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The Lipid Composition of the Egg Shell of the Tick Rhipicephalus sanguineus

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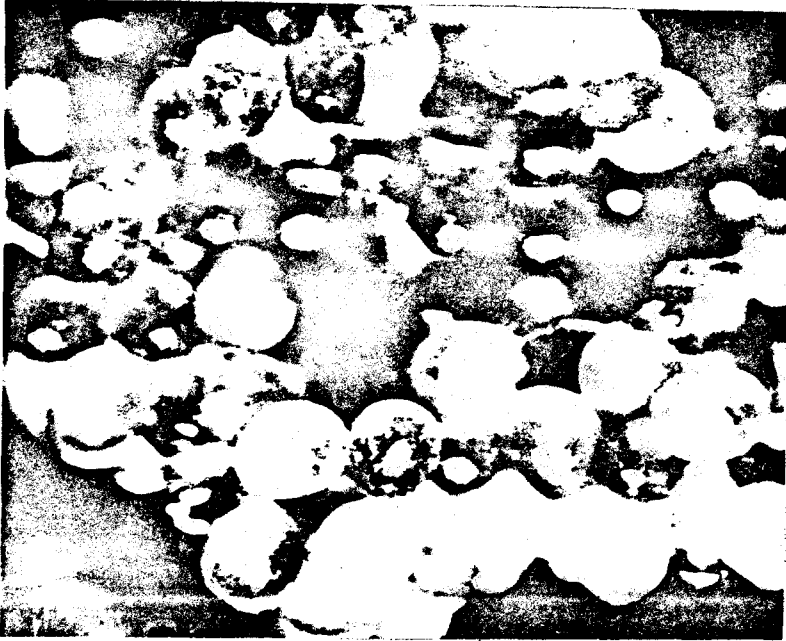The Lipid Composition of the Egg Shell of the Tick

Rhipicephalus sanguineus

by

Anthony F. Molinari

A Thesis Submitted to the Faculty of the Graduate School

of Loyola University in Partial Fulfillment of

the Requirements for the Degree of

Master of Science

September 1970

A PORTION OF AN EGG CLUSTER OF

THE TICK

RHIPICEPHALUS SANGUINEUS

ACKNOWLEDGMENTS

I wish to thank the faculty of the Biology Department for their help and encouragement and also for the use of their facilities. I would also like to thank my fellow graduate students and Miss Claudia Serdiuk for their encouragement and moral support.

I wish to thank Dr. Gordon Sweatman, Tropical Health Department, American University of Beirut and Dr. Willy Burgdorfer, United States Public Health Service, Rocky Mountain Laboratory, Hamilton, Montana, for contributing the Rhipicephalus sanguineus egg shells used in these experiments.

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I would particularly like to thank my advisor Dr. Benedict J. Jaskoski for his guidance in this endeavor and also for his interest in my future academic career.

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INTRODUCTION

It has long been known that ticks are important ectoparasites of animals, but only within the last century was it discovered that the tick can also spread human disease. Ticks have a single source of food, blood, and a direct method of disease transmission is thus available. The harmful effects of ticks are also in the forms of exsanguination, injection of toxic substances, and secondary infection at the site of feeding.

Rhipicephalus sanguineus, the brown dog tick, is found in most parts of the world and is quite common in North America. It is capable of infesting many mammals including cattle, sheep, goats, and particularly dogs, and may spread disease to all of them. R. sanguineus is originally an "Old World" species, but since its introduction to the colonies on imported dogs, it has spread to all warmer parts of North America, particularly the South.

R. sanguineus is capable of transmitting Babesia canis to cattle and dogs. Anaplasma may be spread to cattle, sheep, and dogs causing severe fever and anemia. Coxiella burnetii can be experimentally transmitted by R. sanguineus. Arthur (1962) reports that the main disease transmitted by the tick is "Fievre boutonneuse" caused by the virus Ricketsii conorii, as well as Q-fever and Rocky Mountain Spotted fever.

Present methods of control are directed primarily at the adult tick, usually after infestation has begun. Spraying

the host is the most widely used method, but for larger herds, dipping is more practical. Application of insecticides to fields has also been used. These methods are time consuming and expensive and the effectiveness is quite varied.

Several studies have been made concerning the egg shell structure and morphology as well as the cuticular lipids of insects, but no analysis has been made of the chemical composition of the tick egg shell.

This study was made to determine the nature of the lipids that are in the egg shell of the tick, R. sanguineus in order to gain knowledge of the basic and most vulnerable stage of the life cycle, the egg.

REVIEW OF THE LITERATURE

A considerable amount of material has been written on the physical properties and general morphology of insect egg shells as well as lipid analyses of insect cuticles. The extreme sensitivity of insects and their immature stages to humidity dictates that egg shells be capable of maintaining an adequate internal water supply, while permitting gaseous exchange with the environment, and providing structural support.

The permeability of insect egg shells has been studied by Beament (1948, 1949), Beament and Lal (1957), and Salkeld and Potter (1953). Permeability studies are important in the area of insect control by aqueous insecticides. Ongaro (1933) discovered a wax secretion composed primarily of paraffins surrounding the egg shells of the silk worm _Bombyx_ _mori_. Wigglesworth (1945) and Beament (1945) have associated this phenomenon with the thin wax layer of insect cuticles and its waterproofing properties. The egg shell lipids of _Rhodnius_ _prolixus_, however, are not extracted by lipid solvents and the term used to describe them by Beament is lipidized protein.

The general structure and morphology of insect eggs has been recorded by many including Moscona (1950), Hartley (1961), Hinton (1962), and Lincoln (1965).

Although biochemical analysis has been done on several varieties of insects, the work of Butler (1969) and the

present work are the first to describe the chemical components of a tick egg shell. Thin layer chromatography was used by Butler to determine the amino acid composition of the protein layer of the egg shell of R. sanguineus. The amino acids of the tick were found to be similar to those of the silk worm Bombyx mori, the cricket Acheta domesticus, and the fruit fly Drosophila melanogaster as determined by Tomita (1921), McFarlane (1962), and Wilson (1962) respectively.

Arthur (1948) reported on the layer of the egg shell of Ixodes ricinus secreted by Gene's organ. These eggs were found to rapidly shrivel and dry if they were not allowed to be covered with the secretion of Gene's organ. This layer was found to attract moisture and is presumably of a waxy nature. When the egg clusters were treated with ether, the integrity of the cluster would soon disappear.

The studies of Lees and Beament (1948) showed that a wax secretion from Gene's organ, an organ unique to the ticks, serves to waterproof the eggs. Without this secretion, the eggs of Ornithodorous moubata become completely shrivelled and hard. In other tick species such as I. ricinus, a portion of the external lipid layer was found to be secreted by the common oviduct and the vagina. By interfering with the normal process of Gene's organ in this species, partial waterproofing was still observed. The egg shell lipid layer of O. moubata when extracted with hot chloroform was found to have two fractions in a 6 to 1 ratio: a white waxy solid and a soft

yellow grease. The lipid layers of O. moubata and I. ricinus were measured at 0.47 u. and 1.76 u. respectively.

The outer layer of the egg shell of Metatetranychus ulmi Koch is described by Beament (1951) as a hard, transparent waxy coating of an inert lipoid material that is unsaturated in nature. This lipoid layer has a high melting point, no proteinaceous material, and its function, unlike ticks, is not to waterproof the eggs.

The egg shells of the mite, Petrobia latens Muller were studied by Lees (1961). He described the outer wax layer as having a melting point of 165°C., insoluble in cold benzene or chloroform, or ethanol, but soluble in hot benzene or chloroform. The chain lengths probably range from 16 to 36 as in other hard insect waxes as suggested by Chibnall (1934) and Beament (1945).

MATERIALS AND METHODS

Oviposition was induced in a fully engorged tick by the method of Kohls (from Lutz, 1937). The eggs produced were examined under the dissecting microscope and were found to measure 0.5 mm by 0.3 mm in the form of translucent red ovals. Wigglesworth (1943) found that the pigmentation in the eggs of Ornithodorous moubata is due to the presence of hemoglobin pigments.

The vacated shells of Rhipicephalus sanguineus, which were used in this experiment are translucent white ovals that are split along a thickened area, the "hatching line" as described by Butler (1969). This line was observed when some immature eggs were placed in a drop of Mazola Oil in an attempt to induce development, although they did not split. In all shells used, hatching had occurred and although the shells were split, they were still connected.

A batch of egg shells was first cleaned of macroscopic debris by hand using two sharp dissecting probes under the dissecting microscope. They were then weighed on an analytical balance at 6 mg. The egg shells were then placed in 100 ml triple distilled water and fragmented using a Sonifier Cell Disruptor* for five minutes at which time almost all of the egg shells were broken and had settled to the bottom. The egg shells were removed from the water by centrifugation,

*Heat Systems Co., Melville, Long Island, New York

and then washed three times with triple distilled water.
The small pellet of egg shell fragments produced was drained
by inverting the tube and allowed to dry at room temperature
for one hour. This procedure for cleaning the egg shells
is a modification of the method used by Clegg and Morgan
(1966) for Fasciola hepatica eggs.

The clean dry eggs shells were resuspended in 25 ml
anhydrous ether and transferred to a 125 ml Erlenmeyer flask.
This was shaken on a Burrell Wrist-Action Shaker for ten
minutes and the ether fraction was decanted and stored in
the refrigerator at 3°C. A fresh 25 ml portion of ether
was added to the egg shells and again shaken for 10 minutes.
This ether extraction was repeated three times a day for
three days, the ether fractions being centrifuged, decanted
and collected in a single vessel.

Further extraction of the lipid layer was made using
the method suggested by Schmit and Wynne. The egg shells
were soaked in 10 ml chloroform-methanol (70/30 v/v) solvent.
This was then centrifuged at 2000 rpm for 10 minutes, the
liquid phase being added to the ether soluble lipid fraction.
This was repeated three times.

The lipid-containing solvent was reduced in volume to
dryness using a slow stream of dry nitrogen under the hood.
A small amount of yellow, greasy residue remained which was
dissolved in 15 ml hexane-anhydrous ether (50/50 v/v). This
solution was then washed twice with 10 ml portions of triple

distilled water. The water fraction was allowed to settle
in a separatory funnel for one half hour and removed. The
lipid solvent was then dried over sodium sulfate. The
solvent then contained the major lipid classes. Half of
this sample was stored in the dark at 3^oC. and used for
thin layer chromatographic determination of the lipid classes.

The other half of the lipid-containing solvent was
prepared for gas chromatography by reduction to dryness in
a 25 ml test tube using a stream of dry nitrogen. A fresh
mixture of 9 ml absolute ethanol and 1 ml 33% KOH was added
to the lipids to saponify them. This was then heated on
a water bath for 30 minutes at 55^oC. Fifty per cent HCl
was added to this drop by drop until the solution became
acidic. The fatty acids were then extracted using 10 ml
hexane three times. The hexane fraction was then washed
twice with 10 ml portions of triple distilled water, and
dried for 24 hours over sodium sulfate. The hexane fraction
was then dried over nitrogen in a 25 ml volumetric flask.
Ten ml of 5% perchloric acid in methanol was added and
heated on a water bath for 20 minutes at 55^oC. The methyl
esters thus synthesized are extracted three times with
10 ml hexane-ether (50/50 v/v). They were washed three
times with distilled water to neutralize and then dried
again over sodium sulfate. One ml chloroform containing
50 mg C_{17} was added to the residue as an internal standard.

The gas chromatograph was programmed from 100^oC. to

215°C. at 3°C./minute and had a 6' column of 6% DEGS on 80/100 mesh Diatoport S. Two ul of the above solution was injected into the chromatograph.

Thin layer chromatography was employed to determine the lipid classes using glass plates 20X20 cm and 5X20 cm coated with a layer of Silica Gel 250 u thick. These plates were prepared by the method of Stahl (1965). Twenty-five grams of Silica Gel G and 35 ml triple distilled water were added together and stirred until a smooth homogeneous slurry was obtained. While stirring, an additional 15 ml water was added. This suspension was then added to a TLC aligning tray, and spread across the glass plates which were then allowed to dry. Commercially prepared plates* were also used.

Standard solutions of various lipids from the major lipid classes were prepared. Twenty-five mg of each lipid were weighed directly into small brown vials and dissolved in 5 ml chloroform. These vials were stoppered with corks and stored in the refrigerator at 3°C. The lipid standards used were: trimyristin, tristearin, tripalmitin (neutral triglycerides); myristic acid, palmitic acid, stearic acid, arachidic acid (saturated fatty acids); oleic acid, linoleic acid (unsaturated fatty acids); cholesteryl -acetate, -benzoate, -palmitate, -stearate, -oleate (cholesteryl esters);

*Mallinckrodt 7GF

lecithin ex ovo, L-α-lecithin (synthetic), phosphatidyl ethanolamine (phospholipids); and cholesterol, ergosterol, estrone, testosterone, estradiol 17-B (sterols). All standards used were of the highest available quality.

The cuticular lipids of a single adult tick were extracted by the method of Goodrich (1970). The tick was rinsed in distilled water and allowed to dry. It was then immersed in dichloromethane at room temperature for three minutes.

The solvent systems used to develop the chromatograms were ethylene dichloride, acetic acid, 99:1; n-hexane, diethyl ether, acetic acid, 90:10:1; and petroleum ether, diethyl ether, acetic acid, 80:20:1. In all cases the chromatography chambers were allowed to become saturated with the solvents for 24 hours before the development of the plates.

The distance of the solvent front migration was between 12cm and 15 cm for all chromatograms.

Visualization was accomplished in most cases using the iodine vapor technique. Other methods used include charring with chromic-sulfuric acid, or spraying with 2,7-dichloro-fluorescein, phosphotungstic acid, or ninhydrin.

Lipid standards that did not correspond with the unknown were compared on at least five different chromatograms to be certain that they were not present. Lipids that did appear to be present in the unknown fraction were run at least twenty-five times under all three solvent systems.

Records of the chromatograms were kept in the forms of
tracings and photographs, and a detailed account of each
chromatogram was made.

RESULTS

Use of thin layer chromatography techniques has revealed
the presence of highly mobile non-polar lipids with high Rf
values for the types of solvent systems used. The uppermost
area in the chromatograms of the tick lipids corresponds
with standard solutions of cholesteryl esters, particularly
cholesteryl oleate and cholesteryl palmitate. Refer to
chromatograms 2F4, 1A2 and 2A1. The lower weight cholesteryl
esters have a slightly decreased mobility and do not correspond
with the unknown. Spraying this region with 10% alcoholic
phosphotungstic acid revealed a positive red color that
indicates the presence of cholesteryl esters. Since only
a fraction of this area reacted positively to phosphotungstic
acid, it is certain that other lipids are present in this
area.

Squalene, an unsaturated branched hydrocarbon (C_{30}), has
shown a high mobility corresponding with this area of the
tick lipids, as well as similar tailing effects. The dark
I_2 color is similar to the unknown, lending evidence that
there is a high degree of unsaturation in the tick lipids.
When the charring technique is used to visualize the chrom-
atograms, both the unknown and squalene demonstrated rapid
breakdown in this area. Refer to chromatograms 1A19 and 2A19.

An effort was made to test for saturated hydrocarbons,
that is, long chain alkanes with strong C-H and C-C alpha

bonds, that are not readily attacked by hydrolysis or oxidation. These include mineral oil, lubricants, and paraffin. These saturated hydrocarbons tend to repel the I_2 visualization due to their lack of weak pi bonds and thus have a characteristic faded area that is not present in the egg shell fraction. See chromatograms 1A19 and 2A19.

Natural waxes are not saturated and appear in the unknown. Pure beeswax is a mixture of waxes, the main component being myricyl palmitate. This is the fatty acid ester of myricyl alcohol and palmitic acid. Other bases for these wax esters can be oleic and linoleic acids which have been determined as bases for the lipids in the unknown. It is thus apparent that the uppermost area of the tick egg shell lipids corresponded with natural wax esters, cholesteryl esters, and unsaturated hydrocarbons. See Figures I and II.

Under all types of solvent systems used, lecithin was found to correspond to an area of the unknown that is somewhat less mobile than that mentioned above. Refer to chromatograms 2F4, 2A1 and 1A2. Under long wave ultraviolet light, lecithin and its tailing effects exhibit a light violet color that corresponds with the tick egg shell lipids. Several sources of lecithin were used including synthetic and bovine. It is likely that the fatty acids involved are palmitic and oleic as they would be cleaved from the lecithin molecule during the preparation of the unknown for gas chromatography.

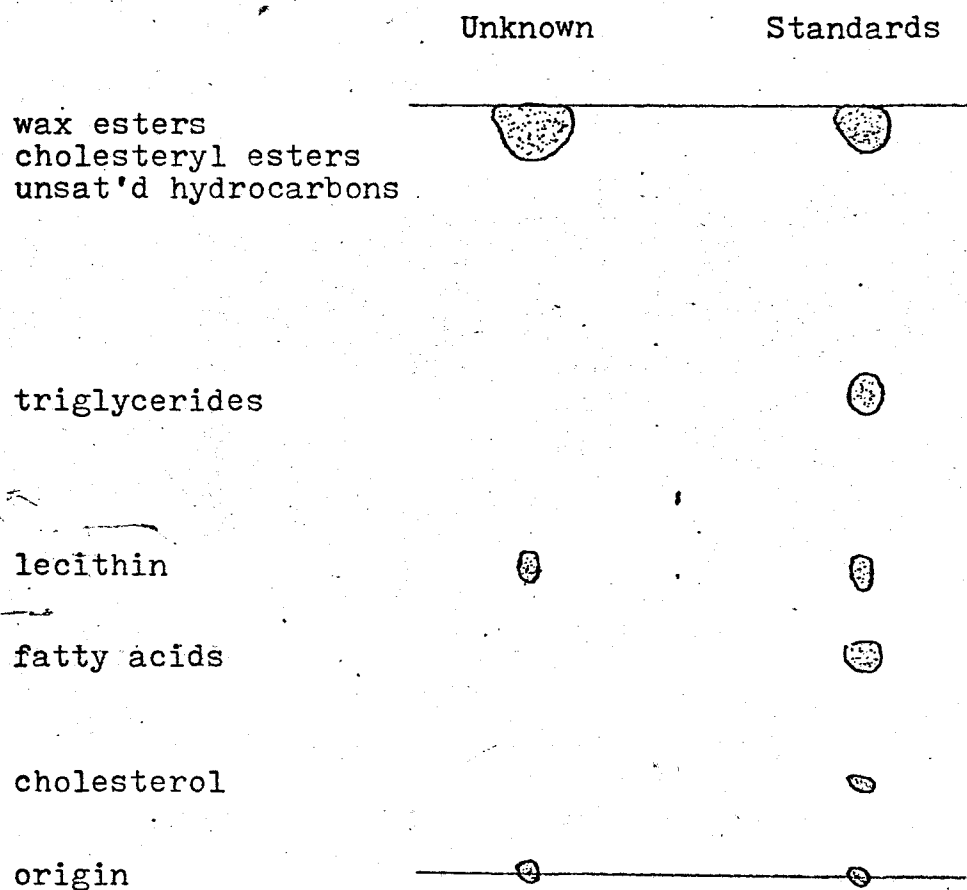Cholesterol and other sterols were repeatedly sought in

the tick fraction and are present in only trace amounts if
at all. Refer to chromatograms 1M31 and 2A19. The area of
the chromatograms where cholesterol would appear if it were
present was sprayed with phosphotungstic acid and results
were negative. It thus appeared that no free cholesterol
is present in the egg shell lipids of the tick.

The use of TLC has demonstrated that only trace amounts
of triglycerides are in the egg shell lipids if at all. Free
fatty acids have been compared to the unknown and no indication
of their presence has been indicated. Refer to chromatograms
1J28, 4J27 and 1F3. The unknown lipids were compared to
several lipids from both classes and negative results were
constant.

Spraying the unknown on the chromatogram with 0.5 per
cent ninhydrin has demonstrated that no amino acids are
present. This indicates the absence of proteinaceous debris
that would be present if the egg shells were not completely
free of embryonic material.

The methyl esters of the total lipid fraction of the tick
egg shells as obtained by extraction and synthesis as mentioned
above indicate the presence of palmitate and oleate, with
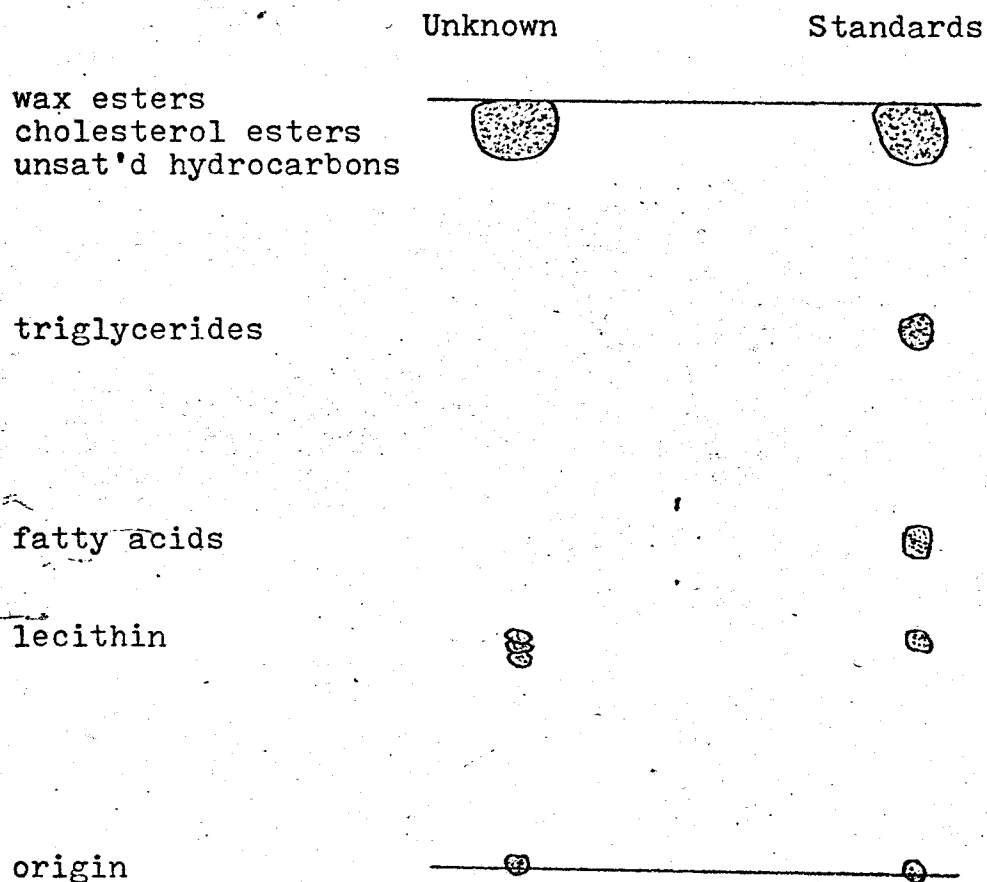smaller amounts of stearate and linoleate. Refer to Figure
III.

LIPID CLASSES OF THE EGG SHELL OF

RHIPICEPHALUS SANGUINEUS

Unknown   Standards

wax esters
cholesteryl esters
unsat'd hydrocarbons

triglycerides

lecithin

fatty acids

cholesterol

origin

Solvent system: petroleum ether   80

ethyl ether   20

acetic acid   1

Figure I

LIPID CLASSES OF THE EGG SHELL OF
RHIPICEPHALUS SANGUINEUS

Unknown   Standards

wax esters
cholesterol esters
unsat'd hydrocarbons

triglycerides

fatty acids

lecithin

origin

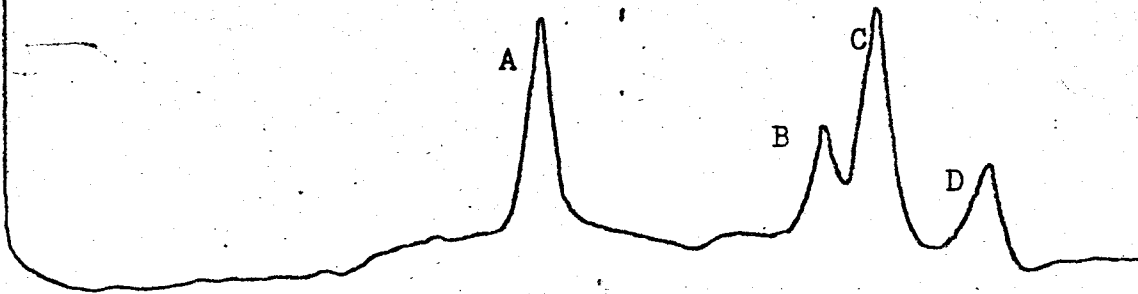Solvent system: ethylene dichloride   99

                           acetic acid   1

Figure II

FATTY ACID COMPONENTS OF EGG SHELL OF

RHIPICEPHALUS SANGUINEUS

A= $C_{16:0}$ Palmitate

B= $C_{18:0}$ Stearate

C= $C_{18:1}$ Oleate

D= $C_{18:2}$ Linoleate

Gas Chromatography

Figure III

DISCUSSION

Recently, Armold (1969), Tartivita (1970), and Jackson (1970) have done extensive investigations on the cuticular lipids of insects. They found that the primary lipid constituent of several species of cockroaches are long chain, saturated hydrocarbons. Cmelik (1969) analyzed the lipids of the Tse-tse fly and found the fatty acids from the neutral lipids to be primarily $C_{16:0}$ and $C_{16:1}$.

Beament (1951) describes the lipid layer of the egg shell of the red spider mite as inert, yet not having a saturated nature. He further indicates that no proteinaceous material is present. Lees and Beament (1948) also indicate a lack of protein material in O. moubata.

The unsaturated nature of the wax esters and cholesteryl esters found to be present in the egg shells of R. sanguineus, and the lack of protein in the lipid layer demonstrated the similarities of the tick and the mite, Metatetranychus ulmi. Koch. The lipids in the cuticles of insects, however, are saturated as in the case of the Australian sheep blowfly Lucilia cuprina as demonstrated by Goodrich (1970). Squalene was chosen as a possible constituent of the lipid fraction of the tick since it is an intermediate in the synthesis of cholesterol. Its highly unsaturated nature also would be consistent with information known about insect lipids.

The fact that cuticular lipids are hard and crystalline as compared with a softer and more viscous wax on the egg

shells of O. moubata and R. sanguineus is consistent with
the ability of the egg lipids to spread more easily than
the cuticular lipids. Lees (1948) also suggests that the
mobile wax of the tick egg shell gradually becomes absorbed
by the shell layer. It was for this reason that such a
rigorous method of lipid extraction was undertaken in the
case of R. sanguineus.

Although the cuticular lipids for this report were
extracted from a single specimen of R. sanguineus, the results
seem to indicate similar chromatographic patterns for the
cuticular lipids and the egg shell lipids. The degree of
similarity between insect egg shell lipids and cuticular
lipids should be investigated further.

SUMMARY

The lipid fraction of the egg shells of <u>Rhipicephalus</u>
<u>sanguineus</u> was analyzed using the techniques of thin layer
and gas chromatography. The outer wax layer was separated
from the inner protein or shell layer by extraction using
anhydrous ether, chloroform-methanol (70/30 v/v), and hexane-
ether (50/50/ v/v).

Using thin layer chromatography, it was shown that the
egg shells contain wax esters, cholesteryl esters, unsaturated
hydrocarbons, and lecithin.

Using gas chromatography, it was found that palmitate
and oleate with lesser amounts of stearate and linoleate are
present.

Free fatty acids, triglycerides, paraffin, and free
cholesterol were not found.

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APPENDIX

TLC No. _____2F4_____

Solvent System _Petroleum ether-_
ethyl ether- glacial acetic
acid _____80:10:1_____

Solvent Distance _____12_____ cm.

Quantity Applied _____-_____

Detection _chromic H₂SO₄_

Date _February 4, 1970_

Time In _____-_____

Time Out _____-_____

TLC No. _____

Date _____

Literature:

Adsorption Layer _____

Thickness _____

Type Recording

Photo _____ Other _____

_____ _____

	Substance		Notes

1. _____

2. cholesteryl palmitate   6

3. lecithin ex ovo   2

4. 2&3   (6,1)

5. 2&3   (8,2)

6. unknown   6

7. synthetic lecithin

8. bovine lecithin   6

9. bovine lecithin   2

10. _____

TLC No. ___1M31___

Solvent System___Ethylene___
___dichloride & 1% Glacial___
___acetic acid___

Solvent Distance ___12___ cm.

Quantity Applied ___-___

Detection___I2___

Date___March 31, 1970___

Time In___-___

Time Out___-___

TLC No._____

Date_____

Literature:

Adsorption Layer_____

Thickness_____

Type Recording

Photo_____Other_____

___-___ ___.___

_____ _____

Substance	Notes
1. phosphatidyl ethanolamine	
2. estrone	
3. cholesteryl oleate	
4. ergosterol	
5. lecithin ex ovo	
6. stearic acid	
7. cholesteryl palmitate	
8. oleic acid	
9. unknown	
10. paraffin	
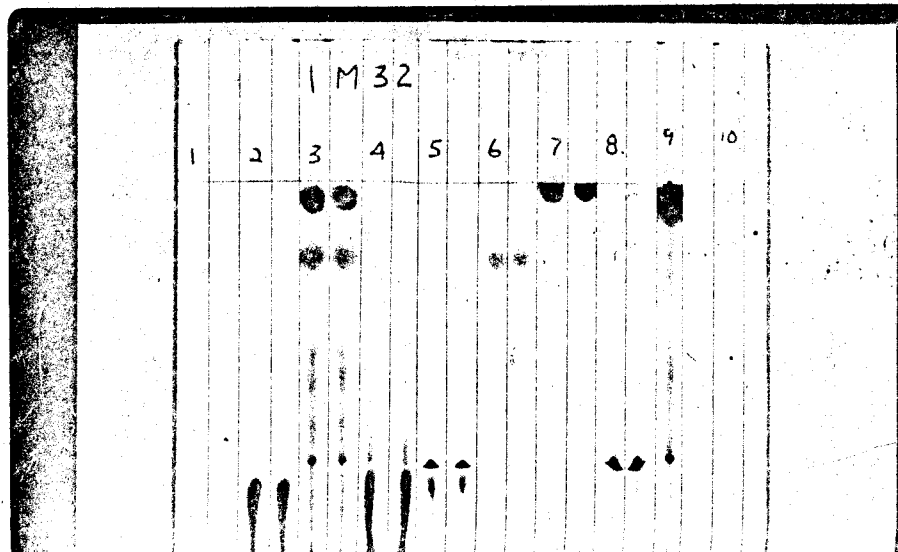
THIN LAYER CHROMATOGRAM

TLC No. __2A1__

Solvent System __Ethylene__
__Dichloride - 1% Glacial__

__Acetic Acid__

Solvent Distance ____12____ cm.

Quantity Applied ____-____

Detection ____I2____

Date __April 1, 1970__

Time In ____-____

Time Out ____-____

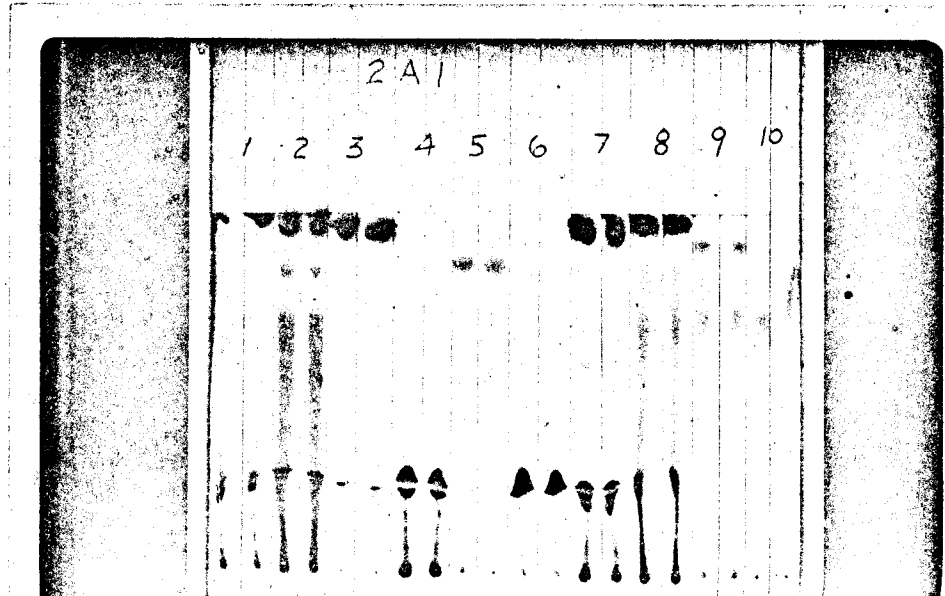TLC No._____

Date_____

Literature:

Adsorption Layer_____

Thickness_____

Type Recording

Photo_____Other_____

_____

Substance

1. cholesteryl palmitate
2. cholesteryl oleate
3. cholesteryl benzoate
4. lecithin ex ovo
5. stearic acid
6. oleic acid
7. chol. palm.-chol. stearate- lecithin
8. unknown
9. tripalmitin
10. trimyristin

Notes

TLC No. _____ 1A2 _____

Solvent System ethylene dichloride & glacial acetic acid

Solvent Distance _____ 12 _____ cm.

Quantity Applied _____ - _____

Detection _____ I_2 _____

Date April 2, 1970

Time In _____ - _____

Time Out _____ - _____

TLC No._____

Date_____

Literature:

Adsorption Layer_____

Thickness_____

Type Recording

Photo_____ Other_____

_____ _____

Substance	Notes
1. cholesteryl oleate	
2. cholesteryl benzoate	
3. cholesteryl stearate	
4. lecithin ex ovo	
5. 1,4,10 (10,1,5)	
6. unknown	
7. arachidic acid	
8. phos. molyb.- phos. tungstic. acid	
9.	
10. trimyristin	

TLC No. _____1F3_____

Solvent System_____80-20-1_____
petroleum ether- ethyl ether-
glacial acetic acid

Solvent Distance _____12_____ cm.

Quantity Applied _____-_____

Detection_____chromic H$_2$SO4_____

Date_____February 3, 1970_____

Time In_____

Time Out_____

TLC No._____

Date_____

Literature:

Adsorption Layer_____

Thickness_____

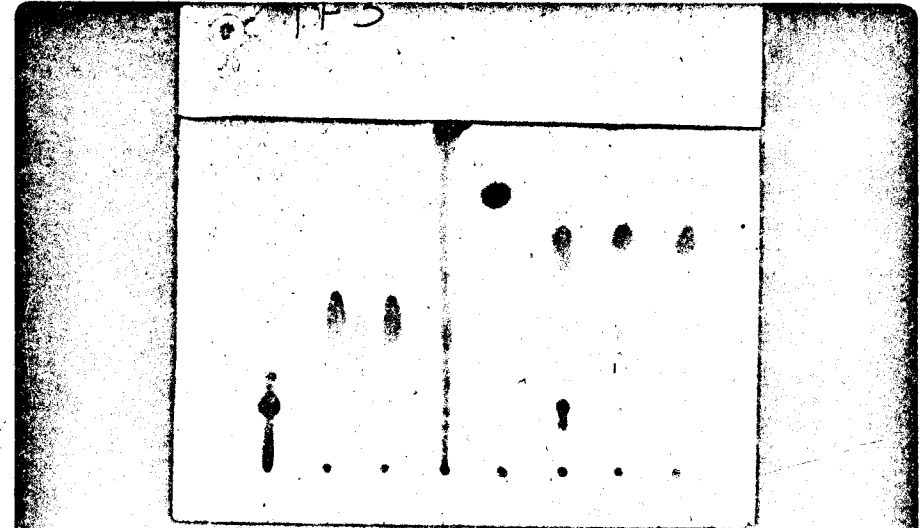**Type Recording**

Photo_____Other_____

_____ _____

Substance

1. _____
2. cholesterol
3. oleic acid
4. myristic acid
5. unknown
6. cholesteryl acetate
7. tristearin
8. tripalmitin
9. trimyristin
10. _____

Notes

TLC No. ___1J28___

Solvent System___90:10:1___
   hexane : ether : glacial

___acetic acid___

Solvent Distance ___15___ cm.

Quantity Applied ___-___

Detection___ chromic-sulfuric acid

Date ___January 28, 1970___

Time In___-___

Time Out___-___

TLC No._____

Date_____

Literature:

Adsorption Layer_____

Thickness_____

Type Recording

Photo_____Other_____

_____

Substance	Notes
1. cholesterol	
2. oleic acid	
3. myristic acid	
4. methyl heptadecanoate	
5. unknown	
6. cholesteryl acetate	
7. tristearin	
8. tripalmitin	
9. trimyristin	
10.	

TLC No. ___4J27___

Solvent System___ethylene___
  dichloride & 1% glacial

acetic acid

Solvent Distance ___15___ cm.

Quantity Applied ___-___

Detection___chromic-sulfuric acid___

Date___January 27, 1970___

Time In___-___

Time Out___-___

TLC No.___

Date___

Literature:

Adsorption Layer___

Thickness___

Type Recording

Photo___Other___

___ ___

Substance	Notes
1. ___cholesterol___	
2. ___unknown___	
3. ___tripalmitin___	
4. ___trimyristin___	
5. ___tristearin___	
6. ___methyl heptadecanoate___	
7. ___myristic acid___	
8. ___oleic acid___	
9. ___cholesteryl acetate___	
10. ___	
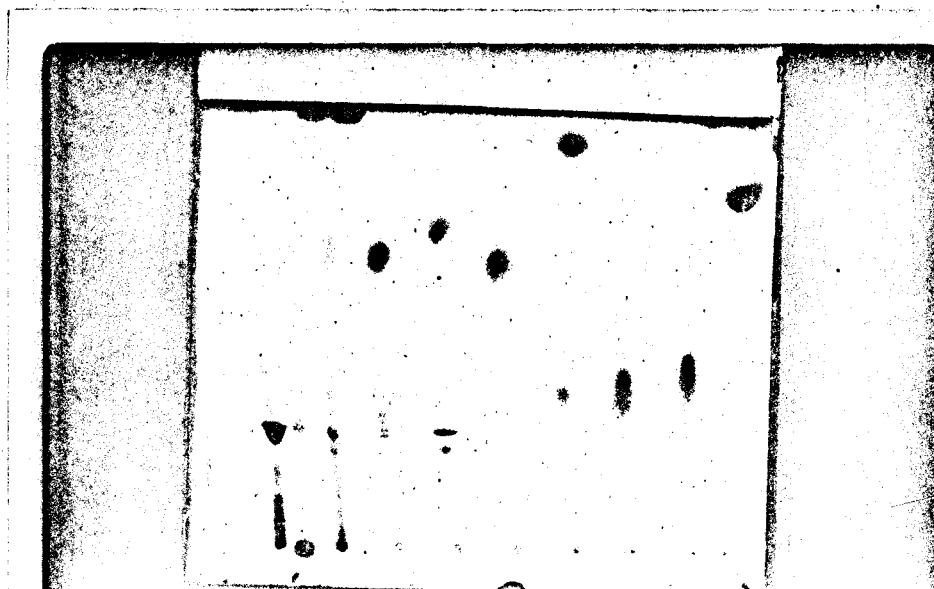
TLC No. ___2A19___

Solvent System___80-20-1___
petroleum ether, ethyl ether-
glacial acetic acid

Solvent Distance ___12___ cm.

Quantity Applied ___-___

Detection___I_2___

Date___April 19, 1970___

Time In___-___

Time Out___-___

TLC No._____

Date_____

Literature:

Adsorption Layer_____

Thickness_____

Type Recording

Photo_____Other_____

_____

	Substance
1.	paraffin&mineral oil (ether)
2.	" (chloroform)
3.	cholesterol
4.	unknown
5.	
6.	squalene
7.	lecithin ex ovo
8.	#1 & peroxide
9.	#2 & peroxide
10.	cholesterol

Notes
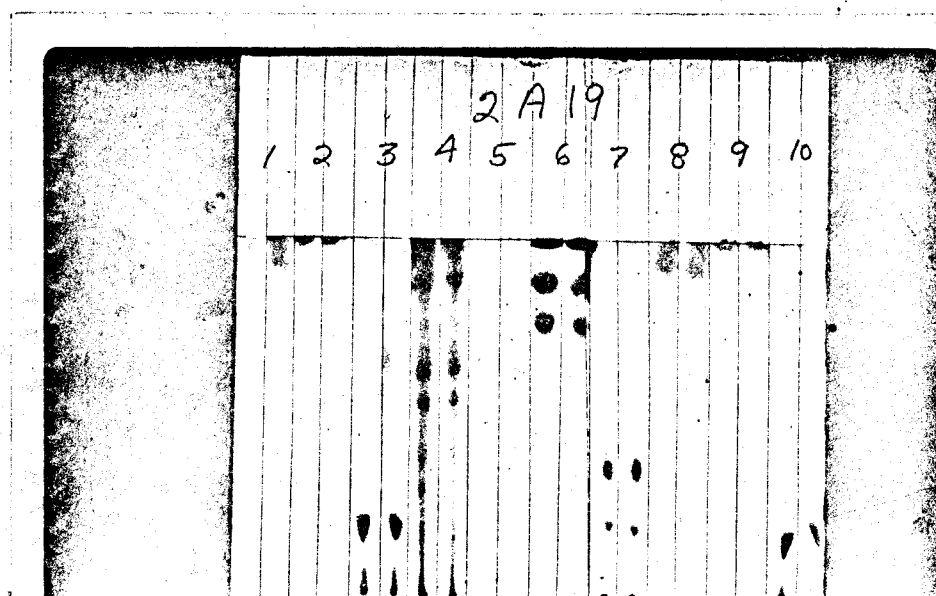
TLC No. _____ 1A19 _____

Solvent System_ ethylene _____
      dichloride & 1% glacial _____
      acetic acid _____

Solvent Distance __ 12 _____ cm.

Quantity Applied _____ - _____

Detection_____ I$_2$ _____

Date_ April 19, 1970 _____

Time In _____ - _____

Time Out_____ - _____

TLC No._____

Date_____

Literature:

Adsorption Layer_____

Thickness_____

Type Recording

Photo_____Other_____

_____ _____ _____

Substance

1. paraffin & mineral oil (ether)
2. _____ " _____ (chloroform)
3. cholesterol
4. unknown
5. _____
6. squalene
7. lecithin ex ovo
8. 1 peroxide
9. 2 "
10. a) cholesterol
    b) oxidized cholesterol

Notes
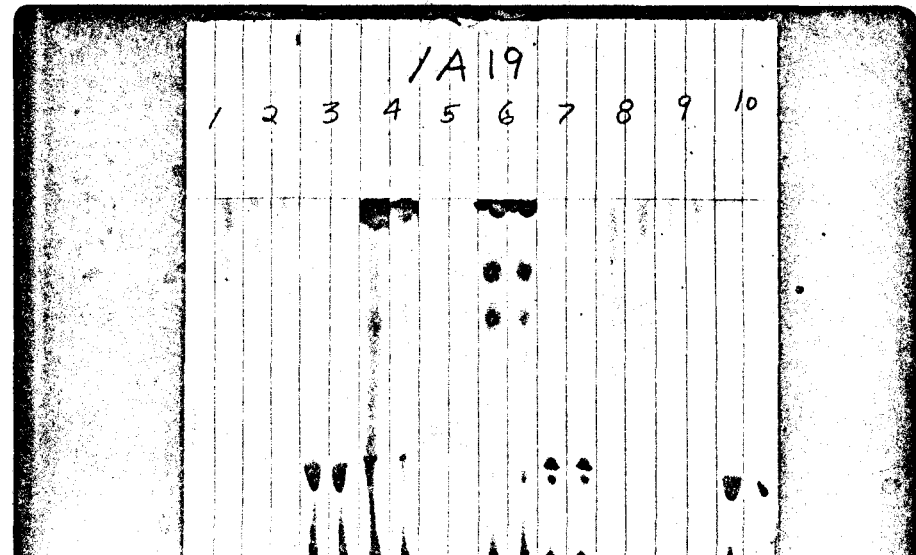
APPROVAL SHEET

The thesis submitted by Anthony F. Molinari
has been read and approved by the director of the thesis.

Furthermore, the final copies have been ex-
amined by the director and the signature which appears
below verifies the fact that any necessary changes have
been incorporated, and that the thesis is now given
final approval.

The thesis therefore is accepted in partial
fulfillment of the requirements for the degree of
Master of Science.

9-6-1970
_____
Date

B. J. Joshoshi
_____
Signature of Advisor