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

Anthony F. Molinari
Loyola University Chicago

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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.

I wish to thank Mr. J. A. Schmit, F & M Scientific Company, Avondale, Pennsylvania, Mr. Paul Hitchco, Department of Biology, Notre Dame University, South Bend, Indiana, and Mr. Montas Farmer, Illinois College of Podiatric Medicine for their gas chromatographic analyses.

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.

Finally, I wish to thank my parents and friends without whose understanding this work would not have been accomplished.
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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 *Rickettsia 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 *Ornithodoros 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 *Ornithodoros 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 egg 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 4°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°C 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°C. 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°C. 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 C₁₇ was added to the residue as an internal standard.

The gas chromatograph was programmed from 100°C, 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-\(\alpha\)-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 12 cm 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-dichlorofluorescein, 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 chromatograms, 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\textsubscript{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

<table>
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<td>wax esters</td>
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</tr>
<tr>
<td>cholesteryl esters</td>
<td></td>
</tr>
<tr>
<td>unsat'd hydrocarbons</td>
<td></td>
</tr>
<tr>
<td>triglycerides</td>
<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>fatty acids</td>
<td></td>
</tr>
<tr>
<td>cholesterol</td>
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</tr>
<tr>
<td>origin</td>
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</table>

Solvent system: petroleum ether 80  
                 ethyl ether 20  
                 acetic acid 1

Figure I
LIPID CLASSES OF THE EGG SHELL OF RHIPICEPHALUS SANGUINEUS

<table>
<thead>
<tr>
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<td>fatty acids</td>
<td></td>
</tr>
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<td>lecithin</td>
<td></td>
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</table>

Solvent system: ethylene dichloride 99 acetic acid 1

Figure II
FATTY ACID COMPONENTS OF EGG SHELL OF
RHIPICEPHALUS SANGUINEUS

A = C\textsubscript{16}:0 Palmitate
B = C\textsubscript{18}:0 Stearate
C = C\textsubscript{18}:1 Oleate
D = C\textsubscript{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 C₁₆:0 and C₁₆:₁.

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 *Q. 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.
The lipid fraction of the egg shells of *Rhipicephalus sanguineus* 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.
BIBLIOGRAPHY


Schmit, J. A. and R. B. Wynne. (No date) Qualitative and Quantitative Lipid Analysis by Gas Chromatography, Methods Bulletin No. 117. F & M Scientific Corporation, Avondale,
Pennsylvania.


APPENDIX
**THIN LAYER CHROMATOGRAM**

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<tr>
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</tr>
<tr>
<td>3. lecithin ex ovo</td>
<td></td>
</tr>
<tr>
<td>4. 2&amp;3</td>
<td>(6,1)</td>
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<td>5. 2&amp;3</td>
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<td>6. unknown</td>
<td>6</td>
</tr>
<tr>
<td>7. synthetic lecithin</td>
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<tr>
<td>8. bovine lecithin</td>
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<td>9. bovine lecithin</td>
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**TLC No.** 1M31

**Solvent System** Ethylene dichloride & 1% Glacial acetic acid

**Solvent Distance** 12 cm.

**Quantity Applied**

**Detection** I$_2$

**Date** March 31, 1970

**Substance**

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
**TLC No. 2A1**

- **Solvent System**: Ethylene Dichloride - 1% Glacial Acetic Acid
- **Solvent Distance**: 12 cm
- **Quantity Applied**: 
- **Detection**: I$_2$
- **Date**: April 1, 1970
- **Time In**: 
- **Time Out**: 

### Substance

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**Notes**

- **Literature**: 
- **Adsorption Layer**: 
- **Thickness**: 
- **Type Recording**: 
- **Photo**: Other

**Thin Layer Chromatogram**

![Image of TLC plate with labeled spots]
**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**

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**Literature:**

- Adsorption Layer
- Thickness

**Type Recording**

- Photo
- Other
<table>
<thead>
<tr>
<th>Substance</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>cholesterol</td>
<td></td>
</tr>
<tr>
<td>oleic acid</td>
<td></td>
</tr>
<tr>
<td>myristic acid</td>
<td></td>
</tr>
<tr>
<td>unknown</td>
<td></td>
</tr>
<tr>
<td>cholesteryl acetate</td>
<td></td>
</tr>
<tr>
<td>tristearin</td>
<td></td>
</tr>
<tr>
<td>tripalmitin</td>
<td></td>
</tr>
<tr>
<td>trimyristin</td>
<td></td>
</tr>
</tbody>
</table>

Solvent System: 80-20-1 petroleum ether-ethyl ether-glacial acetic acid
Solvent Distance: 12 cm.
Quantity Applied: 
Detection: chromic H₂SO₄
Date: February 3, 1970
Time In: 
Time Out: 

Literature:
Adsorption Layer:
Thickness:
Type Recording:
Photo: Other: 

TLC No.: 1FP3
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

Substance

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

Notes
THIN LAYER CHROMATOGRAM

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: 

Substance

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

Notes

Literature:

Adsorption Layer

Thickness

Type Recording

Photo

Other
THIN LAYER CHROMATOGRAM

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

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

Literature:

Adsorption Layer

Thickness

Type Recording

Photo Other

Time In

Time Out

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
## TLC No.

1A19

## Solvent System

ethylene dichloride & 1% glacial acetic acid

## Solvent Distance

12 cm.

## Quantity Applied


## Detection

I2

## Date

April 19, 1970

## Time In


## Time Out


### 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

## Literature:

Adsorption Layer

Thickness

Type Recording

Photo

Other

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## TLC No.


## Date


## Literature:


## Adsorption Layer


## Thickness


## Type Recording


## Photo


## Other


## Notes


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The thesis submitted by Anthony F. Molinari has been read and approved by the director of the thesis. Furthermore, the final copies have been examined 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

[Signature]
Signature of Advisor