein (1973) stated that aging is controlled genetically, and by the environment. A theory of aging based on free radical lipid peroxidation was proposed by Harman (1956). He suggested that a species lifespan is an expression of the genetic control over the rate of oxygen utilization. This rate determines the rate of accumulation of injuries to the mitochondria by free radical reactions. The rate of mitochondrial

damage increases with the rate of oxygen consumption. Tappel (1965) discussed the role of the antioxidants selenium and Vitamin E in inhibiting damage to cells by free radical lipid peroxidation. Harman (1982) sited numerous references where decreased rates of senesence were a result of dietary modifications such as increased consumption of antioxidants. The free radical theory is based on the observation that free radicals cause irreversible membrane damage, or cross linking of biologically important molecules. The initiation step in free radical production is the formation of the superoxide anion (O -) by the addition of a single electron to oxygen (Uhlen,1981).

The free radical theory provides evidence to account for such cellular phenomena as the accumulation of aging pigment (lipofuscin), and the decrease in the number of functionally intact mitochondria (Economos et al., 1980). In a recent study, Fleming et al. (1982) suggested that the initial site of attack in the free radical process is the mitochondrial genome, rather than various membranes as suggested by Harman.

Different morphological, physical and physiological characteristics have been examined in the past as age indicators. Neville (1963) reported a method for aging locusts by counting the daily growth layers of cuticle, and Johnston and Ellison (1982) outlined a method of counting growth layers of apodemes in Drosophila. Hylton

(1966) reported a loss of nuclei and a decrease in nuclear size, along with a loss of cross striations in muscle fibers with increasing age in mosquito flight muscle.

Hodge (1894) noted that nerve cells were more numerous in young than old animals, and that nuclei of the older animals were more pycnotic. An age dependant decrease in nerve lipids was seen by Hofteig et al. (1981). Corbin and Gardiner (1937) noted a decrease in the number of myelinated nerve fibers in the human spinal cord with increasing age, and Wheeler (1982) found a decreased capacity for glutamate (neural transmitter) transport in older animals. Gardiner (1940) considered the presence of aging pigment in spinal ganglia to be associated with increased age. The accumulation of lipofuscin has been well documented in many organisms (Brody, 1960; Samorajski et al., 1964; Reichel et al., 1968; Brizzee and Johnson, 1970; Brizzee, 1974; and Goyal, 1982). Goyal (1982) reported that the pigment accumulates linearly with age. Sulkein and Kuntz (1952) first characterized the accumulating lipofuscin pigment in the ganglion cells of senile dogs as a periodic acid fuschin positive substance containing mucopolysaccharides. Histochemical properties of aging pigment was summarized by Strehler (1964). He characterized lipofuscin as a PAS positive complex of cross-linked polyunsaturated lipid and protein. Brody (1960) found that there is an increase in the percentage of cells containing small amounts of

pigment in the human cerebral cortex with increasing age. After reviewing the literature, Samorajski et al. (1968) concluded that accumulation of age pigment is the most consistent evidence of nerve cell aging.

The process of aging has been widely studied in arthropods. Rockstein (1963) noted a decrease in enzyme activity in the flight muscle of the house fly (Musca domestica) with increasing age. In 1956 he noted a concomitant decrease in the acitvities of acid sodium beta-glycerophosphatase and magnesium activated adenosine triphosphate, as did Press et al. (1966). Simon (1969) studied the house fly and noted changes in the mitochondria of flight muscles. Rockstein and Bhatnager (1965) also noted changes in the size and number of giant mitochondria in the flight muscle of the house fly. Takahashi (1970a,b,c) reported a greater abundance of crystalloid material accumulating within the mitochondria of senescent flies.

The ability of ticks to absorb water from the atmosphere has been established (Lees, 1964a; Sauer and Hair, 1971). Generally, older organisms are less capable of carrying out vital physiological processes. The critical equilibrium humidity (CEH) is defined as the relative humidity at which an organism can absorb water from unsaturated air (Sauer and Hair, 1971). Lees (1946, 1964) found that the CEH of Ixodes ricinus increased as its food reserves became depleted. Fairbanks and Burch

(1970) found that susceptibility to water loss increased with increasing age in both sexes of houseflies, and related this to an increased impairment of the spiracular closing mechanism. Water content was negatively correlated with the percentage of fat content in both sexes of houseflies (Fairbanks and Burch, 1970). Jaworski et al. (1983) found no significant change in water content of Lone Star ticks during the course of a one year laboratory study.

Gilby (1964) noted that the lipid composition of an insect can be variable depending on the physiological state and age of the organism. Total body lipid content is an available energy source that should decrease with age. Tan (1973) found that total cholesterol and triglycerides declined with age while phospholipid content remained constant in the cave roach. Cook (1973) found that total lipid content did not change in a 102 day experiment with Ornithodorus concanensis to alternating periods of dessication and rehydration. Balashov (1972) noted numerous lipid inclusions in gut cells lacking hemoglobin inclusions. He went on to add that fat and glycogen are apparently renewed constantly by slow assimilation of blood proteins.

Several investigators have found that the hemoglobin content of the tick decreases with age (Tatchell, 1964; Balashov, 1972; Cook, 1973; and Jaworski et al. 1983). Sutton and Aurthur (1962) showed that ticks of equal

weight ingested variable amounts of hemoglobin. The blood is ingested primarily in the form of oxyhemoglobin with smaller quantities of methemoglobin (Smit et al. 1977). Smit (1977) reported that oxyhemoglobin was transformed into methemoglobin, whereas Tatchell (1964), and Balashov (1972) both reported the presence of both forms of hemoglobin. Balashov and Raichel (1976) described the histology of the tick gut as it relates to digestion of the blood meal. Balashov (1972) reported that as the hemoglobin is digested, brown black hematin crystals are formed. The hematin crystals often agglutinate into larger inclusions. Using the characteristics of hemoglobin content, gut ceacal distention, and Malpighian tubule content, Balashov catagorized unfed adult ticks in four groups of physiologically similar age that included: Group 1: Recently molted ticks which are in the postmolting development stage, where the terminal ends of digestive cells are filled with hemoglobin and hematin crystals. Group 2: Recently molted ticks that have completed the postmolting process and digestive cells are much smaller and contain many hemoglobin inclusions which are mostly distal and discontinuous. The hematin crystals are scattered throughout the cells and the Malpighian tubules are transparent with medium to large guanine inclusions. Group 3: Ticks that have been active for long periods of time, the caecal diameter is greatly reduced with numerous constrictions and a large lumen.

Digestive cells lack hemoglobin inclusions, and the Malpighian tubules appear beaded. Group 4: Ticks that have been unfed for a long time and the ceacum is very slender with deep constrictions and with an obscure lumen. Digestive cells never contain hemoglobin inclusions but large aggregates of hematin crystals are present. The Malpighian tubules are greatly expanded and appear packed with guanine.

Razumova (1977) classified ticks into various physiological age groups based on the parameters of overall color, flatness, gut ceacal distention and toughness of the cuticle.

Tatchell (1964) reported that the ingestion of blood is accompanied by the destruction of the existing gut epithelium in Argus persicus (Oken). Till (1961) found that the epithelial cells enlarge greatly upon ingestion of the bloodmeal. Balashov and Raikhel (1976) stated that gut cells differentiate during tick feeding. They classed the cells as undifferentiated, digestive (Type I and Type II), and secretory. In argasid ticks, Tatchell (1962, 1964) found that the blood remained unlysed for two or three days while a new epithelium was synthesized. Tatchell went on to divide blood digestion into a rapid and slow phase. The rapid phase persists for one to two weeks while the slow phase continues at a relatively constant rate until the next meal. The amount of blood left after the rapid phase is determined by sex and the

condition of the tick. In the absence of significant fat and glycogen reserves it serves as a food reserve. Cook (1973) found that repeated dessication and rehydration depleted hemoglobin concentrations at a faster rate than ticks kept at elevated humidities.

CHAPTER II

BIOASSAYS AND THE CRITICAL EQUILIBRIUM

Six groups of 5000 ticks, (2500 males and 2500 females in each) , were placed in outdoor cages (Semtner et al., 1973). The first three groups were placed in the field at one month intervals beginning in August, 1981. Another three (Groups 4-7) were placed in the field begining in April, 1982. Another smaller group, Group 5 (2500 ticks) was set out in May, 1982 to study young and old ticks.

Unfed nymphs, previously held at 25° C and 98% relative humidity, were fed on rabbits obtained from local suppliers. Ticks in groups 1 through 4 were fed on rabbits previously unexposed to tick infestation. Ticks in groups 5 through 7 were fed on rabbits that had been parasitized once previously in the laboratory. Therefore, data were analyzed as two separate classes in order to account for possible host resistance effects on results. Replete nymphs were placed in a paper carton and sealed with clear plastic (Handiwrap) fastened by a rubber band. Ticks were stored continuously in environmental chambers (25 C;° 95% R.H.; 14 hours light : 10 hours dark), until

they had molted. Four weeks were required for all nymphs to molt to adults after feeding.

After ecdysis, 5000 ticks (2500 males and 2500 females), were aspirated with an air pump and counted. Ticks were then placed in screened cages (Semtner et. al. 1973) located in a riparian woodlot near the Medical Entomology Laboratory at the Oklahoma State university in Stillwater, Oklahoma.

Temperature and humidity were measured continuously with a Belfort Instrument Hydrothermograph. Temperatures and humidities were also measured at 1, 15, and 30 cm. above the ground near cages with a YSI Tele-Thermometer, and a Cole and Parmer Hygrometer. Precipitation and temperature records were also obtained from a nearby University Weather Station.

Three groups of approximately 5000 ticks were placed in the field at one month intervals beginning in August, 1981. After one day in the field, 300 ticks (150 males and 150 females), were removed for assay and were designated 0 month post-molt ticks. Three hundred ticks were removed from each cage at two month intervals thereafter (Table 1). In later months (10 and 12), only 200 ticks (100 males and 100 females) per group, were removed and assayed. Young ticks (0-8 months) were predessicated at 32% R.H. for 24 hours to remove surface body water prior to assay. Older ticks (10-12 months) were predessicated at 75% R.H. for only 12 hours because

of tick mortality at the lower predessication relative humidities and longer time intervals.

TABLE I

EXPERIMENTAL SAMPLING SCHEDULE. THE FIRST MONTH IN EACH COLUMN REPRESENTS ZERO MONTHS POST MOLT. SUCCEEDING MONTHS REPRESENT MONTHS IN WHICH SAMPLES WERE TAKEN

Groups						
1	2	3	4	5	6	7
AUG'81	SEPT	OCT				
OCT	NOV	DEC				
DEC	JAN82	FEB				
FEB	MARCH	APR	APR	MAY	JLY	AUG
APR	MAY	JNE	JNE	-	SEPT	OCT
JNE	JLY	AUG	AUG	-	NOV	DEC
AUG	SEPT	OCT	OCT	NOV		
			DEC			

Assays consisted of the following: 1). Measuring whole body heme containing compounds. A certified hemoglobin standard (Sigma Chemical Co.) was serially

diluted with Drabkins reagent containing saponin to lyse erythrocytes and a cyanmetheme reagent to oxidize iron in heme to the ferric state. The latter reacts with cyanide ions, from potassium ferricyanide, to form cyanmetheme compounds whose absorbance is measured spectrophotometrically. The standard was diluted from 1600 ug/2000 ul to 1200, 800, 400, 200, and 100 ug/2000 ul. Ten male and ten female ticks (in separate analyses) were weighed and homogenized in 1 ml of Drabkins reagent. After homogenization, another 1 ml of Drabkins reagent was added to the homogenate and centrifuged at 5676 g three times for 15 minutes. The final supernatant was transferred to a clean cuvette and read spectrophotometrically with a Beckman DU spectrophotometer (with a Gilford update) at 540 nm, and compared to the hemoglobin standards prepared in the same way. Results are expressed as hemoglobin equivalents.

2. Total water content. Ten male and ten female ticks were weighed separately, and placed in separate glass containers, and dried at 60 C. Ticks were reweighed at 24 hour intervals until a constant weight was obtained. Total water content was interperated as being the difference between wet weight and final dry weight.

3. Total lipid assay was a modified version of that described by Keleti and Lederer (1974). Ten ticks

of each sex were weighed separately and then homogenized in 1 ml of chloroform. Methanol and water were added to the chloroform homogenate (2:1:1) and vacuum filtered through Whatman #1 filter paper. The beaker was rinsed with chloroform and water (2:1) and the rinse filtered again. Water was added to the filtrate in the same proportion as methanol. The final filtrate was transferred to a graduated test tube vigorously agitated and the contents allowed to separate into two layers; the volume (V1) of the bottom layer (chloroform and lipid) was measured and recorded. The top layer (methanol and water) was removed by a pipette and the remaining volume measured (V2). This was done in order to account for any loss of the chloroform and lipid layer during removal of the top layer. The chloroform-lipid layer was transferred to a preweighed beaker (W1) and chloroform allowed to evaporate under a hood. The beaker with lipid was weighed (W2), washed with a chloroform and then reweighed. Total lipid content was derived from the following : Lipid= (W2-W1) V1/V2 .

4. Critical equilibrium humidity. Nine chambers with different relative humidities (36-96%) were established with saturated salt solutions (Winston and Bates, 1960) (Table 2). Ticks were predessicated at 32% relative humidity for 24 hours to remove surface body water. A screened platform was suspended from

the top of the chamber by wire, and the chamber (1.79 liter) was sealed with two layers of Parafilm. Ten male and ten female ticks, from each group to be assayed, were preweighed. The containers were then placed in the humidity chambers and held constantly at 14 : 10 LD photoperiod and 25° C 2 C. After one week the ticks were removed from the chambers and reweighed. The relative humidities in each chamber were checked during each experiment. It was necessary to reduce the time for holding older ticks (10-12 months) in the humidity chambers to 12 hours to avoid mortality.

Results

The total concentration of heme containing compounds decreased significantly as ticks aged in this study. The average initial concentration was 43.25 ug/mg tick weight in males and 46.80 ug/mg tick weight in females, ranging from 32 to 72 ug/mg in the female and 38 to 61 ug/mg in males of Groups 1-4 (Table 3). The concentration of the heme compounds decreased to 12 ug/mg in females and 24 ug/mg in males after 12 months ($P<.009$ and .007, female, male respectively). Regression analysis indicated that the decrease in heme compounds was linear with respect to time (increasing age) (Figure 1). Interestingly, ticks fed on rabbits previously exposed to ticks (Groups 5-7) did not demonstrate a significantly different heme content in 0

TABLE II

SATURATED SALT SOLUTIONS AND THE RELATIVE HUMIDITIES FOR EACH OF NINE ENVIRONMENTAL CHAMBERS

SOLUTIONS	RELATIVE HUMIDITIES
MgCl	32%
KPO	47%
NaCl	66%
NaCl + sucrose	77%
KCl	84%
Glucose	87%
KNa tartarate	89%
KBr	88%
K SO	93%

and 12 month ticks. However, amounts were significantly less (ranged from 18-19 ug/mg tick weight in females) than those measured for ticks of the same age in Groups 1-4.

The concentration of lipid decreased with increasing age of females of Groups 1-4 ($P < .01$). There was an apparent decrease in lipid concentration in males but variation reduced the statistical

TABLE III

AVERAGE HEME CONCENTRATION (ug/mg TICK WEIGHT + STD DEV) OF EACH SEX AT A PARTICULAR AGE

	Groups 1-4		Groups 5-7	
Age (Mo.)	Male	Female	Male	Female
0	43.3+13.5	46.8+17.7	24.8+1.9	18.6+0.6
2	33.0+14.4	26.1+13.0	37.8***	19.2+1.1
4	29.8+17.2*	19.1+ 8.0	___	18,7+1.3**
6	28.1+10.0	22.5+ 7.6	14.1***	12.2 **
8	21.8+ 8.1	22.3+13.0		
10	17.5+7.5**	23.5+ 7.1		
12	24.4 ***	12.5+ 0.3**		

* = Mean of 3 replicates
** = Mean of 2 replicates
*** = 1 replicate
undesignated = Groups 1-4 4 replicates
Groups 5-7 3 replicates

significance (P=.10) (Table 4, Figure 2). Significant differences were not observed in lipid content between various ticks in Groups 5-7.

Figure 3 illustrates the difference in ability of ticks of various ages to absorb water. There was a decrease in the ability of ticks of 10 and 12 months of

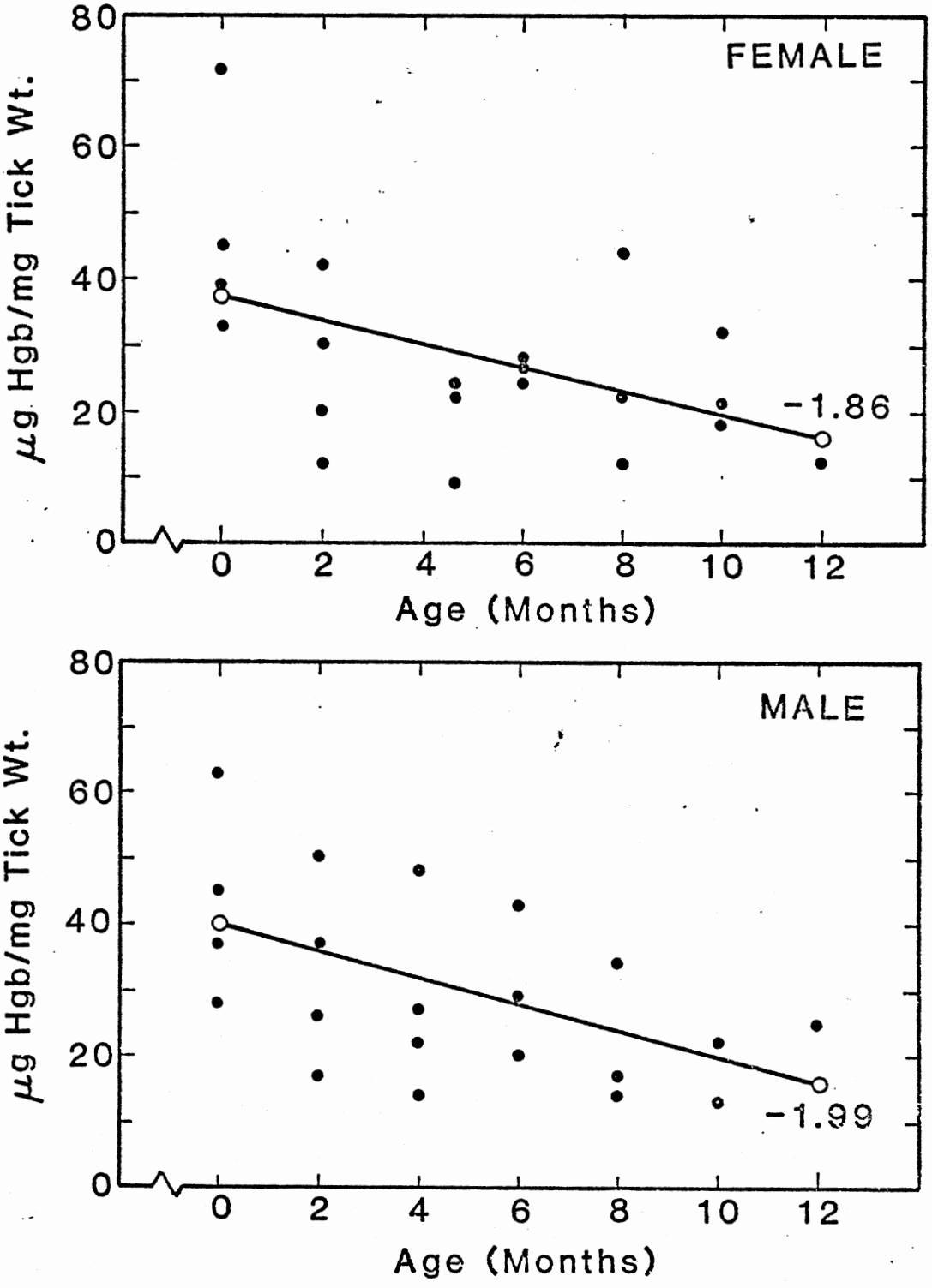

Figure 1. Slopes indicate the decrease in the concentration of heme containing compounds with increasing age in adult Lone Star ticks

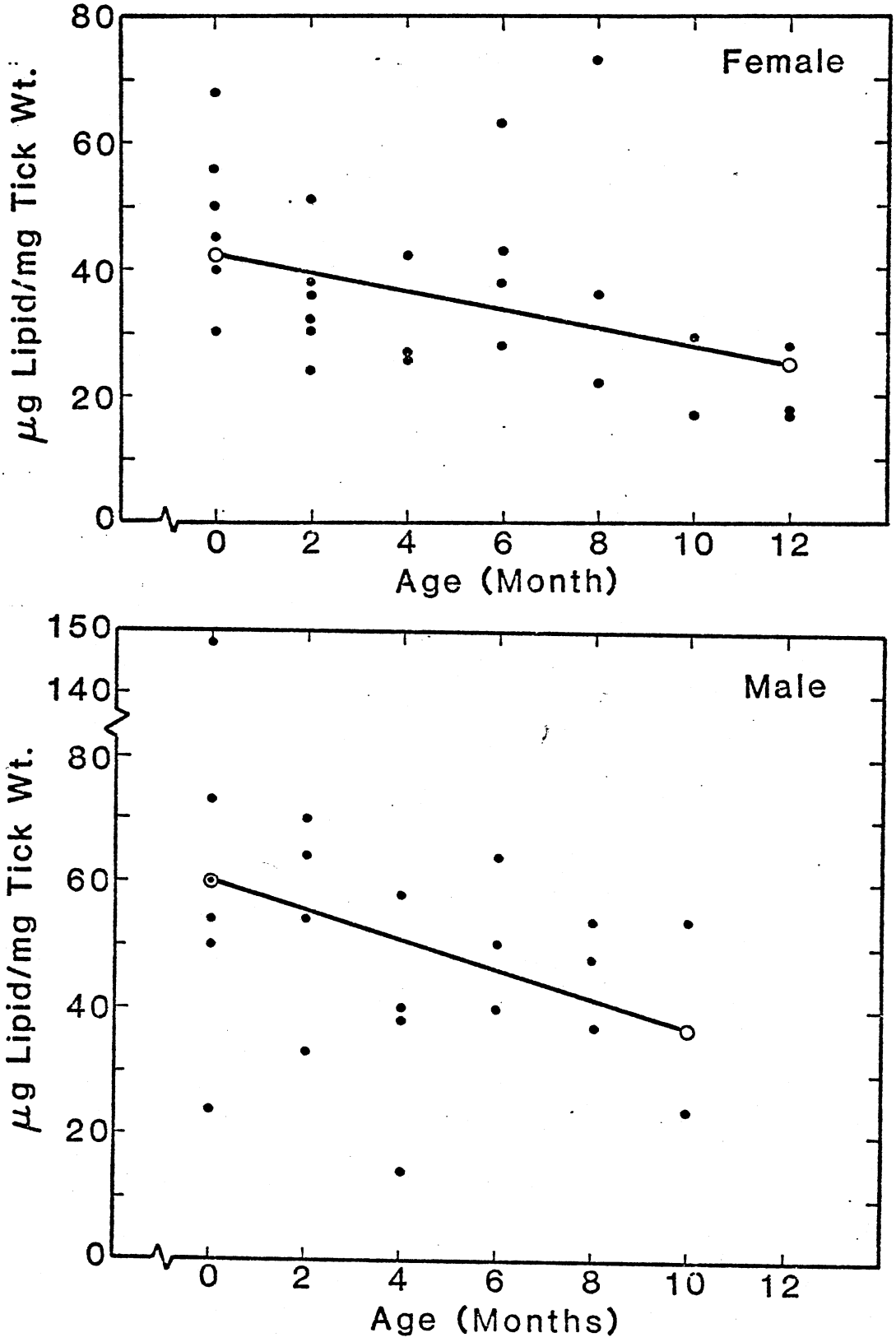

Figure 2. Figures illustrate the decrease in lipid concentration with increasing age for both male and female ticks.

TABLE IV

AVERAGE LIPID CONCENTRATION (ug/mg TICK WEIGHT) ± STD DEVIATION. AVERAGE FOR THE SEX AT A GIVEN AGE

	Groups 1-4		Groups 5-7	
Age (Mo.)	Male	Female	Male	Female
0	587.6±120.3	421.0±146.8	414.4±160.5	410.9±240.3
2	542.3±166.2	352.0±114.7	___	341.8± 37.1
4	378.4±182.4	334.3± 91.3	409.1± 84.2*	437.7± 63.3*
6	499.9±120.0*	422.5±141.6	602.9 **	314.9 **
8	432.8± 89.5	314.2± 91.2*		
10	375.1±216.0**	201.0± 72.6*		
12	___	215.7± 67.1**		

Undesignated = 4 replicates
* = Mean of 3 replicates
** = Mean of 2 replicates

undesignated = 3 replicates
* = Mean of 2 replicates
** = 1 replicate

age to maintain water balance as indicated by an apparent increase in the CEH. Analysis of variance showed no significant net uptake of water vapor in females for the first four months and in males for the first two months.

The water content percent in adult field reared ticks from Groups 1-4 did not change significantly in

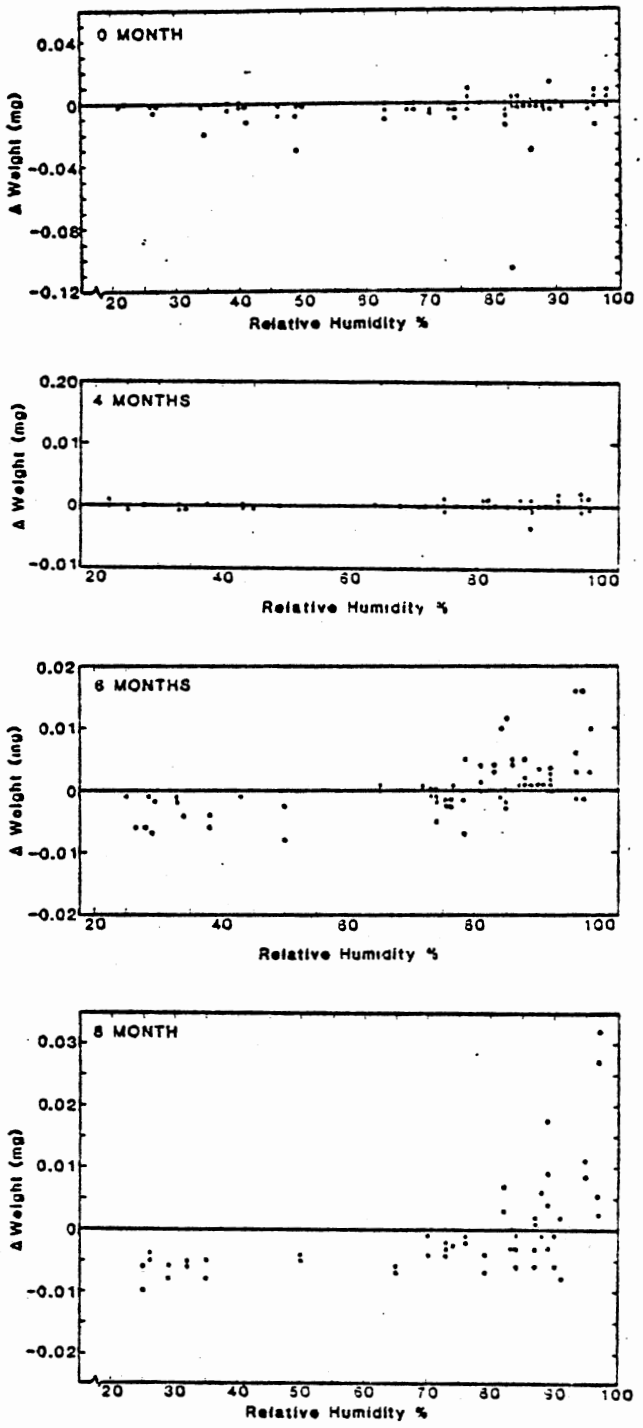

Figure 3. Critical Equilibrium Humidity. Figures illustrate the shift in the ability to absorb atmospheric water

relation to age. However, the water content was significantly different between the sexes (Table 5). In all age groups males had a higher average percent water content than did females (P< .0008 level) (Table 5).

TABLE V

THE AVERAGE PERCENT WATER CONTENT FOR A SEX AT A PARTICULAR AGE

	Groups 1-4		Groups 5-7	
Age (Mo.)	Male	Female	Male	Female
0	65.4±18.9	56.6± 8.8	63.8± 9.1	60.6± 5.0
2	69.7±20.3	66.2±12.9**	___ ***	54.8± 4.1
4	78.9±17.1*	57.9± 7.9	62.2± 4,5**	53.7± 2.4**
6	64.8± 8.2*	58.4± 5.2	___ ***	___ ***
8	69.8± 4.4*	70.6±14.6		
10	70.9±11.2**	62.7± 8.5**		

Undesignated = 4 reps Undesignated = 3 reps.
*= Mean of 3 replicates **= Mean of 2 replicates
= Mean of 2 replicates *= 1 replicate

Discussion

There was no obvious effect of weather on the rate of aging. Although this is an important cue in the seasonal cycle of ticks, this study did not reveal any notable seasonal effect on the aging process. It is important to emphasize that since weather and other climatological factors are so complex, a more detailed study would be necessary to determine the effects on aging in Lone Star ticks.

It was not possible to precisely monitor survivorship because of problems with tick escape from cages during the course of the present study. However, of the ticks which did not escape, none survived longer than one year and females generally outlasted males. Lone Star ticks were reported to survive for up to 3 years in the field in eastern Oklahoma (Semtner et al., 1973). Inability of ticks to survive for longer than one year in the present study may have been caused by catalpa caterpillars which denuded catalpa trees (Catalpa bignonioides), during the summer of 1982 and gradual disappearance of leaf litter in cages. Both conditions probably decreased the relative humidity within micro-habitats in the cages, and the adverse effects of lowered humidity of tick survival is well known (Hoch at al., 1970). Also, Stillwater is approximately 70 miles west of the reported normal

heavy populations of Lone Star ticks (Hair et al., 1975) and so other geographical and/or climatological factors may not have been optimal for long-termed survival.

Hemoglobin and its metabolites represent a major nutrient scource and reserve for adult ticks (Balashov, 1972; Jaworski et al., 1983). This is supported by the decreasing concentration of heme containing compounds (expressed as hemoglobin equivalents) with age in both males and females ($P < .009$ and .007 respectively).

The results show that the lipid concentration decreased significantly in females but not significantly ($P > .05$) in males. This is an important difference between ticks held under field conditions and ticks held under constant laboratory conditions. In studies where ticks were held in the laboratory for one year, no significant change in the lipid concentration was observed in adult ticks (Jaworski et al., 1982). No change in lipid was seen in ticks repeatedly dessicated and rehydrated for 102 days (Cook, 1973).

Water content did not change significantely with increased tick age. The importance of water regulation was further exemplified in critical equilibrium experiments (CEH) where older ticks were more susceptible to water loss and death at lower humidities

than young or newly molted ticks. This may support Lees (1964) conclusion that the CEH of ticks increased with increasing tick age, but because influx and efflux of water were not measured in the present study it is not possible to say if this change was due to an increase in the CEH or change in the ability of the tick to retain water. Scanning electron microscopic examination of ticks of various ages from the field indicated that the integrity of the external surface of the cuticle deteriorates somewhat with increasing age (Chapter 3). Further studies that measure unidirectional flux and water exchange kinetics should be conducted to resolve this question.

The data obtained from Groups 5-7 is of interest because of possible host effects on tick feeding. The range of concentrations of heme compounds in ticks of groups 1-4 were significantly higher than ticks from Groups 5-7. However, the average weights of ticks in the two classes were not significantly different. This suggests that prior exposure of hosts in these experiments was more important in determining the nature than quality of the nymphal ticks meal. It is not known if these differences are important to the overall physiology of the tick.

CHAPTER III

MICROSCOPICAL CHANGES IN ADULT TICKS

Ticks were obtained from cages as previously described, and were examined over the same time intervals (Chapter II).

The midgut from four male and four female ticks were dissected in a 2% gluteraldehyde, cacodylate buffer, at pH 7.2. Methods and schedule for fixing and embedding tissue are outlined in Table 6.

The ticks were dissected on sticky tape in a disposable petri dish. The dorsal cuticle was removed with single eged razors and forceps. Integrity of tissue was maintained by fixing *in situ* with gluteraldehyde placed directly into the hemocoel for two hours.

Fixed and embedded tissue was sectioned on a Sorvall MT-2 ultramicrotome and transferred to 300-mesh copper grids. The grids were post stained with uranyl acetate, and lead citrate and viewed with a Philips 200 transmission electron microscope. Thick sections, prepared from epoxy resin blocks, stained with Mallorys' trichrome were also examined at the light

TABLE VI

FIXATION, DEHYDRATION, AND EMBEDDING PROCEDURES

1). The tissue is fixed with buffered gluteraldehyde in situ. Dissected ticks are fixed in this solution for two hours.

2). Tissue is dissected from tick and placed in a buffered gluteraldehyde solution for an additional hour.

3). The tissue is washed in buffer three times for 15 minutes each.

4). Tissue is post-fixed in 2% OsO_4 for three hours. The solution is OsO_4 and cacodylate buffer (1:1).

5). After post-fixation the tissue is buffer washed three times for 15 minutes each. (This is a good place to stop, store overnight in refrigerator).

6). Dehydration.

a) 50% Ethanol	20 minutes
b) 70% Ethanol	20 minutes
c) 80% Ethanol	20 minutes
d) 90% Ethanol	20 minutes
e) 100% Ethanol	20 minutes
f) 100% Ethanol	20 minutes
g) 100% Ethanol	20 minutes
h) propylene oxide	20 minutes
i) propylene oxide	20 minutes
j) propylene oxide	20 minutes

7). infiltration 1:1 propylene oxide and DER (capped) for 72 hours.

8). Infiltration 1:1 propylene oxide and DER (uncapped) for 24 hours.

9). Embed in 100% DER, place in oven at 60 C for 48 hours.

level to detect changes in gut tissue. Two male and two female ticks from each age group were were prepared for viewing with a scanning electron microscope (SEM). Ticks were sonicated for 20 minutes to remove debris, mounted on a metal stub, and critical point dried. The specimens were then coated with a 2um layer of gold palladium, and viewed with a JOEL JSM 35 SEM.

To investigate lipofuscin (aging pigment), synganglia were removed from the tick and viewed with an Olympus BHz light microscope. The schedule for fixation and embedding was the same as used for midgut tissue except that tissue was embedded in Polybed 812. Sections were stained as described by Huber et al. (1968), Alpert et al. (1959), and Dowson et al. (1981).

Other sections (1um), embedded in DER, and mounted on slides, were placed on a rack, submerged and stained in methylene blue for 2 hours. Slides were removed, rinsed three times in deionized water, and counter stained with indophenol-HCl at room temperature for 15 seconds, dried and viewed with a light microscope.

Gut and synganglion dissected as before in gluteraldehyde or 10% formalin and gluteraldehyde (1:1), dehydrated in increasing concentrations of ethanol, cleared and embedded in Parrafin. Gut and synganlia were embedded in parrafin and sectioned at 4 um. Tissue from all the ages were exposed to 510 nm wavelength light to determine if lipofuscin was present in either tissue.

Gross morphological changes in the gut cecae were measured (length and width) and, after weighing the tick and removing the dorsal cuticle. Three caeca were selected, and their diameters measured. The gut caeca were then photographed while being viewed under a Wilde dissecting microscope at 40X. The caecal diameters were analyzed statistically for possible differences between age groups and sex.

Results

The gross morphology of the gut tissue changed with increasing tick age (Figure 4). The gut of newly molted ticks appeared very dark-black to rich chocolate, and floccular. An undissected tick appears very dark, and the outlines of caeca could not be distinguished through the cuticle. In older ticks, the tissue is lighter brown and appears more homogenous. By 12 months, caeca are less distended and can be clearly seen through the cuticle. The posterior medial cecae diameter (Figure 5) decreased with increasing tick age, but no significant changes were detected in other caeca.

Definite changes in staining affinity of tissue with Mallorys' trichrome was observed (Figure 6). Midgut from newly molted ticks exhibited an intense affinity for for the stain. Tissue near the gut lumen stains a very dark blue to black, while regions more

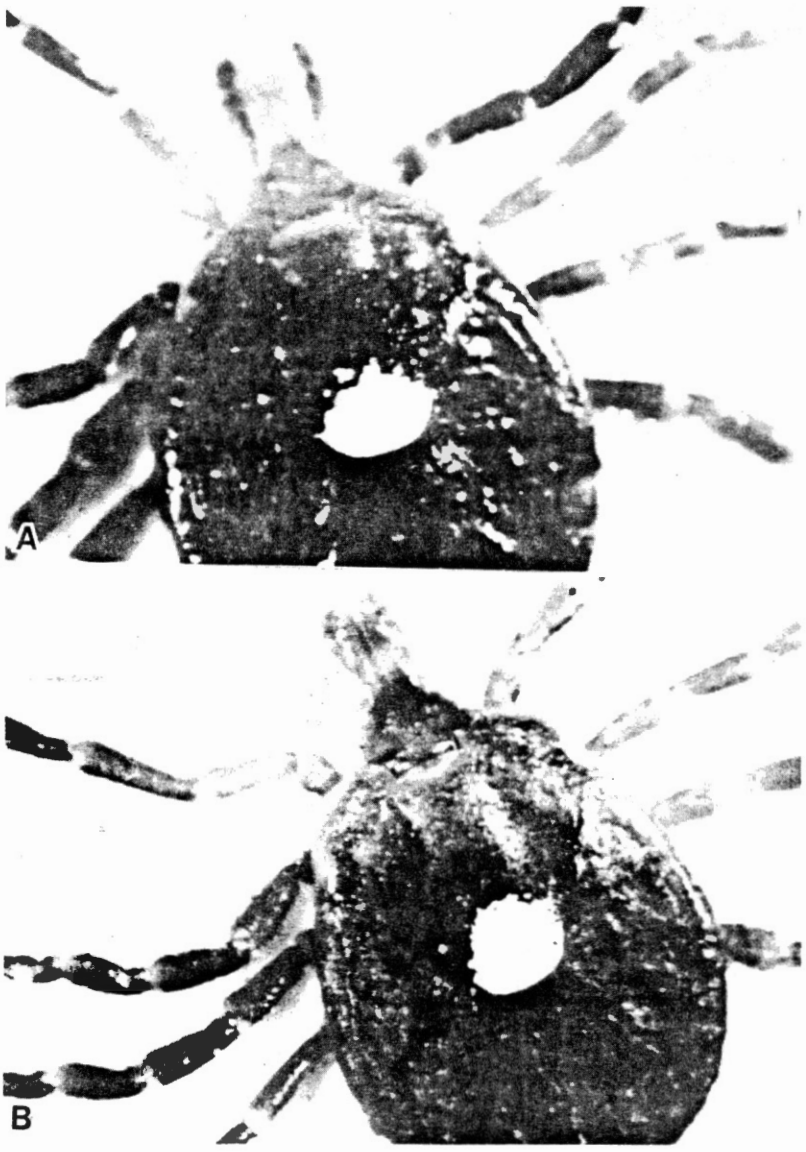

Figure 4. Adult Lone Star Ticks, A). 0 month and B). 12 month post molt. Note the difference in texture and color; X12

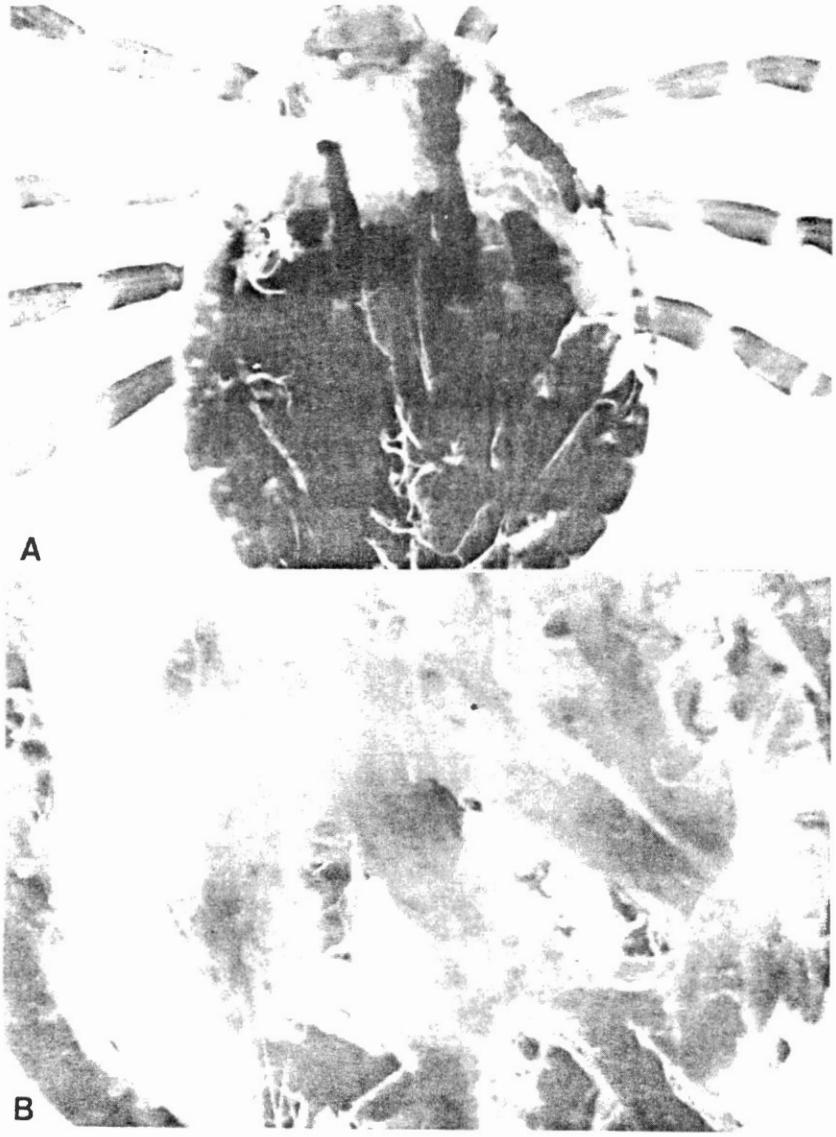

Figure 5. Gut Caeca. A). 0 month female, X12. B). 6 month female, X40. Note the difference in texture and color of the gut caeca

distal stain lighter blue. A Prussian Blue test was positive for iron, supporting transmission electron micrographs showing large inclusions hypothesized to contain phagocytized hemoglobin. Definite differences were observed in staining intensity of gut tissue with increasing tick age (Figure 7). Tissue from newly molted ticks stained bright blue. By 12 months, tissue stained a diffuse purple and darkest in the region of the basement membrane. Stain was virtually absent from the lumenal area.

Examination of tissue sections for lipofuscin under the flourescence microscope did not reveal lipofuscin. However, the microscope was not equipped with the filters needed to pass the proper wavelenth of UV light. Further studies should be conducted to clarify this point.

Changes that occur within the midgut cells with increasing age at the ultrastructural level are indicated in Figures 8-14.

Tissue from newly molted ticks (0 month) is easily distinguished by numerous siderosomes (iron containing residual bodies (Ghadially, 1982)), interspersed with numerous small to medium sized lipid inclusions. Lysosomes were also common in the tissue in association with lipid inclusions. Other intracellular structures, such as mitochondria and glycogen granules were not apparent at this stage.

Figure 6. Mallorys' Stained Gut Tissue, A) Newly molted. B). 6 month, and C). 12 month ticks, X100

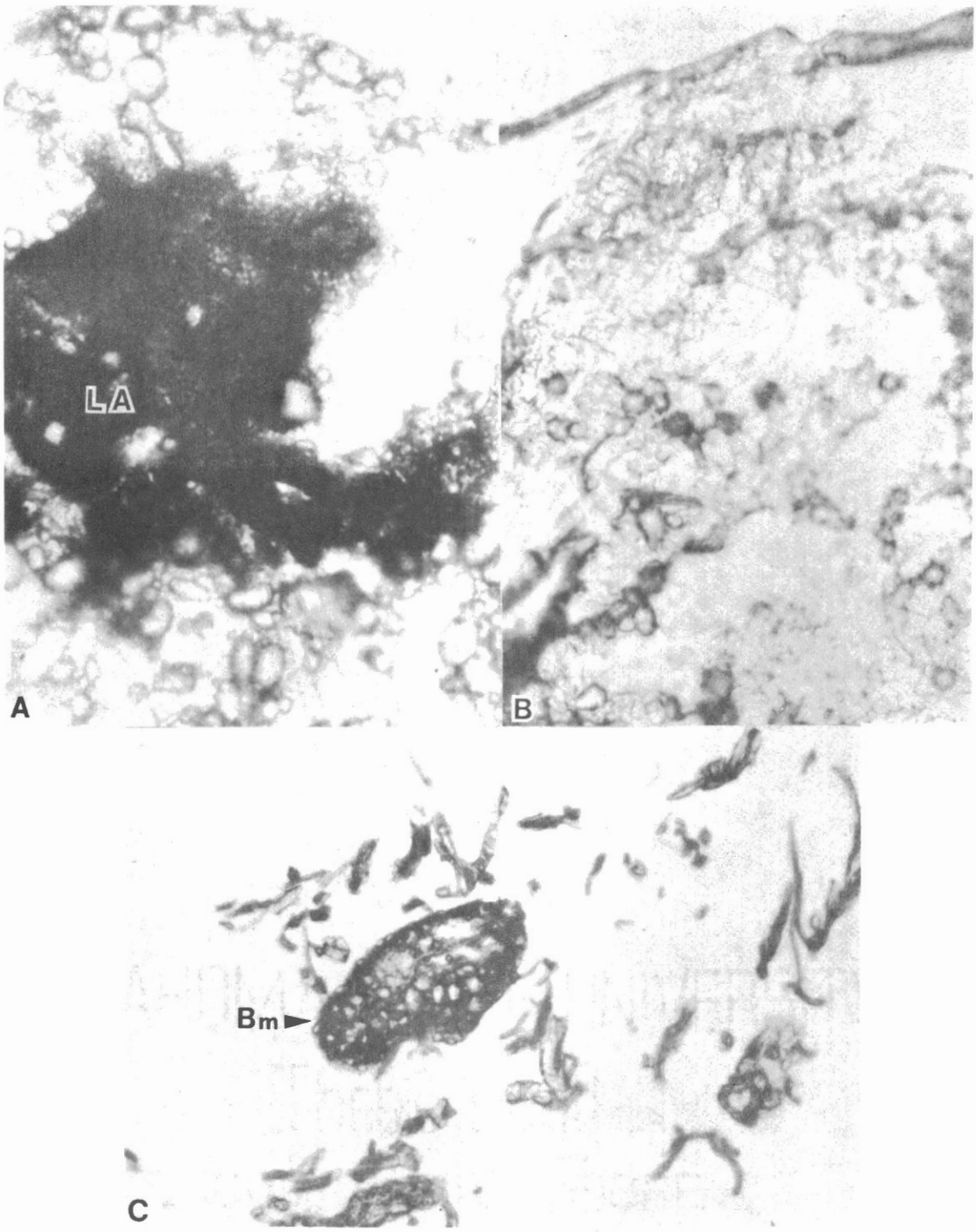

Figure 7. Prussian Blue Stained thick sections. A). Newly molted tick, X400. B). 6 month, X400. C). 12 month, X100. Bm, basement membrane; LA, lumen, and apical portion of cells

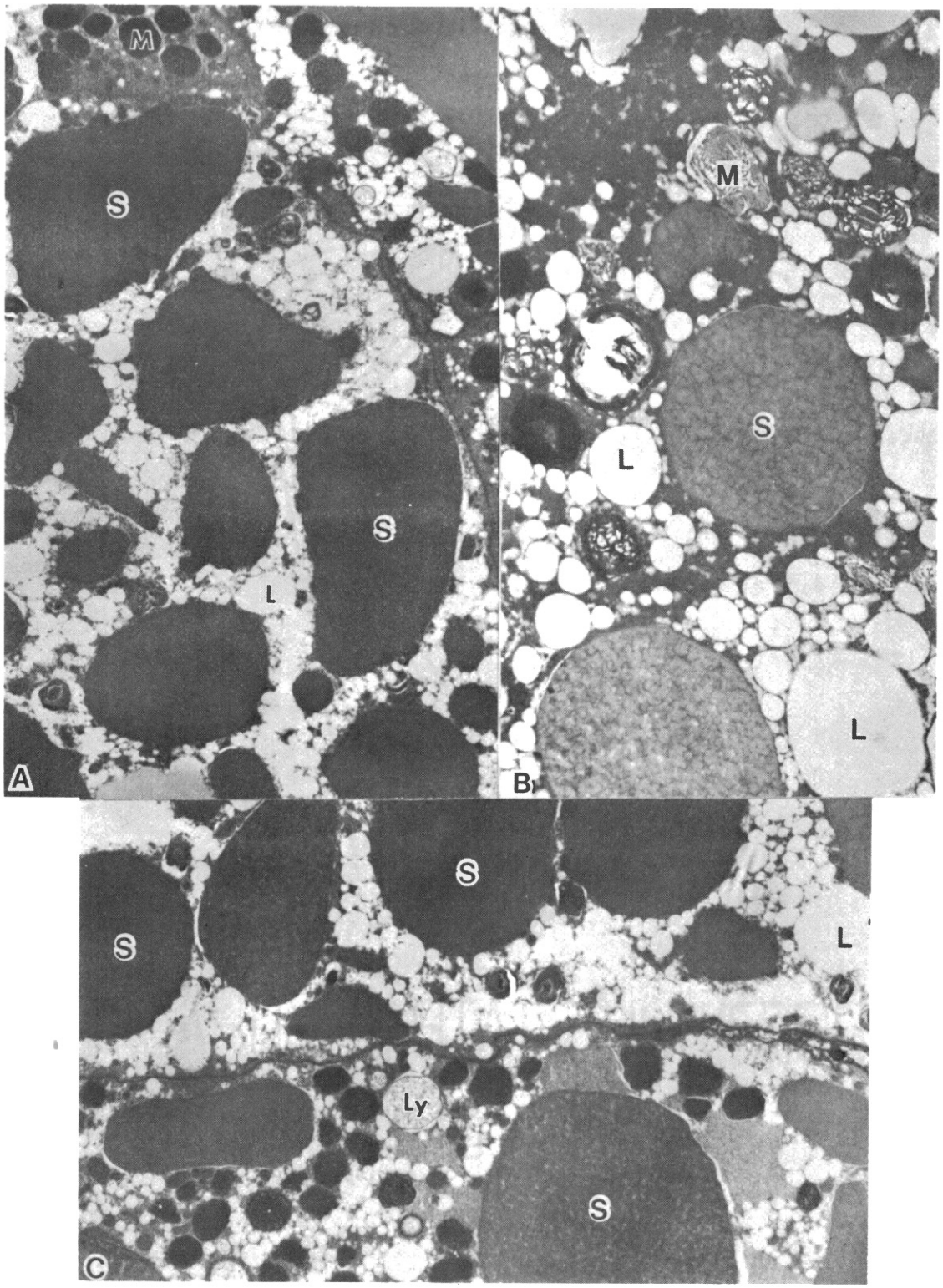

Figure 8. TEM micrograph from newly molted tick. A). L; lipid. S; siderosome. M; myelinosiderosome, X9081. B). X12283 C). Ly; lysosome, X9081

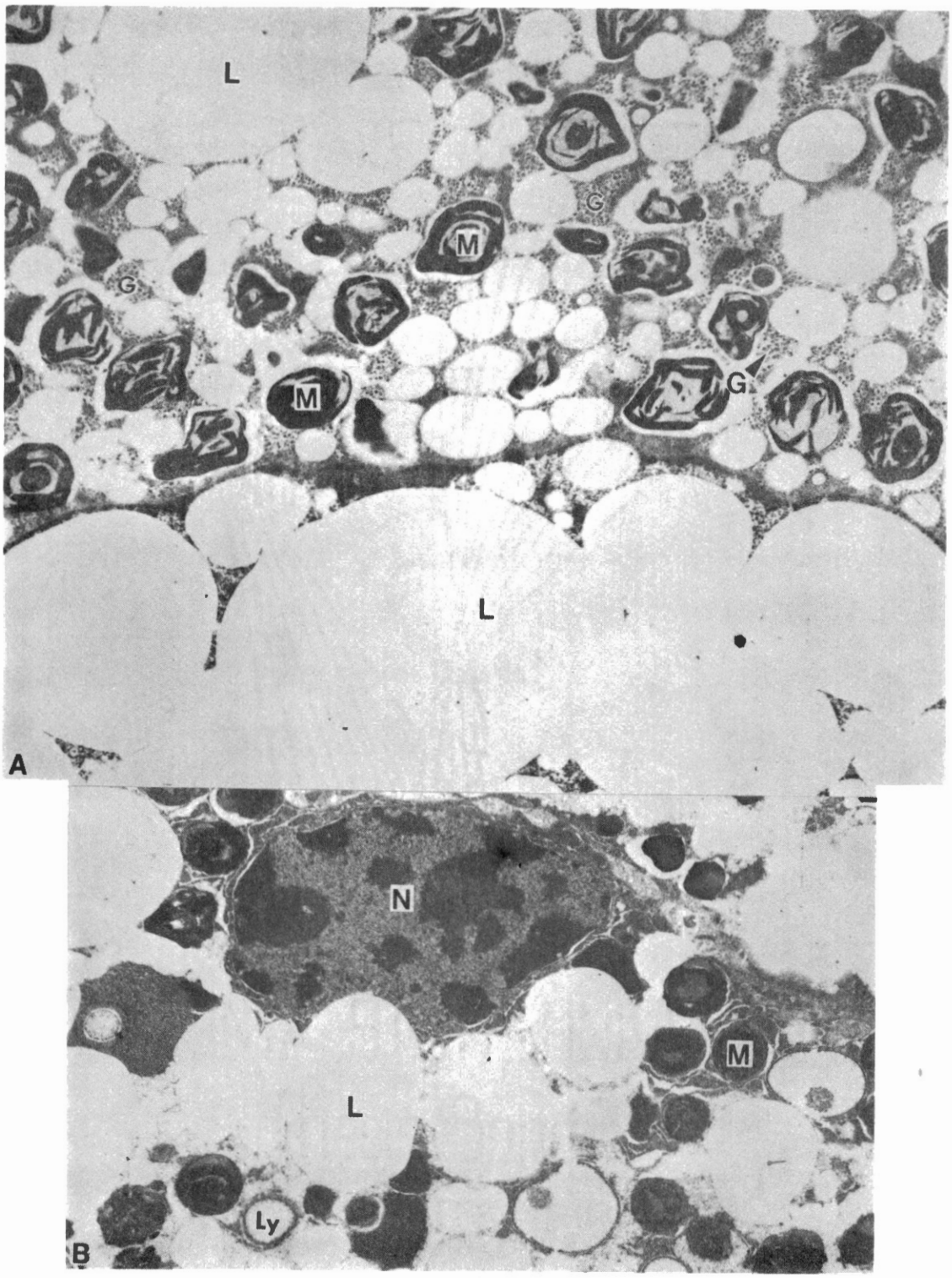

Figure 9. TEM micrograph from 2 month old tick. A). Female, L; lipid. G; glycogen. M; myelinosiderosome, X9081. B). Male. N, nucleus; Ly, lysosome; X16897

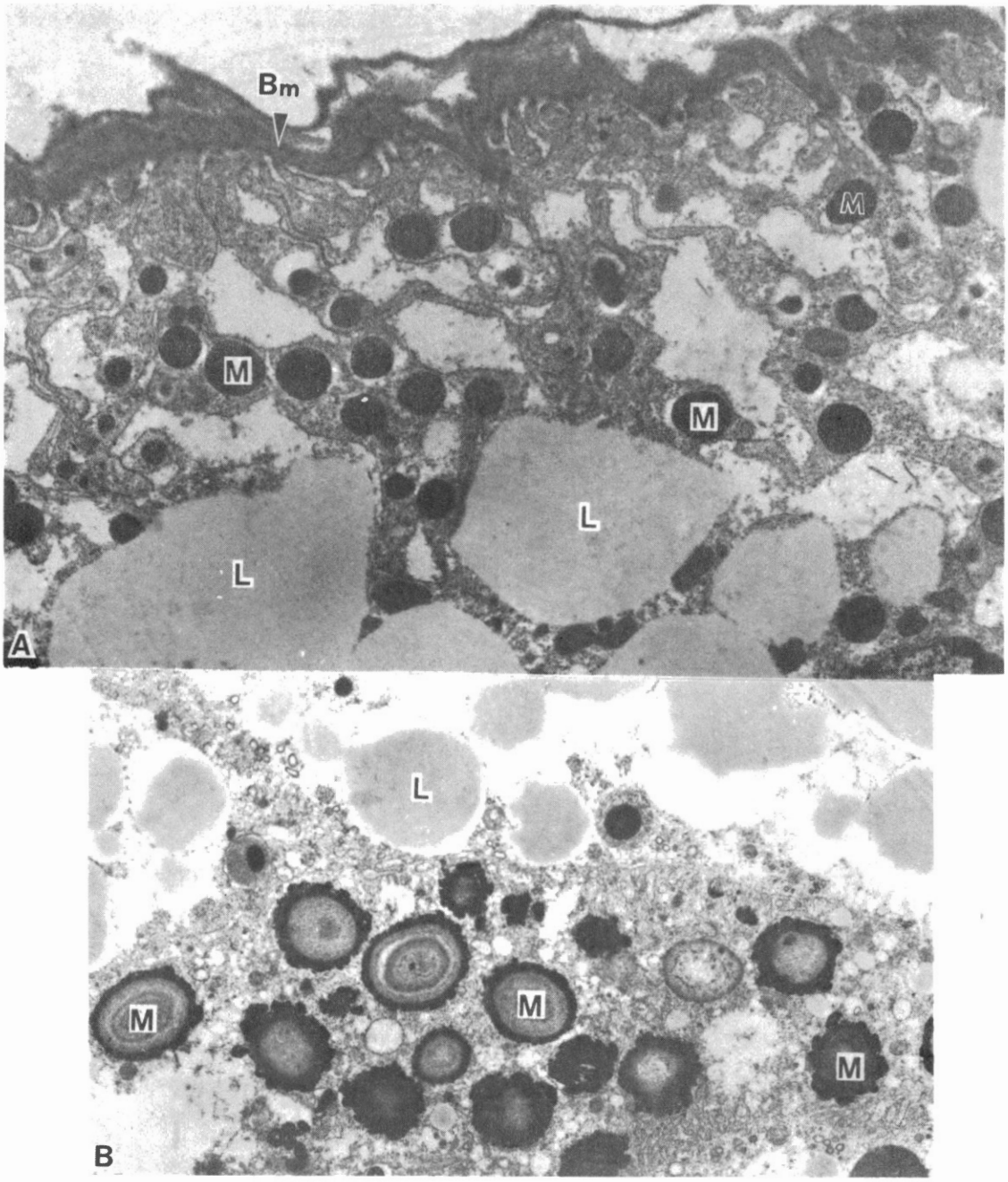

Figure 10. TEM micrograph from 4 month old tick. A). Bm; basement membrane. L; lipid; M, myelinosiderosome, X23520. B). X23520

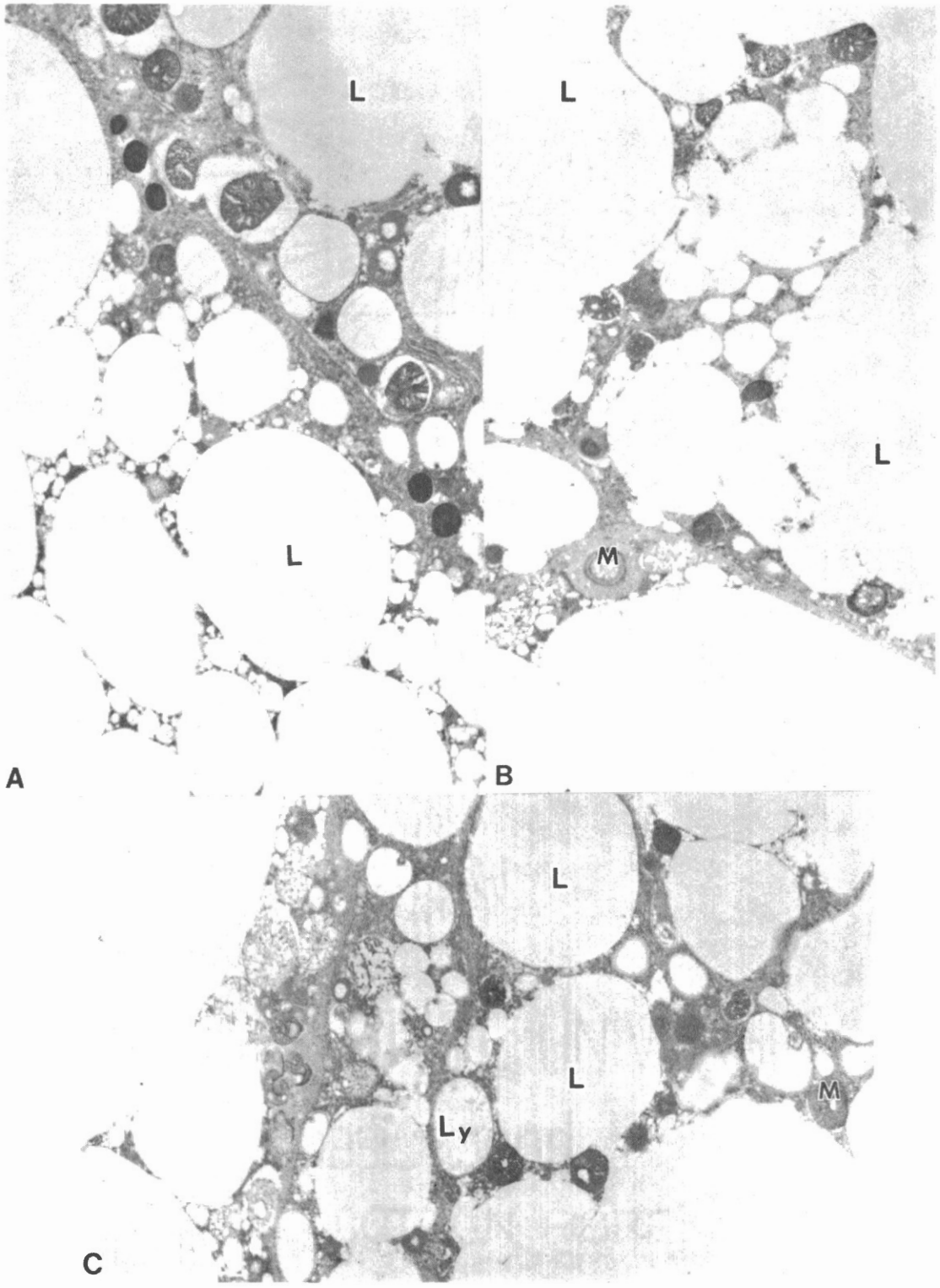

Figure 11. TEM micrograph from 8 month old tick. L; lipid, M; myelinosiderosome, Ly; lysosome, X16987

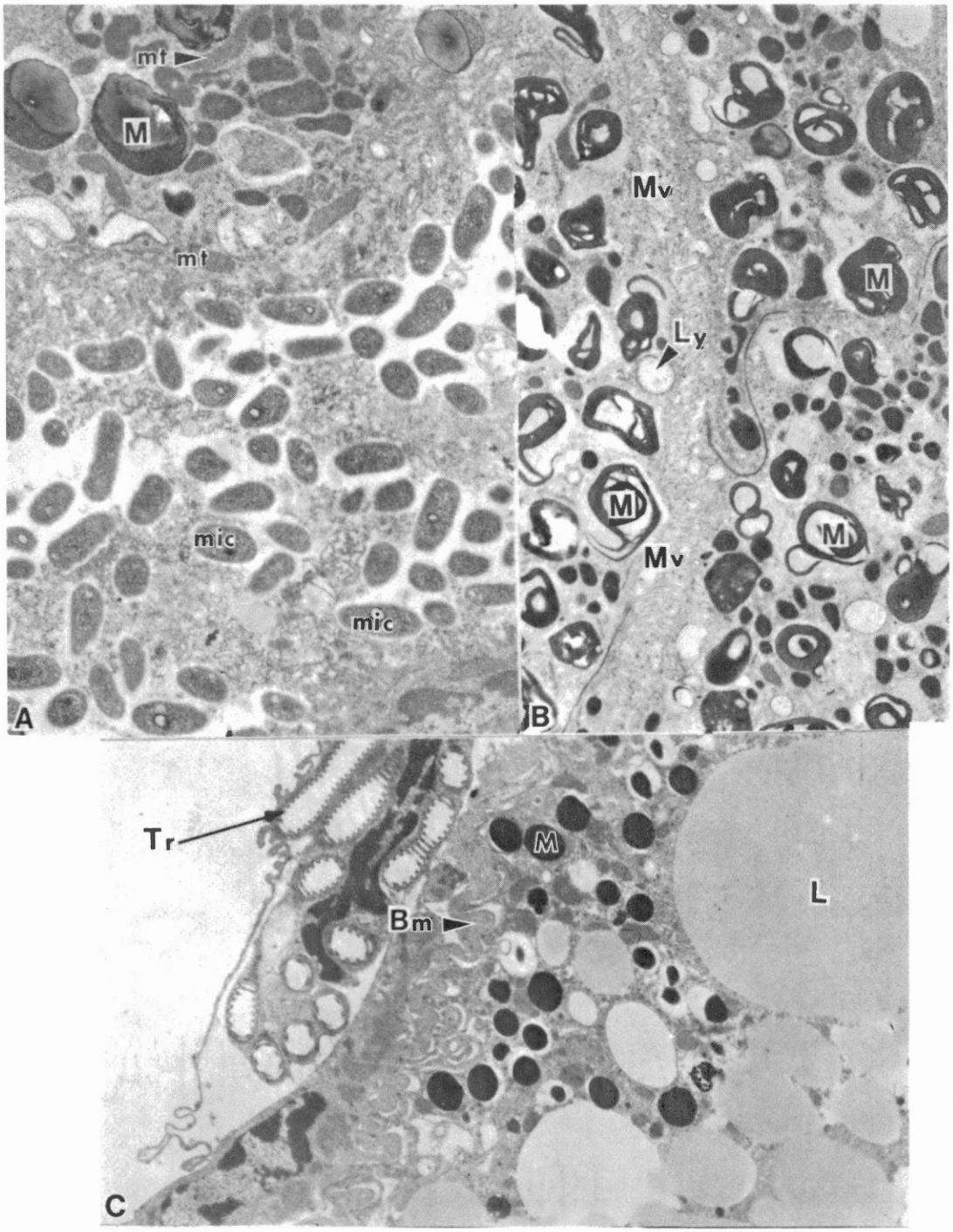

Figure 12. TEM micrograph from 10 month old tick A). Female. mt; mitochondria. mic; microorganism (unidentified). B). Female. Mv; microvilli. M; myelinosiderosome, X23520. C). Male. Tr, trachea; Bm, basement membrane; M, myelinosiderosome; L, lipid, X16987

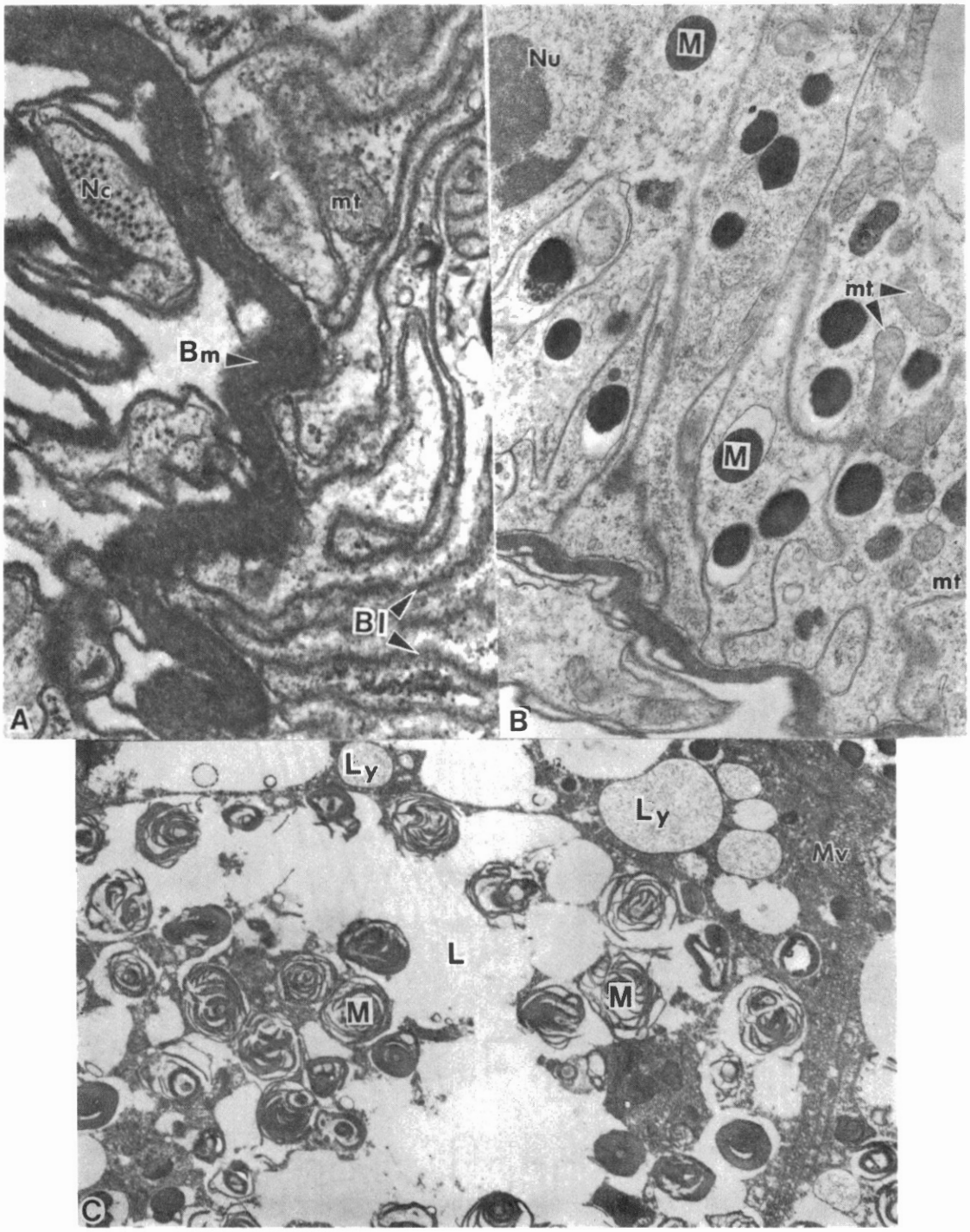

Figure 13. TEM micrograph from 12 month old tick. A). Female. Nc; nerve cord. mt; mitochondria. M; myelinosiderosome. BI; basal infoldings of the plasma membrane, X49000. B). Female. Nu; nucleus, X29400. C). Male. Mv; microvilli. M; myelinosiderosome. Ly; lysosome, X12283.

Tissue from adult ticks 2 months of age had larger, more numerous lipid inclusions interspersed with numerous myelinosiderosomes (residual bodies containing myelin figures and hemosiderin from hemoglobin degradation). Areas with numerous siderosomes were still present.

By 2 and 4 months, midgut tissue exhibited more occasional siderosomes, and more numerous myelinosiderosomes. At this age it was possible to observe modest amounts of basal infoldings of the plasma membrane. Myelinosiderosomes appeared to be crystalline and difficult to section.

Gut tissue from 6 and 8 month old ticks is morphologically similar, with few definitive differences in the the ultrastructural appearance. The tissue is dominated by the presence of large amounts of lipid and scattered myelinosiderosomes. The basement membrane was slightly folded at several locations.

By 10 months, large areas of lipid were still visible, but lipid inclusions did not displace as many intracellular organelles as in tissue from 6 and 8 month ticks. The basement membrane was folded, and numerous small dense myelinosiderosomes were in close proximity to the membrane. Convoluted crystalline myelinosiderosomes further away from the basement membrane apparently undergoing degradation and dispersion were commonly seen. A few smaller, granular

siderosomes were seen at this stage. Apparent lipofuscin inclusions, abundant mitochondria, and glycogen inclusions were also apparent at this stage.

At 12 months basal infoldings of the plasma membrane were quite evident and the basement membrane was convoluted and tightly folded. Lipid inclusions were numerous, but smaller, and commonly associated with a lysosome. The convoluted myelinosiderosomes appeared to be more dispersed.

Scanning electron microscopic examination of the external morphology of adult ticks revealed that the cuticle undergoes an apparent deterioration (Figure 14).

Discussion

In this study we have shown that ultrastructural changes occur in the tick gut that can be used as an indicator of tick age. An estimate of age could also be obtained by examining Mallorys' trichrome stained thick sections, or Prussian Blue stained thick sections. We believe the latter two provide inexpensive, relatively quick methods to estimate the age of ticks. Electron microscopic examination of the tissue could be used to verify the accuracy of the latter two methods.

Seasonal effects on gut ultrastructure are interesting. Group 1 ticks placed in the field in

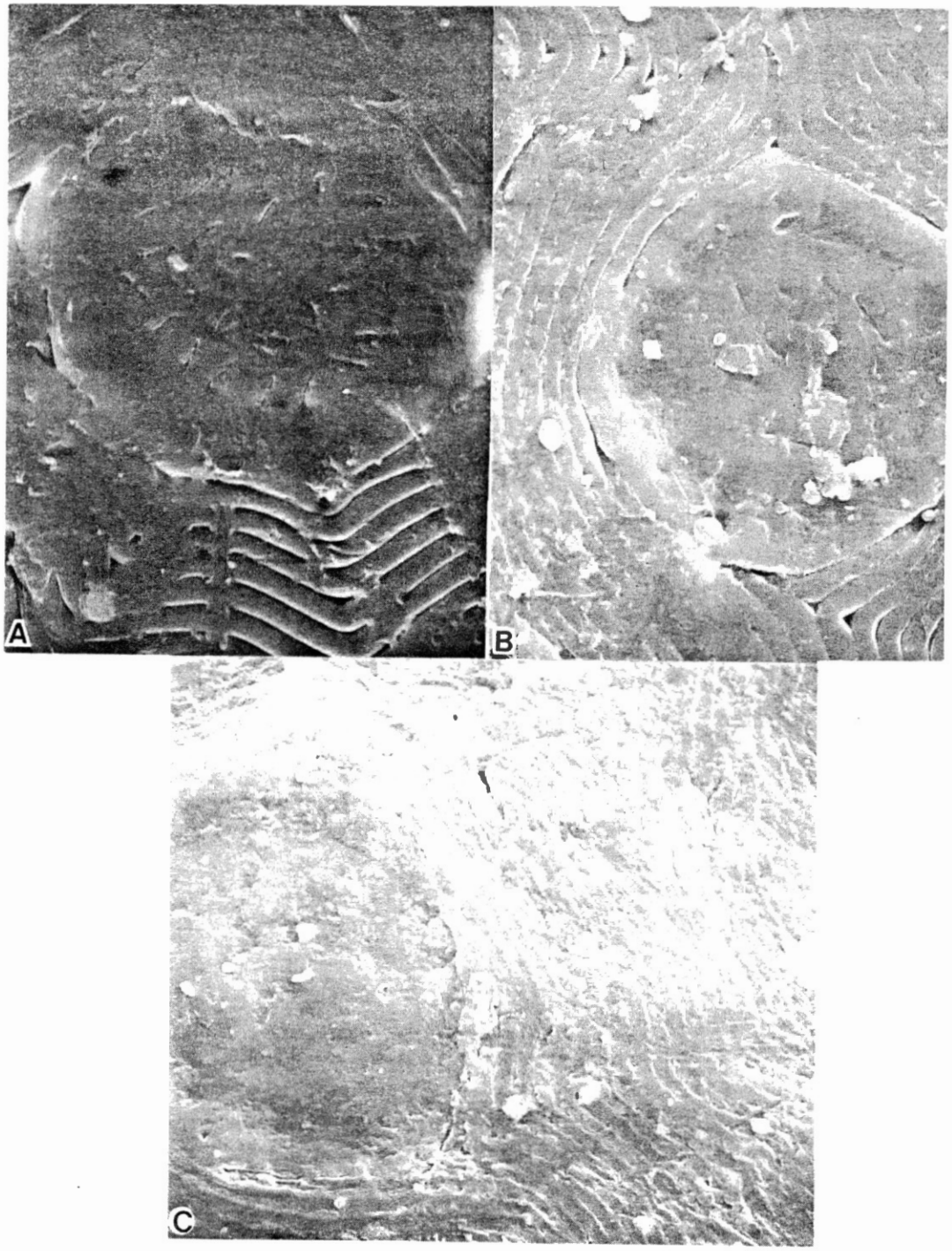

Figure 14. SEM micrographs of adult female alloscutum. A). Newly molted. B). 6 month. C). 12 month, X1000.

August 1981 entered diapause quickly, but gut cells were very similar to Group 4 ticks which were released just prior to peak seasonal activity (May, 1982). However, newly molted ticks of Group 4 exhibited more myelinosiderosomes and areas of heme catabolism. However, after 4 months of age the tissues of these two groups was virtually indistinguishable. The findings suggest that the overall strategy of bloodmeal digestion is to convert the meal to a stable, possibly more efficient storage form such as lipid. This hypothesis is supported by an apparent increase of lipid content in gut cells.

Comparison of results of this study, to those of Jaworski et al. (1983), indicated several important differences. In this study striated myelinosiderosomes began to appear after 8 months. This form did not appear until after 12 months in laboratory reared ticks investigated by Jaworski et al. (1983). After 10 and 12 months in this study, myelinosiderosomes were apparently undergoing further degradation and dispersion. Also, foldings of the basement membrane, and infoldings of the plasma membrane were not as noticeable in the study of Jaworski et al. (1983) as in the present study.

The results illustrate the importance of environmental stress as an important determinant of aging. The apparent prominence of the basal infoldings

at 12 months is interesting, but it is not known if this is a response to physiological processes, or a result of decrease in lipid content. A detailed study of changes in lipid in gut tissue with age would be of interest.

TEM micrographs suggest an important role for lysosomes in the catabolism of stored intracellular blood meal constituents. In general, the majority of the hemoglobin (and its derivatives) appear to be quickly catabolized. Some of the products of hemoglobin catabolism may be used to synthesize lipid stores. Finely granular appearing siderosomes noted in 10 and 12 month ticks appear similar to ferritin described by Ghadially (1982).

The deterioration of the cuticle was most apparent in females because of the folding of the alloscutum. It is possible that this would be a fairly rapid and accurate method of age grading field collected females. Although this study provided no further information on this subject the possible importance of this fact should not be overlooked as a factor affecting the longevity of the adult Lone Star tick.

CHAPTER IV

SUMMARY AND CONCLUSIONS

In an attempt to develop methods to age adult Lone Star ticks, three groups of 5000 ticks were placed in the field at one month intervals beginning in August 1981. Four additional groups were placed in the field at one month intervals beginning in April 1982. Each group was assayed at two month intervals.

The concentration of three body components were monitored during the course of the study, including whole body heme or heme containing compounds, total body lipid, and water content. The ability of the ticks to absorb water from the atmosphere (critical equilibrium humidity, CEH) was also monitored.

Light level, scanning, and transmission electron microscopic examination of the tick midgut and external surface were conducted at the same time intervals as the other experimental assays.

Significant decreases in heme compounds in both male and female ticks, and a significant decrease in the lipid content in the female were demonstrated. There was no significant change in the water content of

male or female ticks during the course of the study. The results of the hemoglobin assay in conjunction with observation of TEM photographs indicate that the majority of the blood meal hemoglobin and its derivatives are rapidly catabolized. The exact fate of these catabolites has not been investigated in detail, but presumably some of them are converted into lipids that are stored in gut cells (high energy source) even though overall body lipid decreases.

The results of the CEH experiments showed a significant decline in the ability of the adult ticks to maintain water balance. The reasons for the shift in the ability of older ticks to maintain their water balance is not clear.

Transmission and scanning electron microscopical examination of adult ticks demonstrated characteristic changes with increasing age. TEM examination of midgut tissue illustrated dramatic changes in the midgut ultrastructure with increasing age. SEM examination of the external morphology of the adult Lone Star ticks revealed an apparent derterioration of the alloscutal portion of the cutticle in females with increasing age.

Light level examination of morphological changes in the tick cuticle and gut caecal distention may provide a rapid economical method for estimating the age of adult, field reared, Lone Star ticks. Flourescence microscope examination of both gut and

synganglia for the presence of lipofuscin was unsuccessful.

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