THE EFFECT OF DIETHYLCARBAMAZINE AND LEVAMISOLE ON THE DEVELOPMENT OF DIROFILARIA IMMITIS IN AEDES TRISERIATUS

Thesis for the Degree of M. S.
MICHIGAN STATE UNIVERSITY
HEIDI KASKA
1977

THE EFFECT OF D
THE DEVEL

An investigatio
gs, diethylcarbama
loping stages of D
e mosquito Aedes t
d D. immitis micr
cond blood meal co
als were given mos
echnique. Dissectio
pproximately two we
evelopmental stages

Analyses of data
atistically signifi
ups showing as muc
mber of larvae deve
ta revealed a bimod
dissections performed
those mosquitoes i
d-stage. Definite
s established during
ested that DEC may s

ABSTRACT

THE EFFECT OF DIETHYLCARBAMAZINE AND LEVAMISOLE ON THE DEVELOPMENT OF *DIROFILARIA IMMITIS* IN *AEDES TRISERIATUS*

By

Heidi Kaska

An investigation was conducted on the effect of two drugs, diethylcarbamazine (DEC) and levamisole, on the developing stages of *Dirofilaria immitis* (dog heartworm) in the mosquito *Aedes triseriatus*. Mosquitoes were infected with *D. immitis* microfilariae and one week later given a second blood meal containing the test drugs. Both blood meals were given mosquitoes using an artificial feeding technique. Dissections of infected mosquitoes took place approximately two weeks after infection and numbers and developmental stages of larvae were noted.

Analyses of data indicated that both drugs produced statistically significant results, with certain treatment groups showing as much as a 44 percent reduction in the number of larvae developing. A closer examination of the data revealed a bimodal distribution of larval numbers in dissections performed over time and fewer numbers of larvae in those mosquitoes in which *D. immitis* development was at mid-stage. Definite modes of action for these drugs were not established during this research but the results suggested that DEC may slow down larval development and

levamisole may kill or inhibit early developmental stages of the larvae.

THE EFFECT OF
THE DEVE

Mi
in partial

De

THE EFFECT OF DIETHYLCARBAMAZINE AND LEVAMISOLE ON THE DEVELOPMENT OF *DIROFILARIA IMMITIS* IN *AEDES TRISERIATUS*

By

Heidi Kaska

A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE

Department of Entomology

1977

To Ed

I am deeply g
son, for his pa
nt throughout my
ny ways during m
rsity and was alw
a person for who
rsonal admiratio
Williams for his he
ding many of the
ve a busy schedul
would also like t
Graduate Committ
r. Donald C. Cres
ch contributed to
ggestions.

Many others pr
research and dese
s. Jean Beemsterboe
ical Clinic at Mic
esponded to my freq
rles Cress of Mic
ch of the Statist

ACKNOWLEDGEMENTS

I am deeply grateful to my advisor, Dr. Harold D. Newson, for his patience, kindness, and constant encouragement throughout my research. Dr. Newson supported me in many ways during my graduate studies at Michigan State University and was always available with help and advice. He is a person for whom I have the highest professional and personal admiration. I am also grateful to Dr. Jeffrey F. Williams for his helpful comments on my work and for providing many of the supplies needed for my research. Despite a busy schedule, he always took time to assist me. I would also like to thank sincerely the other members of my Graduate Committee for their guidance and friendship: Drs. Donald C. Cress, Gary R. Hooper, and Richard W. Merritt. Each contributed to my work by his beneficial criticisms and suggestions.

Many others provided considerable assistance during my research and deserve special credit: Ms. Gail Peel and Ms. Jean Beemsterboer, laboratory technicians at the Small Animal Clinic at Michigan State University, who cheerfully responded to my frequent requests for blood samples; Dr. Charles Cress of Michigan State University and Mr. Michael Bower of the Statistical Institute at Texas A&M University

ac provided stati
f Texas A&M Unive
ime; Dr. Woodbrid
pplied the Ohio
ding of the Unive
m and Alabama st
l hints on mosqu
he University of M
mb membrane in th
ch of these perso

I would especi
ding my stay at M
nd rewarding exper
illingness to teac
rmiling mosquitoes
esearch, and for h
eeded mosquitoes;
lpful suggestions
earing, and for hi
ate student at Mi

Most of all, I
ll the love and su
ars, and for the
his degree.

who provided statistical assistance; Dr. Norman O. Dronen of Texas A&M University who generously provided computer time; Dr. Woodbridge Foster of the Ohio State University who supplied the Ohio strain of Aedes triseriatus; Dr. George B. Craig of the University of Notre Dame who provided the Walton and Alabama strains of A. triseriatus and gave me helpful hints on mosquito rearing; and Dr. Paul R. Grimstad of the University of Notre Dame who suggested using the Natura-Lamb membrane in the artificial feeding of mosquitoes. To each of these persons I extend sincere thanks.

I would especially like to thank my friends for their making my stay at Michigan State University such a pleasant and rewarding experience: Mr. Mori Zaim for his unfailing willingness to teach me the many things I did not know about handling mosquitoes, for his thoughtful comments about my research, and for his generously providing me at times with needed mosquitoes; and Dr. Henry B. Lewandowski for his many helpful suggestions, for his teaching me much about mosquito rearing, and for his making me feel so welcome as a new graduate student at Michigan State University.

Most of all, I would like to thank my husband Ed for all the love and support he has given me over these past two years, and for the sacrifices he has made helping me obtain this degree.

TABLE OF CONTENTS

TA

DISCUSSION

SUMMARY AND CONCLU

TABLE OF CONTENTS (continued)

TABLE OF CONTENTS (continued)

LIST OF TABLES

L

Table

1. Number of la
mitis larvae
strain) from

3. Number of la
mitis larvae
strain) from

4. Number of la
mitis larvae
strain) from

5. Number of la
mitis larvae
strain) from

6. Number of la
mitis larvae
strain) from

7. Number of la
mitis larvae
strain) from

8. Summary chart
third stage D
(Alabama stra
16, grouped a

9. Summary chart
third stage D
(Alabama stra
dissections w
infection, pl
treatment gro

10. Mathematical
of a randomiz
with replicate

11. Final analysis
from Experimen

12. Orthogonal ana
formed on the
11, 12, 14, a

LIST OF TABLES (continued)

Table

3. Results of nor
contrasts) pe
periments 10,

4. Number of obs
group; percen
were 0% and 1
development o

5. Summary of av
and third sta
in total body
dissections o
(Alabama stra
correlation c
from individu

6. Comparisons o
ances (ADV) w
beginning (EV
mitis develop
bama strain)
tal variances

7. Constant valu
for those tre
greater than
gression from

8. Mean number a
larvae in abd
gions of A. t
11, 12, 14, a

LIST OF TABLES (continued)

L

LIST OF FIGURES

Figure

11. Hypothetical
larvae withi
in which a l
is showing e
ment of the

12. Hypothetical
larvae withi
in which a p
in the thora

13. Hypothetical
and third st
seriatus giv
mental rates

14. Hypothetical
and third s
seriatus sh
fects the n
treatment g
larval deve

15. Hypothetica
and third s
seriatus sh
the number
group, give
velopment

ppendix

4-1. Number of la
immitis larv
tus (Michiga

4-2. Number of la
immitis larv
tus (Michiga

4-3. Number of la
immitis larv
tus (Michiga

4-4. Number of la
immitis lar
tus (Michig

4-5. Number of l
immitis lar
tus (Alabam

4-6. Number of l
immitis lar
tus (Alabam

4-7. Number of l
immitis lar
tus (Alaban

4-8. Number of
immitis la
tus (Alaba

4-9. Number of
immitis la
tus (Alaba

4-10. Number of
immitis la
tus (Alaba

LIST OF APPENDICES

LIST

Appendix

B-1. Number of
immitis l
tus (Alab

B-2. Number of
immitis l
strain) r
meal (Con
8 and is
dissectio

B-3. Number of
immitis l
only the
from Expe
according

B-4. Number of
immitis l
strain) f
ing to lo

B-5. Number of
immitis l
strain) f
ing to lo

B-6. Number of
immitis l
strain) f
ing to lo

B-7. Number of
immitis l
strain) f
ing to lo

B-8. Number of
immitis l
strain) f
ing to lo

LIST OF APPENDICES (continued)

LIST OF

Appendix

B-9. Number of late
immitis larva
strain) from
ing to locati

C-1. Analysis of v
Experiments 1
all the data

C-2. Orthogonal ar
formed on the
11, 12, 14, a
cluding zeros

D-1. Calculations
sis performe
10, 11, 12,

LIST OF APPENDICES (continued)

Dirofilari

f the domestic

ediate host.

found in the ri

og, producing

ease. If left

abilitation o

f dog heartwo

actic drugs t

ors" to preven

Although

effect of thes

n the dog, al

effects on dev

osquito. The

squito is ap

nd during thi

blood meal, co

mounts of the

were found to

n the mosquit

idence of the

INTRODUCTION

Dirofilaria immitis (Leidy) is a filarial parasite of the domestic dog for which the mosquito serves as intermediate host. In its mature state, the parasite can be found in the right ventricle and pulmonary artery of the dog, producing a condition known as canine heartworm disease. If left untreated, this disease may result in severe debilitation or death of the animal. Because of this threat of dog heartworm disease, many dog owners administer prophylactic drugs to their dogs daily during the "mosquito season" to prevent development of the parasite in their dogs.

Although considerable research has been done on the effect of these drugs on the development of the heartworm in the dog, almost nothing is known about their possible effects on developing microfilariae in an already-infected mosquito. The developmental period for *D. immitis* in the mosquito is approximately two weeks in temperate climates and during this interval the mosquito may take another blood meal, conceivably one which could contain small amounts of these prophylactic drugs. If certain compounds were found to retard or prevent development of *D. immitis* in the mosquito, the secondary effect of reducing the incidence of the parasite in a mosquito population may be

f considerable

ictic purposes

f the drug on

e an important

To answer

ffect of prop

quito populati

f two drugs,

the developme

<u>ediatus</u>. On

osquitoes we

wo test drug

examined for

both the ini

containing t

n artificia

this thesis

of considerable worth. Thus in choosing a drug for prophylactic purposes in the dog, one might find that the effect of the drug on developing microfilariae in the mosquito would be an important consideration.

To answer some of these questions about the potential effect of prophylactic drugs on parasite levels within a mosquito population, an investigation was made into the effect of two drugs, diethylcarbamazine (DEC) and levamisole, on the development of D. immitis in the mosquito Aedes triseriatus. One week after infection with D. immitis, test mosquitoes were given a blood meal containing one of the two test drugs. These mosquitoes were later dissected and examined for numbers and development of D. immitis larvae. Both the initial infective blood meal and the later drug-containing blood meal were provided to the mosquitoes using an artificial feeding device. The results are reported in this thesis.

D. immitis

alent in regio

Far East, the

bers of mosqui

pical climates

ease, however,

reported to be

North America

the more temp

In the U

prevalent alo

Otto, 1969a

dense. Lind

Mobile, Alab

Other report

populations

discussed by

reported fr

Wallenstei

et al., 197

more season

in Ontario,

LITERATURE REVIEW

Distribution of the Disease

D. immitis has a worldwide distribution but is most prevalent in regions with more tropical climates such as the Far East, the Pacific, and equatorial Africa, where high numbers of mosquitoes persist yearround. Regions with less tropical climates are by no means free from dog heartworm disease, however, and infection rates among dogs have also been reported to be high in certain portions of South America, North America, Australia, and northern Africa, as well as in the more temperate parts of Europe (Soulsby, 1965).

In the United States the disease is, expectedly, most prevalent along the southern Atlantic and Gulf coasts (Otto, 1969a), where mosquito populations are particularly dense. Lindsey (1961) reported that among pound dogs in Mobile, Alabama, 42 percent had circulating microfilariae. Other reports indicating that up to 63 percent of local dog populations had circulating *D. immitis* microfilariae were discussed by Otto (1972). Numerous cases have also been reported from the Middle Atlantic and New England states (Wallenstein & Tibola, 1960; Rothstein et al., 1961; Tritch et al., 1973), where mosquito populations are also high but more seasonal, and heartworm disease has also been reported in Ontario, Canada (Otto, 1969a).

The preval
thr along the
from non-coasta
ases in areas
southern Minne
olated or lo
though cases
reas of the
Otto, 1969a)
the Mississi
resulted fro
However, rec
cine heartwo
states. Al
dence of th
Laubach (19
in-state de

The
described
in 1856.
site in 1
host, nec
the nemat
veloping
port to

The prevalence of the disease inland is usually lower than along the coast, and high rates of infection reported from non-coastal regions (e.g., 30 to 40 percent infection rates in areas of northern and southern Illinois and in southern Minnesota; Otto, 1969a) have usually occurred in isolated or localized areas of high mosquito density. Although cases of heartworm can be found in virtually all areas of the United States east of the Mississippi River (Otto, 1969a), in the past cases in those states west of the Mississippi River have been infrequent and have usually resulted from infected dogs being brought into those areas. However, recent reports indicate that the incidence of canine heartworm disease is on the rise in the arid western states. Alls and Greve (1974) reported a 6.5 percent incidence of the disease in Iowa and, in Oklahoma, Kocan and Laubach (1976) reported adult worms in 7.3 percent of the in-state dogs sampled.

Taxonomy and Biology of the Parasite

The filarial nematode Dirofilaria immitis was first described by Gruby and Delfond in 1843 and named by Leidy in 1856. Fülleborn described the life cycle of this parasite in 1908 and identified the mosquito as the intermediate host, necessary for completion of the developmental cycle of the nematode (Bradley, 1971). Other workers described developing microfilariae in dog and cat fleas thus giving support to the theory that possibly several haemophagous

ropods were r

is (Breinl,

dog heartworm

Newton and

rasite of dog

Dipetalone

out multiple

and cat f

pp. but not

The adu

ole and a

nd white i

nd the adu

arous, wi

within the

hich has

mbryo wi

ischarg

965).

Mi

size

Newto

malle

as was

hich

strai

filar

arthropods were responsible for the transmission of D. immitis (Breinl, 1920). This hypothesis of multiple vectors of dog heartworm continued to receive acceptance until 1956 when Newton and Wright (1956) described another filarial parasite of dogs in the United States belonging to the genus Dipetalonema. This discovery clarified the confusion about multiple vectors of D. immitis and established that dog and cat fleas were apparently vectors of Dipetalonema spp. but not of D. immitis (Bradley, 1971).

The adult worms of D. immitis, found in the right ventricle and adjacent blood vessels of the heart, are slender and white in color. The adult females measure 25 to 30 cm and the adult males measure 12 to 18 cm. The female is viviparous, with fertilized eggs developing into active embryos within the uterus of the female. The vitelline membrane, which has the appearance of a sheath, is stretched over the embryo within the uterus, but is shed before the embryos are discharged by the female into the host's bloodstream (Soulsby, 1965).

Microfilariae are colorless and transparent, and range in size from 307 to 322 μm with a width of 6.7 to 7.1 μm (Newton & Wright, 1956). (Dipetalonema microfilariae are smaller in size.) D. immitis microfilariae lack a sheath, as was mentioned above, and unlike Dipetalonema spp. in which the tail is hooked, D. immitis microfilariae possess straight tails (Newton & Wright, 1956). D. immitis microfilariae possess no intestine or esophagus, but when the

icrofilariae are s

he interior of the

pm staining are a

retory cell, vari

f which are used

f microfilariae (

Although D. i

lood more or less

ver a 24-hour per

icrofilariae bein

inimum period (Ot

orts that the pat

o locale. E.g.,

1944) observed a

t 1630 hours and

1951), in France

t 2000 hours and

as made by Hawki

elated to oxygen

During the

icrofilariae cir

1960b) reported

emain in the sto

fter which they

uthors have rep

he first 24 hou

al end of the M

microfilariae are stained a column of nuclei is visible in the interior of the microfilaria. Other features visible upon staining are a nerve ring, an excretory pore, an excretory cell, various genital cells, and an anal pore, all of which are used in differentiation of the various species of microfilariae (Taylor, 1960a).

Although D. immitis microfilariae are present in the blood more or less continuously, their numbers fluctuate over a 24-hour period with five to fifty times as many microfilariae being found at the maximum period as at the minimum period (Otto, 1969b). Soulsby (1965) discussed reports that the pattern of this periodicity varies according to locale. E.g., in the United States, Schnelle and Young (1944) observed a maximum number of circulating microfilariae at 1630 hours and a minimum at 1100 hours. Euzeby and Laine (1951), in France, found a maximum number of microfilariae at 2000 hours and a minimum at 0800 hours. The suggestion was made by Hawking (1956, 1967) that this periodicity is related to oxygen tension.

During the course of a blood meal, the mosquito ingests microfilariae circulating in the blood of the dog. Taylor (1960b) reported that in Aedes aegypti these microfilariae remain in the stomach of the mosquito for the first 24 hours, after which they migrate to the Malpighian tubules. Other authors have reported that this movement can occur within the first 24 hours. Further development occurs at the distal end of the Malpighian tubules and for the first week

the larvae can
which they dev
first two days
found circula
mately 250 to
ever, the lar
stage larva
first moult
and the secc
tory and int
of infectio
by 20 μm wi
after the s
tubules int
occurs thr
head. Whe
stage larv
1965).

The
days alth
al., 1974
during th
may react
velopmen
to injur
the Malp
ported b

the larvae can be found inside the cells of the tubules after which they develop within the lumen of the tubules. For the first two days larvae appear very much like microfilariae found circulating in the blood of the dog and measure approximately 250 to 300 μm by 8 to 10 μm. By the fourth day, however, the larvae shorten and thicken to form a "sausage" stage larva measuring 220 to 240 μm by 20 to 25 μm. The first moult occurs at about the eighth day of development and the second stage larvae possess certain elementary excretory and intestinal cells. By approximately the tenth day of infection, the third stage larva develops, measuring 500 by 20 μm with the principle organs already formed. Shortly after the second moult the larvae work their way from the tubules into the body cavity of the mosquito. Movement then occurs through the thorax and into the cephalic spaces of the head. When found in the head, this third or "infective" stage larva may measure 900 μm (Taylor, 1960a,b; Soulsby, 1965).

The infective larvae can remain in the head for several days although many of them may be lost from the head (Ho et al., 1974). (It has been suggested that this loss may occur during the act of obtaining a sucrose meal.) The mosquito may react to the developing larvae by allowing complete development of the parasites, succumbing to the parasites due to injury inflicted during the migration of the larvae into the Malpighian tubules or into the body cavity, or, as reported by Brug (1932) and Kartman (1956), reacting

physiologically
stage of develop
velopment withou

Infective l
the dog) while
ae actually "in
from the mouthpa
small droplet of
the dog. The la
the dog and into
ure made by the
1953a; McGreevy e
wo moults in th
develops in the
tissues (Kume &
generally occur
57, 1965) at wh
1 cm. Followi
heart, the worm
lease microfila
are usually not
initial inocula
worms varies, s
roduced microf

Over 60 sp
laboratory to h
of D. immitis,

physiologically by encapsulating the larvae during an early stage of development (Soulsby, 1965). Lack of larval development without encapsulation can also occur.

Infective larvae are passed to the definitive host (the dog) while the mosquito feeds. The infective larva is not actually "injected" into the animal but, rather, escapes from the mouthparts of the mosquito and may be found in a small droplet of liquid which is deposited on the skin of the dog. The larva then works its way through the skin of the dog and into the bloodstream, generally through the puncture made by the mosquito at the site of feeding (Kartman, 1953a; McGreevy et al., 1974). The infective larva undergoes two moults in the dog (Orihel, 1961) during which time it develops in the submuscular membranes and subcutaneous tissues (Kume & Itagaki, 1955). Migration to the heart generally occurs two to three months after infection (Soulsby, 1965) at which time the worms vary in size from 3.2 to 11 cm. Following arrival in the right ventricle of the heart, the worms reach maturity after which the females release microfilariae into the blood. Circulating microfilariae are usually not found for at least eight months after the initial inoculation. Although the longevity of the adult worms varies, some adult females reportedly have lived and produced microfilariae for more than five years (Otto, 1969a).

Over 60 species of mosquitoes have been shown in the laboratory to be capable of supporting the developing stages of D. immitis, though there is considerable variation among

nsceptible mo

evelopment of

ar support, a

hat may occur

f D. immitis

he Anopheles

ulex genera.

more limited

mals) and who

he developmen

f larger mos

Although

ase of dogs,

arnivores su

1944; Erickso

arely, in n

1975) and ma

ere found i

Medway & Wie

in humans, i

the nematode

1974). In

have been r

is nearly a

outine che

susceptible mosquito species in the length of time needed for development of the parasite, the number of larvae which each can support, and the degree of development of the parasite that may occur. At identical temperatures the development of D. immitis occurs somewhat more rapidly in mosquitoes of the Anopheles genus than in mosquitoes of either Aedes or Culex genera. Species of smaller mosquitoes which possess a more limited stomach capacity (and ingest smaller blood meals) and whose Malpighian tubules are small, may support the development of fewer numbers of larvae than the species of larger mosquitoes (Soulsby, 1965).

Other Hosts

Although canine heartworm disease is primarily a disease of dogs, D. immitis has been shown to occur in other carnivores such as foxes, wolves, coyotes, and cats (Coffin, 1944; Erickson, 1944; Otto, 1972; Sharp, 1974), and, more rarely, in non-carnivores such as beavers (Foil & Orihel, 1975) and marine animals such as seals. (Ninety adult worms were found in the heart of a male harbor seal in California; Medway & Wieland, 1975.) Although infection also may occur in humans, it is generally felt that complete development of the nematode cannot occur in man (Center for Disease Control, 1974). In all, more than 40 human cases of dirofilariasis have been reported (Otto, 1972), and presence of the worm is nearly always discovered inadvertantly, such as on a routine chest x-ray, where immature worms are detected in

the lung or pulmon

lesion (Navarrete

such human cases

Holden (1974) rep

mary symptoms d

hemotherapy clea

As one would

microfilariae wer

peared to reach

tion in a human

In a patient wit

parent infection

Dog heartwo

examination of

odified Knott'

ther microfilar

and x-rays may

Jackson, 1969a

filariae provid

the disease (nu

sequent congest

Treatment

the infection

tion of a propl

different stag

the lung or pulmonary artery in the form of a small "coin" lesion (Navarrete, 1972; Robinson et al., 1974). Although such human cases are usually asymptomatic, Feldman and Holden (1974) reported a case of a female patient with pulmonary symptoms diagnosed as having presumptive D. immitis. Chemotherapy cleared up the symptoms.

As one would expect in the cases described, circulating microfilariae were not demonstrated as the worms never appeared to reach sexual maturity. One recent case of infection in a human with circulating microfilariae was reported in a patient with lupus erythematosus, however, but the apparent infection cleared up without treatment (Green, 1975).

Diagnosis and Treatment

Dog heartworm disease is most frequently detected by examination of a blood sample from the dog using either the modified Knott's or a filtration technique to determine whether microfilariae are present. Additionally, heartsounds and x-rays may also be used in the diagnosis of the disease (Jackson, 1969a,b) because the number of circulating microfilariae provides no real indication of the severity of the disease (number of adult worms in the heart and subsequent congestion thereof).

Treatment of active cases involves first eliminating the infection and then preventing reinfection by administration of a prophylactic drug. Different drugs are used for different stages of the disease as a drug used to treat one

stage of the d

stage. Many d

often too toxi

tive heartworm

sodium which c

microfilariae a

three weeks (O

then is recomme

in dogs treated

h., Inc., 1967

Prophylaxi

of DEC from two

"to season" a

with further re

every six month

veloped. Admin

of circulating

allergic or tox

amisole (levam

is currently be

microfilaricide

and in prophyla

However, discuss

for the dog and

stage of the disease may not be effective against another stage. Many drugs used to treat an active infection are often too toxic to be used routinely for prophylaxis. Active heartworm infections are treated with Thiacetarsamide sodium which causes the adult female worms to release their microfilariae all at once and then slowly die in two to three weeks (Otto, 1969b). Dithiazanine iodide or Stibophen is recommended to destroy the circulating microfilariae in dogs treated in the above manner (Otto, 1969b: Merck and Co., Inc., 1967).

Prophylaxis currently is limited to daily administration of DEC from two months prior to two months after the "mosquito season" at a dosage of 1.5 mg/lb (Jackson, 1969c), with further recommendations that Thiacetarsamide be given every six months to kill any adult worms that may have developed. Administration of DEC to a dog with high numbers of circulating microfilariae can produce severe, presumably allergic or toxic, reactions (Desowitz et al., 1975). Tetramisole (levamisole), a new broad-spectrum anthelmintic, is currently being tested for its potential use both as a microfilaricide in the treatment of active heartworm disease and in prophylaxis (Tulloch et al., 1970) of the disease. However, discussion continues as to the proper dosage levels for the dog and the side effects and contraindications.

Diethylcar
ıamy-4-methylpi
in 1947 by Hewi
ared for use a
ilized in a 5
itrate tablet
rade name Cari
in man) and has
stages of Wuche
nd other filar
arwood, 1969;

Long and
Alford (1972)
latively non-t
that oral admi
and reached a
wo hours afte
cleared from t
found in the
three times th
a testing labo
period of two
udged by rat
al chemistry
athology. S
DEC had no si

Pharmacology of Diethylcarbamazine

Diethylcarbamazine, the generic name for 1-diethylcarbamy-4-methylpiperazine, was first reported as a filaricide in 1947 by Hewitt et al. (1947). Although DEC was first prepared for use as a hydrochloride, it is now most commonly utilized in a 50 percent biologically active dihydrogen citrate tablet form. This compound is marketed under the trade name Caricide (for use in dogs) and Hetrazan (for use in man) and has been used in the treatment of developing stages of Wuchereria bancrofti, W. pacifica, Brugia malayi, and other filariases for the past twenty years (James & Harwood, 1969; Bryan & Southgate, 1976).

Long and short term studies discussed by Burkhart and Alford (1972) on DEC's pharmacology have shown that it is relatively non-toxic to vertebrates. Plasma assays showed that oral administration of the drug was rapidly absorbed and reached a peak concentration in the plasma approximately two hours after dosage, after which the drug was rapidly cleared from the blood. No detectable trace of the drug was found in the plasma after 24 hours. Daily administration of three times the recommended dose (9.9 mg/kg) to test dogs by a testing laboratory of the American Cyanamid Company for a period of two years produced no evidence of toxicity as judged by rates of survival, food intake, hematology, clinical chemistry, organ weights, and gross and microscopic pathology. Studies through F1 and F2 generations showed that DEC had no significant long term effects on reproduction.

In studies

ingham (1955a)

nd broken down

the ring intac

n the degradat

chain occurred,

were excreted a

. immitis s

vitro, such upt

rowding medium

In vitro s

ingham (1955b)

active against

et al. (1950) d

showed no ill e

heart blood dra

animals with th

dermis atropary

ment solution

the proboscis

Such resu

Bangham, 1955

by immobilizin

ying their su

ration of mi

treatment with

et al. (1976)

In studies with cotton rats, hooded rats, and monkeys, Bangham (1955a) showed that DEC was metabolized very rapidly and broken down into four metabolites, each with the piperazine ring intact. Partial demethylation was an early step in the degradation, after which further stripping of the side chain occurred, until finally piperazine and methylpiperazine were excreted as the end products. Although microfilariae of D. immitis showed uptake of the drug after one hour in vitro, such uptake was only one-third the amount of the surrounding medium (Bangham, 1955b).

In vitro studies with DEC and its metabolites by Bangham (1955b) showed that all of these compounds were inactive against Litomosoides carinii microfilariae. Hawking et al. (1950) demonstrated that microfilariae of D. repens showed no ill effects after having been placed in citrated heart blood drawn one-half to two hours after dosing the animals with the drug. In the same study, Anopheles maculipennis atroparvus infected with D. repens fed on a one percent solution of DEC in glucose had well-developed larvae in the proboscis after 14 days.

Such results led Hawking et al. (1950) and later authors (Bangham, 1955b) to speculate that DEC acts as a filaricide by immobilizing the microfilariae in the bloodstream or modifying their surfaces for later removal by phagocytosis. Examination of microfilariae and host tissues before and after treatment with DEC in cases of onchocerciasis have led Gibson et al. (1976) to speculate from observable changes in

microfilarial cuticle that DEC alters the mucopolysaccharide or collagen of the cuticle unmasking the worm to the vertebrate host and allowing the body to recognize it as foreign.

Pharmacology of Levamisole

The anthelmintic activity of tetramisole hydrochloride (dl-2,3,4,6-tetrahydro-6-phenyl imadazole (2,1-b) imazole hydrochloride) was first reported by Thienpoint et al. (1966). Investigators concluded that it was the levorotatory isomer of this drug which was biologically active and it is this isomer (l-tetramisole or levamisole) which is now in use in the United States as a general anthelmintic for sheep, cattle, and swine. The hydrochloride form is generally given in oral administrations of the drug whereas levamisole phosphate solution is marketed as an injectable anthelmintic (Bradley, 1976).

Although levamisole's primary use is in treatment of the many internal parasites of livestock, recent studies have shown that it may also be effective in treating certain types of cancer in man and animals and in certain immune deficiency or autoimmune diseases such as rheumatoid arthritis (Tripodi et al., 1973; Schuermans, 1975; Grob, 1976). Its effect in these diseases is apparently to stimulate the immune system.

Preliminary investigations have indicated that levamisole shows promise as a D. immitis microfilaricide and adulticide. Mills and Amis (1975) stated that daily doses of 10 mg/kg of levamisole cleared microfilariae from the bloodstream after 7 to 11 days of treatment, when the levamisole treatment

as given to

treatment.

uced circul

adult worms

to 1200 mg c

Other s

effective ag

Lazar and Na

sole (1 to 5

terri microf

days of leva

mg/kg of DEC

reduction in

Duke (1974)

at 10 mg/kg

microfilaria

reduction in

bancrofti patie

in microfila

used to trea

and cats (Jo

In vitr

against micr

certain nema

the percent

effective la

minutes and

was given to dogs three weeks after Thiacetarsamide sodium treatment. Tulloch and Anderson (1971, 1972) reported reduced circulating microfilariae levels and elimination of adult worms in infected dogs each dosed with a total of 1100 to 1200 mg over approximately six weeks.

Other studies have indicated that levamisole is also effective against developing stages of other filarial worms. Zaman and Natarajan (1973) showed that oral doses of levamisole (1 to 50 mg/kg) eliminated circulating Breinlia sergenti microfilariae in slow lorises after only one to four days of levamisole treatment, whereas treatment with 50 mg/kg of DEC over a similar time period resulted in only reduction in the numbers of circulating microfilariae. Duke (1974) found that levamisole injected intramuscularly at 10 mg/kg for 15 days in a chimpanzee greatly reduced microfilariae counts and Zaman and Lal (1973) found marked reduction in microfilariae numbers in two Wuchereria bancrofti patients treated with levamisole. Similar reductions in microfilarial counts were obtained when levamisole was used to treat Brugia malayi in man (O'Holohan & Zaman, 1974) and cats (Joon-Wah et al., 1974; Rogers & Denham, 1976).

In vitro studies have shown that levamisole is effective against microfilariae and third stage infective larvae of certain nematodes. Rogers and Denham (1976) showed that a one percent concentration of levamisole can kill third stage infective larvae of Brugia pahangi in vitro within five minutes and that a 0.04 percent solution can kill the

infective larvae within 50 to 60 minutes. These authors also reported that 0.5 to 1.0 percent solutions of levamisole can kill B. pahangi microfilariae in vitro within 15 minutes whereas 0.04 percent and even 0.01 to 0.03 percent concentrations can kill the microfilariae within 24 hours. Unpublished data reported by the same authors (Rogers & Denham, 1976) showed that significantly higher concentrations of DEC are required to affect B. pahangi microfilariae. More than 0.2 percent DEC was required to kill all B. pahangi microfilariae in 24 hours in vitro, whereas 0.04 percent levamisole killed microfilariae in the same amount of time. A 0.7 percent levamisole concentration killed microfilariae in six hours whereas 0.3 percent DEC was needed to produce similar results. (Hawking et al. (1950) reported that a 4 mg/ml concentration of 50 percent biologically active DEC was ineffective against microfilariae of D. repens in vitro.)

Studies on the effect of levamisole on Brugia pahangi in Aedes aegypti indicated that when the drug was given to mosquitoes in sugar solution, concentrations as low as 0.08 percent resulted in stunted third stage or sausage stage larvae; greater concentrations reduced the number of larvae per infected mosquito (Rogers & Denham, 1976). Similarly, Gerberg et al. (1972) found that a 0.1 percent solution of levamisole administered in sucrose solution to A. aegypti mosquitoes infected with D. immitis eliminated or prevented complete development of the nematode. Such studies show that levamisole is apparently directly effective against

nematodes rather than having to be first activated by metabolic processes in the dosed animal.

Clearance tests in pigs have shown no traces (at 0.05 PPM sensitivities) of levamisole in the blood of an animal dosed with 8 mg/kg after 24 hours (Johnson et al., 1972).

The most accepted explanation of levamisole's activity is that levamisole probably acts by inhibiting the succinic dehydrogenase pathways of the nematode (Van den Bossche & Janssen, 1967; PharmIndex, 1973) resulting in helminth paralysis and dislodgement. Such paralysis preceded by hyperactive coiling of Breinlia sergenti adults was reported by Natarajan et al. (1974) and has also been associated with increased muscular tone of the worms. Coles and Jenkins in personal communication to Rogers and Denham (1976) reported that in vitro 0.01 percent concentrations of levamisole caused reversible paralysis of the adult Nippostrongylus brasiliensis (an internal parasite) when the worms were left in the drug. The authors postulated that this recovered motility resulted from recovery of activity of a neuroreceptor in the worm. (The drug at high doses stimulates the receptor for a time but a point is reached where the receptor can no longer be stimulated and the worm recovers its motility.)

Although levamisole appears to be as effective as DEC against developing stages of D. immitis and other filarial worms, controversy exists regarding its mammalian toxicity. Single oral dosages as low as 5 mg/lb have resulted in

vomiting, diarrhea, lack of appetite, and "distress" in treated dogs (Jackson, 1972), although these symptoms may appear only after the first dosage. Administration of the d and l isomers at levels of 15, 20, and 25 mg/kg/day to cats infected with developing stages of Brugia pahangi also produced salivation and "distress" for several hours after dosing (Rogers & Denham, 1976). Alford in Jackson (1972) reported that daily administration of the dl isomers of tetramisole (which is slightly less than 50 percent biologically active) to dogs at levels of 30 mg/kg, 15 mg/kg, and 7.5 mg/kg resulted in four out of six female dogs developing hemolytic anemia after 13 weeks. No changes were seen in similarly treated male dogs, however, and attempts by Alford to duplicate his results were unsuccessful.

Artificial Feeding Technique

Current artificial feeding techniques involve the use of a membrane to cover the blood source and an apparatus to hold the blood and membrane and possibly regulate such factors as temperature of the blood, etc. The blood used in the artificial feeding of mosquitoes is usually taken from the preferred host of that particular mosquito species (e.g., in the case of A. aegypti, human or mammalian blood; in the case of Culex territans, amphibian blood). Fresh, whole blood is usually used and is always either heparinized or defibrinated. However, hemolysed or frozen blood, erythrocyte extract (Rutledge et al., 1964), or outdated human

blood (as described by Tarshis, 1959) can be prepared and used with success.

Types of membranes used in such artificial feeding techniques have ranged from fresh ones, such as rat skin (Woke, 1937), rabbit skin (Yoeli, 1938), and chicken skin (Bishop & Gilchrist, 1944), to artificial or prepared membranes, such as the Baudruche membrane (a bovine intestinal preparation) used by Greenberg (1949) and the Trojan brand NaturaLamb condom membrane used by Dr. Paul Grimstad of the University of Notre Dame (personal communication). Currently, most workers find these latter two membranes most satisfactory because of their ease of preparation and storage.

Kartman (1953b) and later authors such as Wade (1975) demonstrated that mosquitoes can successfully be infected with filarial worms using the artificial feeding technique and that anticoagulants apparently have no observable effect of the development of such larvae. The advantages to infecting mosquitoes artificially with such inoculants as filarial worms are not only reduced cost and experiment simplification, but also, as pointed out by Weiner and Bradley (1970), the titer of the infected blood can be regulated by dilution with "normal" blood.

Microfilariae from such nematodes as D. immitis are seemingly quite hardy. Bemrick et al. (1965) showed that blood containing microfilariae could be stored for up to four months at -68°C and then thawed and fed artificially

x mosquitoes.

infective stage

Perumdurai et al

be transported

taining microfi

successfully in

velopment of th

Most of th

ques has involv

membrane and th

et al. (1974) i

other suitable

the tubes, plac

the cage. Refi

egulation of s

ture (if and wh

non-colloidal s

of the blood.

developed by Ru

warm water to

start temperatu

be autoclaved a

infectious ager

1975) have mod

method for stir

to mosquitoes. Development of the microfilariae to the infective stage in such mosquitoes was near normal levels. Ponnudurai et al. (1971) reported that filarial worms can be transported between laboratories by mailing blood containing microfilariae at room temperature and later can be successfully introduced to susceptible mosquitoes with development of the worms going to completion.

Most of the variability in artificial feeding techniques has involved the feeding apparatus used to house the membrane and the blood. A simple system described by Wills et al. (1974) involves placing the blood in test tubes or other suitable containers capped with membrane and inverting the tubes, placing them either over the mosquito cage or in the cage. Refinement of the technique has concentrated upon regulation of such variables as the homogeneity of the mixture (if and when the blood were mixed with non-soluble or non-colloidal substances) (Behin, 1967) and the temperature of the blood. A frequently cited system now in use is that developed by Rutledge et al. (1964) involving circulating warm water to maintain the blood or other material at a constant temperature. This apparatus, shown in Figure 1, can be autoclaved and is especially designed for the feeding of infectious agents to mosquitoes. Other authors such as Wade (1975) have modified such feeding apparatuses to include a method for stirring the blood mixture.

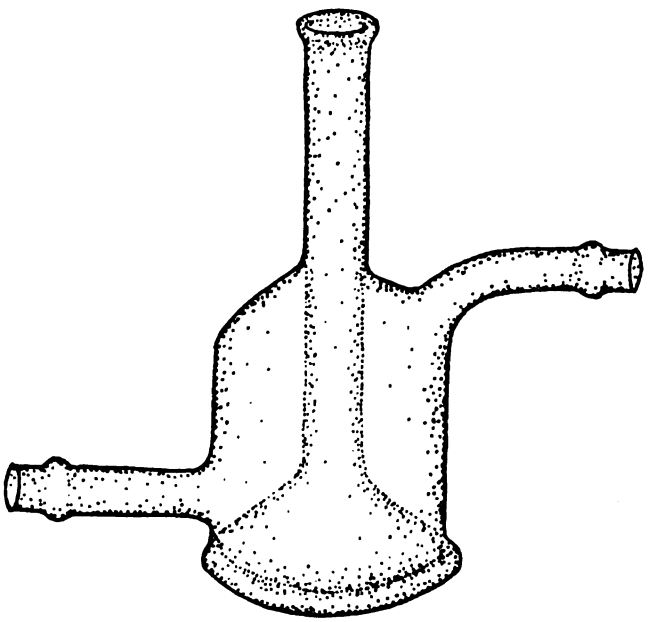

Figure 1. Apparatus for artificial feeding of mosquitoes. (Adapted from a figure by Rutledge et al., 1964.)

The proced

) an initial

ation on mosqu

itis in the mo

nd (2) perform

esigned to det

evamisole on d

Ho et al.

rom the head

o insure that

ults here, all

ent were perf

ice constraint

ould be disse

y been conduc

icrofilariae,

ents involving

. irritis gen

ug to the mo

Hawking et al

osquitoes in

METHODS AND MATERIALS

Proposed Research

The procedure followed during this research involved (1) an initial period of gathering certain background information on mosquito feeding behavior, development of D. immitis in the mosquito strain chosen, and research techniques, and (2) performing several replicates of a single experiment designed to determine the effect of diethylcarbamazine and levamisole on developing D. immitis in the mosquito.

Ho et al. (1974) reported loss of infective larvae from the head or mouthparts of infected mosquitoes over time. To insure that such losses would not affect experimental results here, all dissections from a single replicate experiment were performed over a 24-hour time period. (Given this time constraint, no more than approximately 80 mosquitoes could be dissected in one day.) Investigations have previously been conducted on the effect of DEC and levamisole on microfilariae, infective larvae, or adults in vitro. Experiments involving the effects of these two drugs on developing D. immitis generally have involved administration of the drug to the mosquitoes in sucrose or other sugar solutions (Hawking et al., 1950; Gerberg et al., 1972). The use of mosquitoes in screening anti-filarial and anti-malarial drugs,

ministering t

to be quite eff

gan; Keegan, l

owever, in thi

osquitoes in a

training the d

the drug in suc

the blood inges

goes directly t

to the diverti

difficulties o

on-infected d

and because of

Man mosquitoe

both infected

rugs using a

ificial feed

Two spec

during these

ides aegypt

State Univer

s. aegypti h

elopment o

Moreevy et

that D. imm

administering the compounds in such a manner, has proved to be quite effective and relatively inexpensive (Terzian, 1947; Keegan, 1968; Gerberg, 1971; Gerberg et al., 1972). However, in this research the drugs were given to infected mosquitoes in a second blood meal to better simulate their obtaining the drugs under natural conditions. (Provision of the drug in such a manner may be vitally important in that the blood ingested by the mosquito during the blood meal goes directly to the midgut whereas sucrose solution goes to the diverticulum or crop.) Additionally, because of the difficulties of obtaining and maintaining infected dogs and non-infected drug-dosed dogs for the mosquitoes to feed upon and because of the variability of feeding rates reported when mosquitoes feed directly upon dogs, mosquitoes were both infected with D. immitis microfilariae and given the drugs using a modification of the previously described artificial feeding apparatus.

Mosquito Rearing

Two species and five strains of mosquitoes were used during these experiments. Initially, the ROCK strain of Aedes aegypti, maintained in our laboratory at Michigan State University, was chosen for use in this research as A. aegypti had been shown to successfully support the development of D. immitis (Taylor, 1960b; Gerberg et al., 1971; McGreevy et al., 1974). However, early experiments indicated that D. immitis would not develop in this particular strain

f mosquito. (Th

irely surprising

aintained in the

t sometimes los

filarial worms fo

A native str

he Michigan stra

nd obtained from

State University

esearch. Experi

s immitis develo

olony of this Mi

mself in the lab

s priseriatus co

rains from the

f Notre Dame wer

r. Woody Foster,

provided by Dr.

usceptibility to

evelopment in al

as chosen for us

ription of each

hese techniques

des aegypti (RO

The colony

esticide Researc

ersity was origi

of mosquito. (This problem with the ROCK strain was not entirely surprising, as strains of mosquitoes which have been maintained in the laboratory for some time have been known to sometimes lose their ability to support development of filarial worms for unknown reasons; Taylor, 1960b.)

A native strain of A. triseriatus (hereafter called the Michigan strain) initially chosen because of expediency and obtained from treeholes in wooded areas on the Michigan State University campus, was then considered for use in this research. Experiments indicated that this strain supported D. immitis development. However, when my particular subcolony of this Michigan strain failed to mate and maintain itself in the laboratory, one strain from an established A. triseriatus colony at the Ohio State University and two strains from the Vector Biology Laboratory at the University of Notre Dame were obtained. The Ohio strain (provided by Dr. Woody Foster, OSU) and the Walton and Alabama strains (provided by Dr. George Craig, UND) were all tested for susceptibility to D. immitis. Although D. immitis completed development in all three of these strains, the Alabama strain was chosen for use in the remainder of this research. A description of each mosquito strain and its rearing and maintenance techniques appears below.

Aedes aegypti (ROCK strain)

The colony of A. aegypti mosquitoes maintained at the Pesticide Research Center laboratory at Michigan State University was originally obtained from Dr. George Craig of the

University of Notre Dame. Dr. Craig's laboratory initially received this strain from Dr. D. W. Jenkins of the Rockefeller Institute in 1959. This strain of A. aegypti is considered the best laboratory strain available because of its fecundity and vigor. These mosquitoes are relatively large and uniform and are used in numerous laboratories as the "typical" strain of mosquito (University of Notre Dame, 1974).

Eggs of this strain were obtained from existing colonies at MSU and after embryonation were hatched in distilled water and transferred to enamel rearing pans (10"x 16"x 2½"), 350 mosquitoes per pan. Diet during this larval stage consisted of Tetramin, a commercially available fish food. Rearing pans were kept in an insectary maintained at 78±2°F and 80±10% relative humidity. Pupation began approximately seven days after hatching. Adults emerged two days after pupation and were thereafter kept in 12"x 12"x 12" mosquito cages and maintained on 10 percent sucrose solution absorbed onto cotton. Two days after the infective blood meal, the experimental group of mosquitoes was provided with oviposition materials (filter paper placed in a beaker containing approximately one inch of water). (Although not all authors allow blood-fed mosquitoes to oviposit it was felt that nearly natural conditions should be maintained during this research. Additionally, as mosquitoes were expected to receive a second blood meal, it was felt that allowing them to oviposit after the first blood meal might increase the number which fed during the second blood meal.) Mosquitoes kept separately

for stock purposes were allowed to feed every two weeks on an immobilized guinea pig and eggs were later collected.

Aedes triseriatus (Michigan strain)

This strain of A. triseriatus was initially obtained from larvae collected from treeholes in wooded areas on the campus of Michigan State University. A successful colony was maintained in the insectary at the Pesticide Research Center by Mr. Mori Zaim but my attempt to maintain a subcolony failed because the adults would not mate. Attempts to encourage mating behavior by lowering the temperature in the insectary to 72°F, by reducing the male:female ratio from 1:1 to 1:2, by increasing the size of the cage in which the mosquitoes were being maintained from 12"x 12"x 12" to 24"x 24"x 24", and by repositioning the lighting in the insectary proved unsuccessful. General maintenance techniques and blood meals were provided in a manner similar to that described above, for A. aegypti, although larval rearing pans contained 250 larvae each and water used for hatching was first deoxygenated with nitrogen gas.

Aedes triseriatus (Alabama strain)

Eggs from this strain of mosquito were obtained from Dr. George Craig of the University of Notre Dame in September 1976. Dr. Craig received this strain from H. Schoof of the Calhoun Technical Development Laboratory, U. S. Public Health Service, Savannah, Georgia, in July 1969, but the strain was reported to be initially collected from Alabama in the 1930s and had been maintained in the laboratory ever since that

time (University of Notre Dame, 1974; personal communication with Dr. Craig, 1976). This strain of mosquito is noted for its high fecundity and is considered to be an excellent laboratory mosquito (University of Notre Dame, 1974).

Rearing procedures for this strain of A. triseriatus differred somewhat from those for the two previously discussed strains. After embryonation, eggs of this strain were hatched in distilled water primed with a small amount of Difco nutrient broth powder to reduce the oxygen content of the water. Shortly after hatching, mosquitoes were transferred to enamel rearing pans, approximately 250 mosquito larvae per pan, and were fed Tetramin fish food during the course of their development. Rearing pans were kept in the insectary, maintained at 72±2°F and 80±10% relative humidity. Pupation began about 11 days after hatching and the pupal period lasted approximately two days. The adults used for stock purposes were placed in a 24"x 24"x 24" cage whereas mosquitoes which were used for experimental purposes were placed in 18"x 18"x 18" cages. Approximately three days after the experimental group of mosquitoes received their infective blood meal, oviposition materials were placed in the cage and eggs were collected. As genetic selection of experimental mosquitoes was to be avoided, these eggs were later discarded.

Aedes triseriatus (Walton strain)

Eggs of this strain were also obtained from Dr. Craig of the University of Notre Dame and were collected by R. Beach at the Izaac Walton Preserve, St. Joseph Co., Indiana in

ne 1969. T

ished by fo

the mating

974). This

n the mann

Aedes trise

This m

Ohio State

at the med

versity ap

personal

strain was

research

Alabama s

The

meals du

sources

with sma

(Figure

in 10 m

to prev

the blo

and the

turning

or "li

June 1969. The University of Notre Dame colony was established by forced copulation for two generations after which time mating occurred naturally (University of Notre Dame, 1974). This strain of mosquito was reared and maintained in the manner described above for the Alabama strain.

Aedes triseriatus (Ohio strain)

This mosquito, obtained from Dr. Woody Foster at the Ohio State University in September 1976, was established at the medical entomology laboratory of the Ohio State University approximately 15 months prior to our obtaining it (personal communication with Dr. Foster, 1976). This Ohio strain was reared and maintained during the course of this research in the manner described for the A. triseriatus Alabama strain.

Artificial Feeding Apparatus

The apparatus used to provide mosquitoes with blood meals during the course of this research heated stationary sources of water which were in direct contact with the blood with small electric light bulbs (7-watt "night lights") (Figure 2). Such lights, wired in parallel, were enclosed in 10 ml beakers sealed against a board with latex caulking to prevent electric shock. The temperature of the water and the blood was monitored with a small aquarium thermometer and the temperature of the water was regulated by manually turning the lights on and off. (A variable voltage regulator or "light dimmer" could also be used to control the

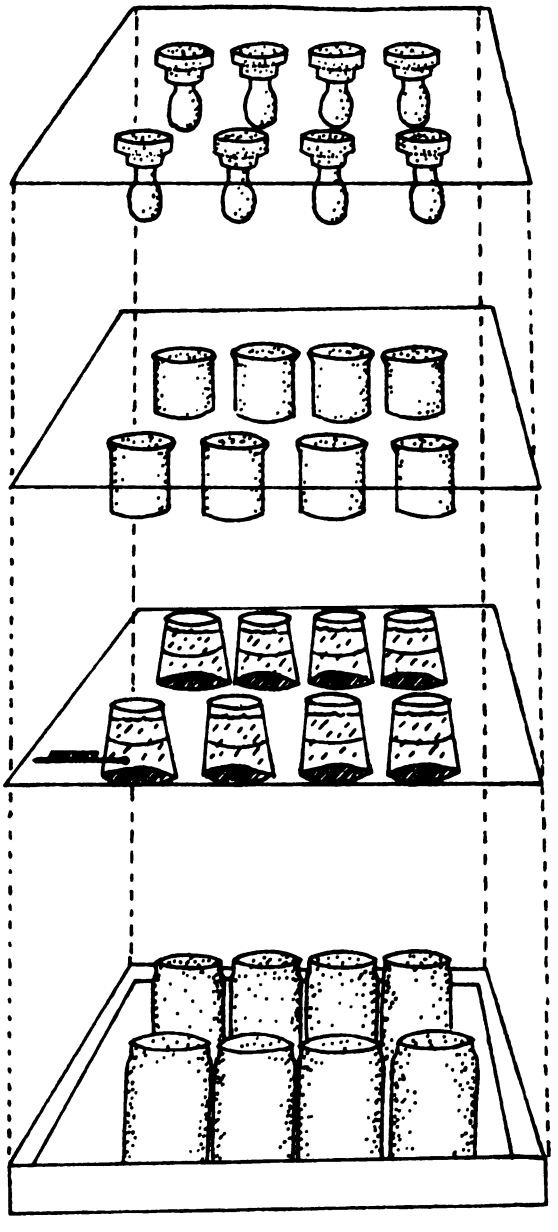

Figure 2. Artificial feeding apparatus used to feed mosquitoes in this research.

ater temp

provides e

without th

ransporte

Membra

The a

sed durir

. immitis

lood meal

tested.

fed female

irated f

bout 50

hen secu

nd eight

eeding a

or holdi

ith the

mall pie

ipped to

ately 3

membrar

rd also

verted ar

eating a

beaker sh

water temperature.) The cost of such an apparatus which provides eight feeding stations is no more than seven dollars, without the dimmer, and it is lightweight and can be easily transported.

Membrane Preparation and Artificial Feeding Setup

The artificial feeding apparatus shown in Figure 2 was used during this research both to infect mosquitoes with D. immitis microfilariae and to provide them with a second blood meal containing various dosages of the two drugs tested. On the day of the first blood meal, previously unfed female mosquitoes, approximately one week old, were aspirated from their cage and placed in 24 pint jars, with about 50 female mosquitoes per jar. Mosquito netting was then secured over the top of these jars with rubber bands and eight jars at a time were placed in position under the feeding apparatus containing the blood. The containers for holding the blood consisted of eight small plastic cups with the bottoms cut out (open cylinders) (Figure 3). A small piece of Saran Wrap was loosely stretched across the lipped top of the cup and secured with rubber bands. Approximately 3 ml of blood was poured into this section of the cup. A membrane was then tightly stretched over this blood source and also secured with rubber bands. The cup was then inverted and water was poured into the upper section. The heating apparatus, consisting of the light bulbs and glass beaker shield section, was then placed in the water and the

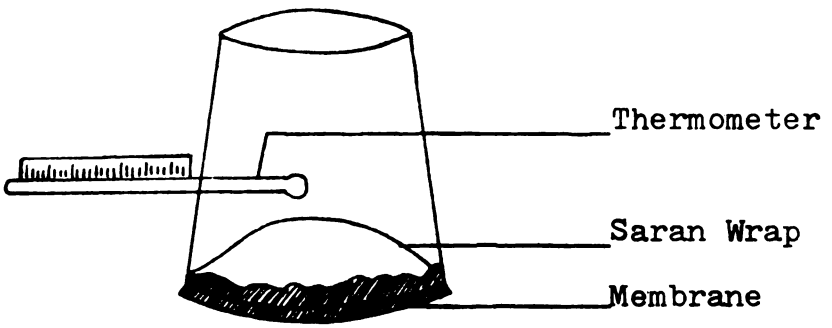

Figure 3. Feeding cup for use in the artificial feeding apparatus shown in Figure 2.

temperature was monitored with the aid of a small aquarium thermometer. Throughout the feeding period, the blood was maintained at approximately 102°F (dog body temperature) by turning the light bulbs on and off.

Mosquitoes generally landed upon the membrane surface within the first five minutes after placement of the entire apparatus on the netting-covered jars containing the mosquitoes. Feeding soon ensued and within 15 minutes most of the mosquitoes which were to feed engorged and dropped to the bottom of the jar. Jars were removed from under the feeding apparatus after 20 minutes and the procedure was repeated.

During the early course of this research, fresh bovine small intestine obtained from a local slaughterhouse, was used as a membrane source. The intestine was cut into 2"x 2" portions, placed in a pan containing saturated sodium chloride solution and soaked for approximately five minutes to help loosen the inner mucosa and the remainder of the gut contents. The inner mucosa was then removed by gently scraping it with the blunt edge of a scissors blade. The remaining outer membrane was then placed in a saturated sodium chloride solution and soaked for another five minutes. Any remaining inner mucosa was gently rubbed away with the finger and the membrane left was then rinsed several times in distilled water. The remaining outer membrane was tan colored, opaque, quite elastic and flexible, and was very easy to work with. Remaining portions of unprepared gut

were then i

also found

time, and i

Later,

membranes.

sheep gut a

cow gut mem

branes invol

they were p

three or fo

ms.

During

were sorted

gas generat

carefully s

new holding

consuming a

ches would

manner, so

after being

from the fe

into cage

forged mosq

destroyed.

ches for t

were then frozen for later preparation and use. It was also found that the membranes could be prepared ahead of time, and frozen for later use.

Later, Trojan brand NaturaLamb condoms were used as membranes. These condoms are commercially prepared from sheep gut and are quite flexible and much thinner than the cow gut membrane described above. Preparation of these membranes involved carefully washing off the lubricant in which they were packaged. Each membrane was then divided into three or four sections, ready for use in the feeding apparatus.

Sorting the Mosquitoes

During the early experiments, blood-fed mosquitoes were sorted after being anesthetized with carbon dioxide gas generated from dry ice. Engorged mosquitoes were carefully separated from unengorged ones and placed in a new holding cage. This technique was extremely time-consuming and it was impossible to insure that no mosquitoes would escape or become damaged when sorted in such a manner, so the following sorting technique was soon adopted. After being offered the first blood meal, all mosquitoes from the feeding jars were placed in a 12"x 12"x 12" mosquito cage and unengorged mosquitoes or incompletely engorged mosquitoes were then aspirated from that cage and destroyed. The cage was carefully checked visually several times for the presence of any non-fed mosquitoes.

On th

:oes were

:hen place

:ngorged a

:ribed and

:ized ice

:om. Thos

:ood blood

:nd were r

:ion. Ques

:oes were

Blood

:rom dogs

:t the Col

:f blood w

:Mariae (

:lood cont

:xed Husk

:hes and

:usky's bl

:hich cont

:er 1000

:y when i

:econd blo

:ounts of

On the day of the second blood meal, infected mosquitoes were first transferred from their cage into jars and then placed under the feeding apparatus. Mosquitoes that engorged at this time were separated in the manner described and placed into small, especially prepared, pint-sized ice cream cartons, no more than 25 mosquitoes per carton. Those mosquitoes which did not engorge during this second blood meal were used as one of the two control groups and were replaced into their original cage for later dissection. Questionably engorged or partially engorged mosquitoes were destroyed.

Blood Source and Drug Concentrations

Blood used during these experiments was obtained from dogs kept for blood donation or research purposes at the College of Veterinary Medicine at MSU. Two types of blood were used: blood containing circulating microfilariae (from a Basset hound named Scotch) and normal blood containing no microfilariae (from a mixed-breed dog named Husky). Both male dogs were kept indoors at all times and neither dog received medication of any kind. Husky's blood was used both to "dilute" Scotch's blood, which contained a very high microfilariae count (600 to over 1000 microfilariae per 20 μl, depending on the time of day when it was drawn), and to provide mosquitoes with a second blood meal, at which time it was mixed with small amounts of the test drugs. Dilution of Scotch's blood

as done bec
oes fed upo
bury et a
). Addi
irculating
ered feedi
ingested by
than that ac
14 concen
sed during
On the
each dog was
s an antico
he mosquito
ml of th
feeding cups
then distrib
These prepar
ented to fe
For th
nd it was
brought
rations of
al, 2.5 m
he eight f
0.9 perc
d each of

was done because of reported of high mortality among mosquitoes fed upon dogs with a high microfilaremia (Travis, 1947; Duxbury et al., 1961; Weiner & Bradley, 1970; Kuntz & Dobson, 1974). Additionally, microfilariae tend to settle when not circulating (that is, they settle on the membrane of the inverted feeding apparatus) and the number of microfilariae ingested by artificially fed mosquitoes might be much higher than that acquired during natural feeding. For this reason, a 1:4 concentration of Scotch's blood to Husky's blood was used during the course of these experiments.

On the day of the infective blood meal, blood from each dog was collected in 5 ml vacuum tubes containing EDTA as an anticoagulant. Such blood was immediately brought to the mosquito laboratory and combined. Approximately 2.5 to 3.0 ml of this blood mixture was poured into each of eight feeding cups, the blood being continually shaken to insure even distribution of microfilariae throughout the medium. These prepared cups were then capped with membrane and presented to female mosquitoes in the manner described previously.

For the second blood meal, only Husky's blood was used and it was drawn in the manner described above, immediately brought to the laboratory, and mixed with various concentrations of the test drugs. At the time of this second blood meal, 2.5 ml of blood (final volume) was placed in each of the eight feeding cups. Two of them contained small amounts of 0.9 percent saline (mixed with the blood) as a control, and each of the remaining cups contained a 10^{-3}, 10^{-4}, or

$^{-5}$ percer

ration of

Little

levamisc

fter norma

hat the cc

search adeq

A norm

s consider

ord (1972)

he citrate

hich would

eight. Le

re monito

ration pea

the oral

t al. (194

se of DEC

the same

al dose.

iven to a

.33 ng/l c

he plasma.

Rogers

dicated t

10^{-5} percent concentration of either DEC or levamisole. Preparation of these drug concentrations is described below.

Drug Concentrations and Preparation

Little information was available on the amount of DEC or levamisole that would be present in the blood of a dog after normal prophylactic treatment. However, it is felt that the concentration of the drugs used during this research adequately represents the entire range of probability.

A normal dose of DEC used for prophylaxis of D. immitis is considered to be 3.3 mg/kg (drug base). Burkhart and Alford (1972) reported about dosing dogs with 45.4 mg/lb of the citrate form of DEC (50 percent biologically active) which would be equivalent to 50 mg/kg (base drug) of dog weight. Levels of DEC in the plasmas of such dosed dogs were monitored after dosing and after two hours the concentration peaked at 5 μg/ml plasma (5 mg/l), or one-tenth of the oral dose given. Similarly, the results of Harned et al. (1948) showed that one-fifth to one-tenth of an oral dose of DEC administered intravenously produced approximately the same results (as judged by dog symptomology) as the oral dose. Therefore if a normal dose of 3.3 mg/kg were given to a dog, after two hours one would expect to find 0.33 mg/l of the drug (3.3×10^{-5} percent concentration) in the plasma.

Rogers and Denham (1976) in dosing cats with levamisole indicated that an oral dose of 10,000 mg/kg of levamisole

uld be necessar

e drug in the b

uld therefore p

ug in the blood

at a dose of 1

ophylaxis again

rapolating fro

pect to find 2.

ecause Gerberg e

ncentration of

f D. immitis in

f 5 mg/kg of the

dog all at once

ly 65 mg/l of

ight percent of

10^{-3} percent con

l. Thus for t

ercent concentr

r use in these

The source

ections was Car

Na citrate. Ea

tain 100 mg of

ighed out. Th

.5 percent sali

f the drug base

en distributio

would be necessary to produce a one percent concentration of the drug in the blood. A 1 mg/kg oral dose of levamisole would therefore produce a 10^{-4} percent concentration of the drug in the blood. Tulloch and Anderson (1972) suggested that a dose of 1 mg/lb, administered orally, is adequate for prophylaxis against D. immitis in the dog. At this dosage, extrapolating from Rogers and Denham's findings, one would expect to find 2.2×10^{-5} percent levamisole in the blood. Because Gerberg et al. (1972) showed that a 10^{-3} percent concentration of levamisole affected the developing stages of D. immitis in mosquitoes, and, because even if a maximum of 5 mg/kg of the drugs were to enter the bloodstream of a dog all at once the concentration found there would be only 65 mg/l of blood or 6.5×10^{-3} percent (given that eight percent of an average-sized dog's body is blood), a 10^{-3} percent concentration as an upper limit is not illogical. Thus for the reasons presented, 10^{-3}, 10^{-4}, and 10^{-5} percent concentrations of each of these drugs were chosen for use in these experiments.

The source of DEC for preparation of the various concentrations was Caricide® tablets, each containing 400 mg of DEC citrate. Each pill actually weighed 0.948 grams and to obtain 100 mg of the base drug, 0.474 grams of the pill were weighed out. This amount of DEC was placed in 1000 ml of 0.9 percent saline to produce a 10^{-2} percent concentration of the drug base and sonicated for five minutes to insure even distribution of the drug. (Saline was used as the

diluent for

would lyse r

the ml of th

diluted into

ml 10^{-4} per

of these thr

hog blood.

the blood we

provided by

ml of blood

The te

available i

stock, as t

widespread

according t

material co

EDL, a 90

ns of 100

formulatio

was placed

a 10^{-2} per

were made

and 0.25 m

0.25 ml of

$^{-4}$, and

percent sa

diluent for these concentrations because sterile water alone would lyse red blood cells when added to the dog blood.) One ml of the 10^{-2} percent concentration was then serially diluted into 9 ml of sterile saline twice to produce 10^{-3} and 10^{-4} percent concentrations. One-quarter ml of each of these three concentrations was placed into 2.25 ml of dog blood. Thus the final concentrations of the drug in the blood were 10^{-3}, 10^{-4}, and 10^{-5} percent. A control was provided by placing 0.25 ml of 0.9 percent saline in 2.25 ml of blood.

The tetramisole used during this research was only available in the liquid form, used for treatment of livestock, as this particular drug is not yet authorized for widespread use in dogs by the Food and Drug Administration. According to the package instructions, one mg of the liquid material contained the equivalent of 182 mg of levamisole HCl, a 90 percent active compound (Jackson, 1972), or 164 mg of 100 percent active compound. Six-tenths ml of this formulation, containing approximately 100 mg of levamisole, was placed in 1000 ml of sterile saline solution to produce a 10^{-2} percent concentration of the drug. Serial dilutions were made to obtain 10^{-3} and 10^{-4} percent concentrations, and 0.25 ml of each of these concentrations was placed into 2.25 ml of blood to obtain final concentrations of 10^{-3}, 10^{-4}, and 10^{-5} percent levamisole. One-quarter ml of 0.9 percent saline was placed into 2.25 ml of blood as a control.

Througho

the drugs wer

tion of the c

found in the

during each

were thoroug

pipettes wer

same step of

were made.

were kept s

proximately

Dissec

quitoes wer

effect on

quito. On

mosquitoes

tions were

before the

seen. At

was kille

the carto

any other

found ins

to identi

clean sl

were rem

Throughout these experiments, new concentrations of the drugs were prepared every two weeks to preclude deterioration of the compounds (although this phenomenon had not been found in the literature). Pipettes were used only once during each step of the drug concentration preparations and were thoroughly washed before reuse at a later time. These pipettes were marked and the same pipette was used at the same step of preparation each time the drug concentrations were made. Prepared mixtures were shielded from light and were kept stored, when not in use, in a refrigerator at approximately 40°F.

Dissection Techniques

Dissections of experimentally-treated infected mosquitoes were made to determine whether the drugs had any effect on the development of *D. immitis* larvae in the mosquito. On the day of dissection, cartons containing the mosquitoes which had ingested the various drug concentrations were drawn at random and then completely dissected before the drug and dosage the mosquitoes had received was seen. At the time of dissection the mosquito to be examined was killed by knocking it several times against the side of the carton. (Freezing the mosquitoes or treating them in any other similar manner reduced the motility of the larvae found inside, which in turn made the larvae more difficult to identify and count.) The dead mosquito was placed on a clean slide under a dissecting microscope and legs and wings were removed.

In the mann

gut of the mosq

slide in a drop

empty anterior

reased open and

mainder of the

was the head, a

apart. The he

pharyngeal are

found larvae i

so particular

Many infective

quito and cou

very carefull

complete, eac

scope and th

were found i

of the thora

larvae were

two areas w

No lar

nation beca

one day. M

cause in s

s. immitis

The crite

stages of

In the manner described by Jones (1967), the entire gut of the mosquito was removed and placed on a separate slide in a drop of distilled water. The terminal and now-empty anterior abdominal segments of the mosquito were teased open and any remaining contents expelled. The remainder of the abdomen was broken away from the thorax, as was the head, and the thorax was opened and muscles teased apart. The head was teased open and the buccal cavity and pharyngeal area very carefully examined. (Burton (1963) found larvae in such unusual places as palpi and antennae, so particular care was taken in examining the entire head.) Many infective larvae were found in the labium of the mosquito and could be overlooked, so the labium was always very carefully slit longitudinally. After dissection was complete, each slide was examined under the compound microscope and the results recorded. Because so many larvae were found in the cervical region and in the anterior part of the thorax, it was difficult to ascertain whether these larvae were in the head or the thorax so counts from these two areas were added together.

No larval stain was used during the microscopic examination because so many mosquitoes had to be examined during one day. Methylene blue, a quick stain, was not used because in some cases its use reduced the motility of the D. immitis larvae and made them more difficult to locate. The criteria used for determining the various developmental stages of the larvae was their size. Late second stage

larvae (post "sausage" stage) and early and late third stage larvae were added together in counts, though notation was made as to the approximate stage of each larva identified (Iyengar, 1957; Taylor, 1960b).

Experimental Procedures

Nine trials or experiments were conducted to gather background information for this research. Tasks included refining experimental procedures and techniques, determining the best mixture of Scotch's blood and Husky's blood to use in infecting mosquitoes, examining development of *D. immitis* in the mosquito, and determining the best post-infection day for dissection of the mosquitoes. Although the procedures used in Experiments 1 through 9 varied somewhat from experiment to experiment, they were generally similar. Previously unfed female mosquitoes of the same age group were offered a dilution of microfilaria-infected blood in the artificial feeding device. (After Experiment 7, the Natura-Lamb membrane was used instead of the cow gut membrane in this device.) To reduce mosquito mortality, mosquitoes were not provided with this first infective blood meal until they were approximately one week old (Duxbury et al., 1961; Weiner & Bradley, 1970; Intermill, 1973). Following the 20 minute feeding period, engorged mosquitoes were sorted either by anesthetization (Experiments 1 and 2) or by aspiration (Experiments 3 through 9). Infected mosquitoes were kept in the insectary at 78±2°F and 80±10% relative humidity, except

here otherwi

osquitoes in

fected mosqui

Results secti

osquitoes re

ere utilized

ith D. immi

ny kind, an

ceived a sec

Seven e

f DEC and l

quito. For

ale mosquit

a 1:4 dilut

tificial fe

After feedi

into 12"x l

elative hu

aining the

oes in th

ontrol gr

issection

4, and 1

or Exper

After infe

quitoes w

quitoes c

where otherwise mentioned. A second blood meal was provided mosquitoes in Experiments 4, 5, and 6, and dissections of infected mosquitoes occurred at intervals specified in the Results section of this thesis. In those experiments where mosquitoes received a second blood meal, two control groups were utilized: one in which the mosquitoes had been infected with D. immitis but had not received a second blood meal of any kind, and a second group in which infected mosquitoes received a second blood meal containing no drug.

Seven experiments were conducted to determine the effect of DEC and levamisole on developing D. immitis in the mosquito. For Experiments 10 through 16 previously unfed female mosquitoes, approximately one week old, were offered a 1:4 dilution of microfilariae-containing blood in an artificial feeding apparatus, using the NaturaLamb membrane. After feeding, engorged mosquitoes were sorted by aspiration into 12"x 12"x 12" cages and maintained at 72±2°F and 80±10% relative humidity. One week later a second blood meal containing the test drugs was offered to the infected mosquitoes in the same manner as the infective blood meal. Two control groups were used as in Experiments 4, 5, and 6. Dissection of the mosquitoes from Experiments 10, 11, 12, 14, and 15 took place on post-infection day 16. Dissections for Experiment 13 mosquitoes took place early, on day 14 after infection, and late dissections for Experiment 16 mosquitoes were performed on day 17. Occasionally not all mosquitoes could be dissected on the appointed day. In such

cases, the extra mosquitoes belonged to Control group 2 and were usually dissected during the first 12 hours of the following day. (The results of these late Control group 2 dissections were listed separately from the dissections performed on time.)

Back

Experiment 1

Scotch's bl

1:5, and 1:11 co

offered to Aedes

mine what effect

and subsequent m

are shown in Tab

percent for 337

After feedi

separate cages a

had been fed. F

of dead mosquito

the results appe

quitoes was low

dead per day) al

after infection

Dissections

the first week a

toes (1 to 2 per

tion. Microfila

distributed thro

RESULTS

Background Information Experiments

Experiment 1

Scotch's blood was mixed with Husky's blood in 1:1, 1:5, and 1:11 concentrations. These concentrations were offered to Aedes aegypti (ROCK strain) mosquitoes to determine what effect, if any, each might have on feeding rates and subsequent mosquito mortality. Mosquito feeding rates are shown in Table 1. The overall feeding rate was 78.2 percent for 337 females fed and 94 females not fed.

After feeding, engorged mosquitoes were placed in three separate cages according to the concentration of blood they had been fed. Examinations of these cages for the presence of dead mosquitoes were made over the next several days and the results appear in Table 2. Mortality among infected mosquitoes was low after the first week of infection (1 to 2 dead per day) although mortality during the first six days after infection ranged from 68.5 to 82.6 percent.

Dissections were performed of dead mosquitoes during the first week after infection and of the surviving mosquitoes (1 to 2 per day) almost daily up to 19 days after infection. Microfilariae or early first stage larvae were seen distributed throughout the Malpighian tubules within 24

Table 1. Feeding rates for A. aegypti (ROCK strain) in Experiment 1.

Jar No.	Conc. Received	Engorged Females	Unfed Females	% Females Fed
1	1:1	32	6	84.2
2	1:1	30	8	78.9
3	1:1	20	3	86.9
4	1:1	33	1	97.1
Total	1:1	115	18	86.5
5	1:5	31	16	65.9
6	1:5	26	12	68.4
7	1:5	40	1	97.6
8	1:5	30	10	75.0
9	1:5	3	5	37.5
Total	1:5	130	44	74.7
10	1:11	4	12	25.0
11	1:11	36	8	81.8
12	1:11	23	6	79.3
13	1:11	22	4	84.6
14	1:11	7	2	77.8
Total	1:11	92	32	74.2

Table 2. Mortality rates of *A. aegypti* (ROCK strain) fed upon dog blood containing three different concentrations of *D. immitis* microfilariae.

Cage No.	Blood Conc. Received	Day Post-Infection	No. Mosqs. Living	Cumulative % Mortality
1	1:1	0	115	0
		1	67	41.7
		2	40	65.2
		3	36	68.7
		4	34	70.4
		5	33	71.3
		6	31	73.0
2	1:5	0	130	0
		1	102	21.5
		2	75	42.3
		3	61	53.1
		4	52	60.0
		5	44	66.2
		6	41	68.5
3	1:11	0	92	0
		1	61	33.7
		2	36	60.9
		3	26	71.7
		4	22	76.1
		5	20	78.3
		6	16	82.6

hours after the infective blood meal. By the fourth day, some larvae, shortened in length and enlarged in width, could be seen in the distal ends of the tubules. Later dissections showed that development past the "sausage" stage never did occur and most of the larvae were encapsulated by the mosquito in the earliest stages of development. In six mosquitoes dissected 19 days after the initial infective meal four had no larvae visible at all and two contained several encapsulated larvae each.

Experiment 2

A. aegypti (ROCK strain) mosquitoes were allowed to feed on a 1:2 concentration of blood containing microfilariae, and feeding rates and D. immitis development in the mosquito were examined. The overall feeding rate for this experiment was 52.6 percent (103 females fed and 93 females unfed). Seventy-two hours after the blood meal, 48 of the 103 engorged mosquitoes had died. Results of dissections performed on the 13th and 17th days after the infective blood meal were similar to those in Experiment 1: the nematode apparently did not develop in this strain of mosquito.

Experiment 3

Both A. aegypti (ROCK strain) and A. triseriatus (Michigan strain) mosquitoes were allowed to feed on a 1:4 dilution of D. immitis-containing blood and both feeding rates and nematode development were observed. Results are reported in Table 3.

Table 3. Feeding rates of A. aegypti (ROCK strain) and A. triseriatus (Michigan strain) in Experiment 3.

Jar No.	Mosquito Species	Engorged Females	Unfed Females	% Females Fed
1	A. aegypti	8	7	53.3
2		10	11	47.6
3		18	20	47.4
4		9	12	42.9
5		12	8	60.0
6		20	6	23.1
7		7	24	22.6
8		11	24	31.4
9		9	5	64.3
10		21	52	28.8
11		17	15	53.1
12		5	14	26.3
13		17	22	43.6
14		14	10	58.3
15		23	18	56.1
16		29	10	74.4
17		15	23	39.5
Total		295	281	51.2
18	A. triseriatus	13	21	39.4
19		12	11	52.2
20		15	21	41.7
21		7	12	36.8
22		24	8	75.0
23		9	7	56.3
Total		80	80	50.0

Mortality of engorged mosquitoes was extremely low. (The aspiration method of sorting engorged mosquitoes was used here.) Three days after infection only 10 out of 296 infected A. aegypti and four out of 80 infected A. triseriatus mosquitoes had died. By the 19th day a total of 19 A. triseriatus mosquitoes had died.

A. aegypti were dissected on days 3, 4, and 6 after infection. As in Experiments 1 and 2, D. immitis had not developed beyond the "sausage" stage. Encapsulation of early first stage larvae was again seen in many of the mosquitoes dissected.

A. triseriatus dissections, performed on days 3, 4, 6, 8, 10 through 14, and 17 post infection, revealed definite progressive development of the nematode. Sausage stage larvae were seen on the third day and subsequent dissections showed continued larval development so that by the eighth day many late second stage larvae could be seen in the Malpighian tubules. By the tenth day, early third stage larvae were present in the tubules, and by day 11 many third stage larvae were observed in the abdominal hemocoele and thorax. By day 12, many infective larvae were found in the head and proboscis regions and any larvae still remaining in the Malpighian tubules were in at least the late second or early third stages of development. Dissections done after the 12th day showed a progressive decrease in the number of larvae found in the Malpighian tubules. A summary of A. triseriatus dissections appears in Table 4.

Table 4. Average number of late second and third stage D. immitis larvae per A. triseriatus (Michigan strain).

Day Post-Infection	No. Mosqs. Dissected	No. Larvae in Abdomen	No. Larvae in Head/Thorax	Total No. Larvae
8	2	21.0	0	21.0
10	4	19.3	0	19.3
11	2	8.5	0.5	9.0
12	6	3.8	16.2	20.0
13	4	3.3	7.0	10.3
14	4	2.0	9.0	11.0
17	4	1.0	6.3	7.3

The maximum number of infective stage larvae was found in the head region on post-infection day 12; therefore, it was decided that the best day for dissection, under the experimental conditions used here, was the 12th day after initial infection of mosquitoes. It was also decided that A. aegypti would no longer be used in this research.

Experiments 4 and 5

The purpose of these two experiments was to evaluate laboratory procedures used to provide the second blood meal before research proceeded on the major objective of the study. Both groups of A. triseriatus (Michigan strain) mosquitoes used in these experiments were hatched and reared together. Experimental procedures and techniques used were identical, with both groups of mosquitoes being allowed to feed on a 1:4 concentration of Scotch's blood. Feeding rates at the first blood meal for each experiment appeared to be about average (that is, about 50 percent fed); however,

the number of mosquitoes available for attempted feeding was low. Only 53 of approximately 100 mosquitoes from the Experiment 4 group and 35 of approximately 75 mosquitoes from the Experiment 5 group engorged.

Six days later, a second blood meal containing various concentrations of the experimental drugs was made available to the groups of infected mosquitoes. Feeding rates this time were quite low: of the 38 original Experiment 4 mosquitoes which were still alive at the time of this second feeding, only 9 took a blood meal; and of the 30 remaining Experiment 5 mosquitoes, only 5 fed this second time.

Dissections from both Experiments 4 and 5 were begun on the 12th day after infection and the number of late second and third stage larvae recorded for each of the test and control groups. Results are shown in Tables 5 and 6. (See Appendix A for data on individual observations.)

Experiments 6 and 7

The purpose of these two experiments was to evaluate the experimental procedures involved in providing mosquitoes with a second drug-containing blood meal and gather data on infection levels within individual mosquitoes. The results of these experiments are discussed together because mosquitoes from each of these two groups were hatched on the same day and reared together. Infection of Experiment 6 and 7 mosquitoes on 1:4 concentrations of Scotch's blood took place five days apart, although otherwise identical experimental procedures were used for both experiments.

Table 5. Number of late second and third stage D. immitis larvae in A. triseriatus (Michigan strain) from Experiment 4.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	-	-	-	-
DEC 10^{-4}	12	2	5.0	2.0
DEC 10^{-5}	12	1	30.0	-
Lev 10^{-3}	-	-	-	-
Lev 10^{-4}	12	1	3.0	-
Lev 10^{-5}	12	1	6.0	-
Control 1*	12	3	16.7	12.8
Control 2*	12	6	27.8	5.4
Control 2*	13	12	19.4	2.8

Table 6. Number of late second and third stage D. immitis larvae in A. triseriatus (Michigan strain) from Experiment 5.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	-	-	-	-
DEC 10^{-4}	-	-	-	-
DEC 10^{-5}	12	1	19.0	-
Lev 10^{-3}	12	1	8.0	-
Lev 10^{-4}	-	-	-	-
Lev 10^{-5}	-	-	-	-
Control 1*	12	1	15.0	-
Control 2*	13	16	14.1	1.4

*In these and following tables, Control 1 refers to the control group containing infected mosquitoes taking a second blood meal containing saline rather than a drug and Control 2 refers to the control group containing infected mosquitoes not taking a second blood meal at all.

Only 34 mosquitoes from Experiment 7 took an infective meal and for this reason these mosquitoes were not given a second blood meal. Dissections of the Experiment 6 mosquitoes, which did take a second blood meal, were begun on day 12 after infection and the results are shown in Table 7. Experiment 7 mosquitoes were dissected 14 days after infection and the results appear in Table 8. Additional data on the numbers of second and third stage larvae from individual mosquitoes can be found in Appendix A.

Experiment 8

In this experiment, evaluations were made of feeding rates and developmental success of D. immitis for the Alabama strain of A. triseriatus. The Alabama strain was infected with a 1:4 concentration of Scotch's to Husky's blood and kept at 75±2°F. An estimated 75 to 80 percent of the females fed during the 20 minute feeding period. Mortality of infected mosquitoes over the next 15 days was relatively low.

Dissections of infected mosquitoes, performed on days 3, 5, 7, 9, 12, and 13 after infection, indicated the same, progressive development of D. immitis that was described in Experiment 3. Dissections performed on days 15 and 16 indicated that on these days most larvae were in the head/proboscis region and, after the 13th day, few larvae were still in the Malpighian tubules. Results of these dissections are shown in Table 9. A more detailed account of the number of larvae observed in individual dissections appears in Appendix B.

Table 7. Number of late second and third stage D. immitis larvae in A. triseriatus (Michigan strain) from Experiment 6.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	12	2	12.5	4.5
DEC 10^{-4}	12	2	3.5	2.5
DEC 10^{-5}	12	3	10.7	3.8
Lev 10^{-3}	12	4	4.8	1.6
Lev 10^{-4}	12	8	9.3	2.3
Lev 10^{-5}	12	1	9.0	-
Control 1	12	7	10.3	1.5
Control 2	13	17	15.8	3.1
Control 2	14	27	10.9	1.1
Control 2	15	13	10.7	1.8

Table 8. Number of late second and third stage D. immitis larvae in A. triseriatus (Michigan strain) from Experiment 7.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	-	-	-	-
DEC 10^{-4}	-	-	-	-
DEC 10^{-5}	-	-	-	-
Lev 10^{-3}	-	-	-	-
Lev 10^{-4}	-	-	-	-
Lev 10^{-5}	-	-	-	-
Control 1	-	-	-	-
Control 2	14	20	13.4	1.5

Table 9. Average number of late second and third stage D. immitis larvae per A. triseriatus (Alabama strain) from Experiment 8.

Day Post-Infection	No. Mosqs. Dissected	No. Larvae in Abdomen	No. Larvae in Head/Thorax	Total No. Larvae
12	14	2.1	0	2.1
13	6	7.3	2.2	9.5
15	12	1.0	3.7	4.7
16	10	1.8	2.9	4.7

Experiment 9

Four strains of A. triseriatus (Walton, Alabama, Ohio, and Michigan) were compared for feeding rates and susceptibility to D. immitis development. Mosquitoes of each strain were fed a 1:4 concentration of infected blood and maintained in the insectary at 72±2°F.

Dissections were performed on days 2, 5, 12, 13, 15, and 16 after infection and no real variation in progressive rates or levels of nematode development was apparent although two Michigan and one Walton strain mosquitoes contained some encapsulated first stage larvae. Third stage, pre-infective larvae, were observed in the Malpighian tubules of all mosquitoes dissected on the 12th day after infection and by the 13th day, infective larvae were found in the heads of the Alabama, Ohio, and Walton strains. A progressive shift in the numbers of larvae from the Malpighian tubules to the head region was seen in the later dissections. (Because of the few number of infected Michigan strain mosquitoes, this

strain was not dissected until day 15.) A summary of the dissection results is shown in Table 10. A more detailed account of these dissections appears in Appendix B.

The temperature in the insectary during this experiment ($72\pm2^{o}F$) was lower than it had been in previous experiments ($78\pm2^{o}F$) and development of the nematode occurred at a slower rate. Consequently, the optimum day for dissection of infected mosquitoes maintained at the lower temperature was no longer day 12 after infection. Examination of dissection data indicated that post-infection day 15 or 16 was best for dissection because peak numbers of third stage larvae were found in the head regions on these days.

Table 10. Average number of late second and third stage *D. immitis* larvae per *A. triseriatus* in Experiment 9.

Mosq. Strain	Day Post-Infection	No. Mosqs. Dissected	No. Larvae in Abdomen	No. Larvae in Head/Th.	Total Larvae
Alabama	12	4	21.3	0	21.3
	13	5	6.8	6.8	13.6
	15	10	2.7	11.3	14.0
	16	19	0.8	10.9	11.7
Walton	12	4	14.5	0.5	15.0
	13	5	9.6	0	9.6
Ohio	12	5	11.6	0	11.6
	13	4	7.8	0	7.8
Michigan	15	8	2.1	12.3	14.4

Observations on the survival rates and feeding activities of the Alabama, Walton, and Ohio strains of A. triseriatus indicated little or no differences. Dissections of the spermathecae from week-old females of these strains indicated that successful mating had occurred and hatch rates of eggs of each strain were very similar. However, the Alabama strain was chosen for the remainder of this project because I had on hand many more embryonated eggs of this strain than of the Ohio and Walton strains.

Summary of Background Information Experiments

The results of the first nine experiments dictated the direction of the remainder of this research. The ROCK strain of A. aegypti was eliminated when it was determined that it did not support D. immitis development. The Michigan strain of A. triseriatus allowed complete development of this nematode but was not used because the females in my particular subcolony were not inseminated. Differences among the Ohio, Walton, and Alabama strains of A. triseriatus appeared to be slight, but the Alabama strain was chosen over the others for the remainder of this research because more eggs were on hand. A 1:4 concentration of Scotch's infected blood to Husky's "normal" blood was used because this concentration resulted in the development of high numbers of D. immitis larvae (8 or more per mosquito) with little mosquito mortality. The amount of blood needed when using this mixture (4 ml of Scotch's blood and 20 ml of Husky's blood) also was the maximum that could be safely drawn from each dog for the experiments conducted each week.

The temperature in the insectary was lowered to 72±2°F to accommodate the experiments of some other graduate students and this extended the developmental time of D. immitis in the test mosquitoes. As a result, dissections during the drug feeding experiments were made on the 16th rather than the 12th day post-infection. The NaturaLamb membrane was used instead of the laboratory-prepared cow gut membrane because it was easier to prepare and its use resulted in increased mosquito feeding rates. Finally, aspiration was chosen as a sorting technique as it was less time-consuming than the carbon dioxide anesthetization procedure and it produced lower mosquito mortality.

Drug-Feeding Experiments

Experiments 10 through 16

Dissection results for each experiment are shown in Tables 11 through 17. Details of individual dissections appear in Appendix A.

Summary of Drug-Feeding Experiments

A summary of Tables 11 through 17, showing the means for each treatment group from the various trials, is presented in Table 18. (The results of late Control group 2 dissections were not used to prepare Table 18.) An estimated mean of 6.18 for the missing Experiment 10 observation in Table 18 was calculated using the formula

$$\frac{t(T) + b(B) - S}{(b - 1)(t - 1)} ,$$

Table 11. Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 10.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	16	3	9.33	0.67
DEC 10^{-4}	16	3	2.33	1.33
DEC 10^{-5}	-	-	-	-
Lev 10^{-3}	16	11	6.45	1.34
Lev 10^{-4}	16	6	10.67	5.43
Lev 10^{-5}	16	6	12.83	1.96
Control 1	16	15	9.27	1.76
Control 2	16	10	11.60	1.63
Control 2	17	10	11.60	1.67
Control 2	18	10	12.33	4.64

Table 12. Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 11.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	16	12	8.25	1.50
DEC 10^{-4}	16	18	7.67	1.08
DEC 10^{-5}	16	9	6.56	1.99
Lev 10^{-3}	16	4	6.50	1.94
Lev 10^{-4}	16	7	9.86	2.39
Lev 10^{-5}	17	9	9.33	1.93
Control 1	16	30	7.77	1.19
Control 2	17	63	8.00	0.75

Table 13. Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 12.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	16	6	10.33	1.76
DEC 10^{-4}	16	6	6.17	1.28
DEC 10^{-5}	16	11	4.91	0.96
Lev 10^{-3}	16	5	7.60	2.01
Lev 10^{-4}	16	8	7.63	2.63
Lev 10^{-5}	16	6	14.83	4.22
Control 1	16	14	12.79	2.06
Control 2	16	18	10.11	1.72

Table 14. Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 13.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	14	10	7.20	1.43
DEC 10^{-4}	14	11	7.91	0.72
DEC 10^{-5}	14	14	8.21	0.96
Lev 10^{-3}	14	6	7.50	1.64
Lev 10^{-4}	14	4	7.50	1.04
Lev 10^{-5}	14	10	8.90	1.16
Control 1	14	17	6.88	0.98
Control 2	14	21	9.05	1.04

Table 15. Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 14.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	16	5	9.80	2.63
DEC 10^{-4}	16	7	11.14	1.53
DEC 10^{-5}	16	10	6.90	1.70
Lev 10^{-3}	16	7	7.43	1.91
Lev 10^{-4}	16	6	8.33	1.33
Lev 10^{-5}	16	5	8.60	3.56
Control 1	16	17	10.82	1.15
Control 2	16	14	12.79	2.16

Table 16. Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 15.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	16	19	6.32	1.24
DEC 10^{-4}	16	12	6.83	1.50
DEC 10^{-5}	16	13	5.31	0.88
Lev 10^{-3}	16	16	4.19	0.78
Lev 10^{-4}	16	13	6.08	1.41
Lev 10^{-5}	16	11	7.00	1.17
Control 1	16	28	7.54	1.49
Control 2	16	9	8.89	2.37

Table 17. Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 16.

Treatment Group (%)	Day Post-Infection	No. Mosqs. Dissected	Mean No. Larvae	Standard Error
DEC 10^{-3}	17	5	6.40	2.52
DEC 10^{-4}	17	4	2.75	1.03
DEC 10^{-5}	17	6	2.83	1.08
Lev 10^{-3}	17	8	2.38	0.96
Lev 10^{-4}	17	8	4.00	0.80
Lev 10^{-5}	17	6	3.67	0.95
Control 1	17	8	2.88	0.81
Control 2	17	6	6.50	0.76

Table 18. Summary chart of number of late second and third stage D. immitis in A. triseriatus (Alabama strain) from Experiments 10 through 16, grouped according to dissection day.

Treatment Group (%)	Exper. 10	Exper. 11	Exper. 12	Exper. 14	Exper. 15	Exper. 13	Exper. 16
DEC 10^{-3}	9.33	8.25	10.33	9.80	6.32	7.20	6.40
DEC 10^{-4}	2.33	7.67	6.17	11.14	6.83	7.91	2.75
DEC 10^{-5}	-	6.56	4.91	6.90	5.31	8.21	2.83
Lev 10^{-3}	6.45	6.50	7.60	7.43	4.19	7.50	2.38
Lev 10^{-4}	10.67	9.86	7.63	8.33	6.08	7.50	4.00
Lev 10^{-5}	12.83	9.33	14.83	8.60	7.00	8.90	3.67
Control 1	9.27	7.77	12.79	10.82	7.54	6.88	2.88
Control 2	11.60	8.00	10.11	12.79	8.89	9.05	6.50

where T = the sum of all other weekly values for the DEC 10^{-5} percent treatment group, B = the sum of all other values for the various treatment groups of Experiment 10, t = the total number of treatment groups, including control groups (8), b = the total number of trials or experiments (5), and S = the sum of all values shown for all experiments and all treatment groups (Snedecor, 1956). Treatment means from only Experiments 10, 11, 12, 14, and 15 and total means for each treatment group from those five trials are shown in a second summary table, Table 19. (The overall mean for the 10^{-5} percent concentration of DEC was calculated using the estimated mean of 6.18 for the missing value from Experiment 10.)

Table 19. Summary chart of means of late second and third stage D. immitis in A. triseriatus (Alabama strain) from experiments in which dissections were performed on day 16 after infection, plus overall means for each treatment group.

Treatment Group (%)	Exper. 10	Exper. 11	Exper. 12	Exper. 14	Exper. 15	Overall Means
DEC 10^{-3}	9.33	8.25	10.33	9.80	6.32	8.81
DEC 10^{-4}	2.33	7.67	6.17	11.14	6.83	6.83
DEC 10^{-5}	(6.18)	6.56	4.91	6.90	5.31	(5.97)
Lev 10^{-3}	6.45	6.50	7.60	7.43	4.19	6.43
Lev 10^{-4}	10.67	9.86	7.63	8.33	6.08	8.51
Lev 10^{-5}	12.83	9.33	14.83	8.60	7.00	10.52
Control 1	9.27	7.77	12.79	10.82	7.54	9.64
Control 2	11.60	8.00	10.11	12.79	8.89	10.28

Graphs of the overall treatment group means for diethylcarbamazine and levamisole shown in Table 19 are presented in Figures 4 and 5.

Analysis of Experimental Results

The results of the drug-feeding experiments were analyzed in two phases, each dealing with a major question about this research and each involving several separate analytical steps. To determine whether any drug treatment group showed a significant difference in D. immitis numbers which developed, an analysis of variance, an orthogonal analysis, and a non-orthogonal analysis were performed. To determine whether individual stages of D. immitis larvae were affected by diethylcarbamazine or levamisole and whether the larvae in all mosquitoes of a test group were synchronized (at the same stage of development), frequency distributions, correlation coefficients, "developmental variances", power curves, and developmental cycles were examined. Each of these analyses is presented below.

Phase One Analysis

(1) Analysis of variance. The research performed for this thesis was classified as a randomized block design experiment with replications in subclasses (Dr. Charles Cress, Michigan State University, personal communication). Because the number of observations in the subclasses was disproportionate and there was a missing observation from Experiment 10, the computer was used to perform the analysis of variance

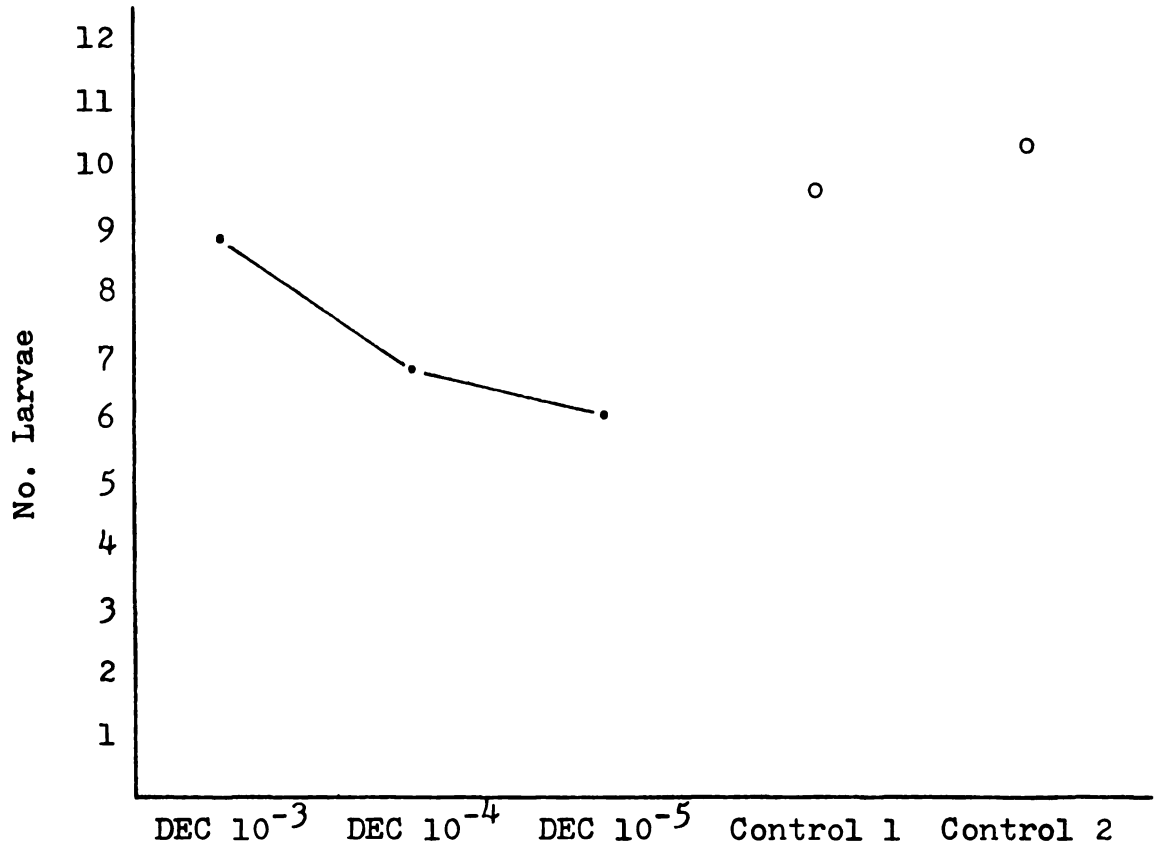

Figure 4. Overall means from Experiments 10, 11, 12, 14, and 15 of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) for DEC treatment groups (%) and controls.

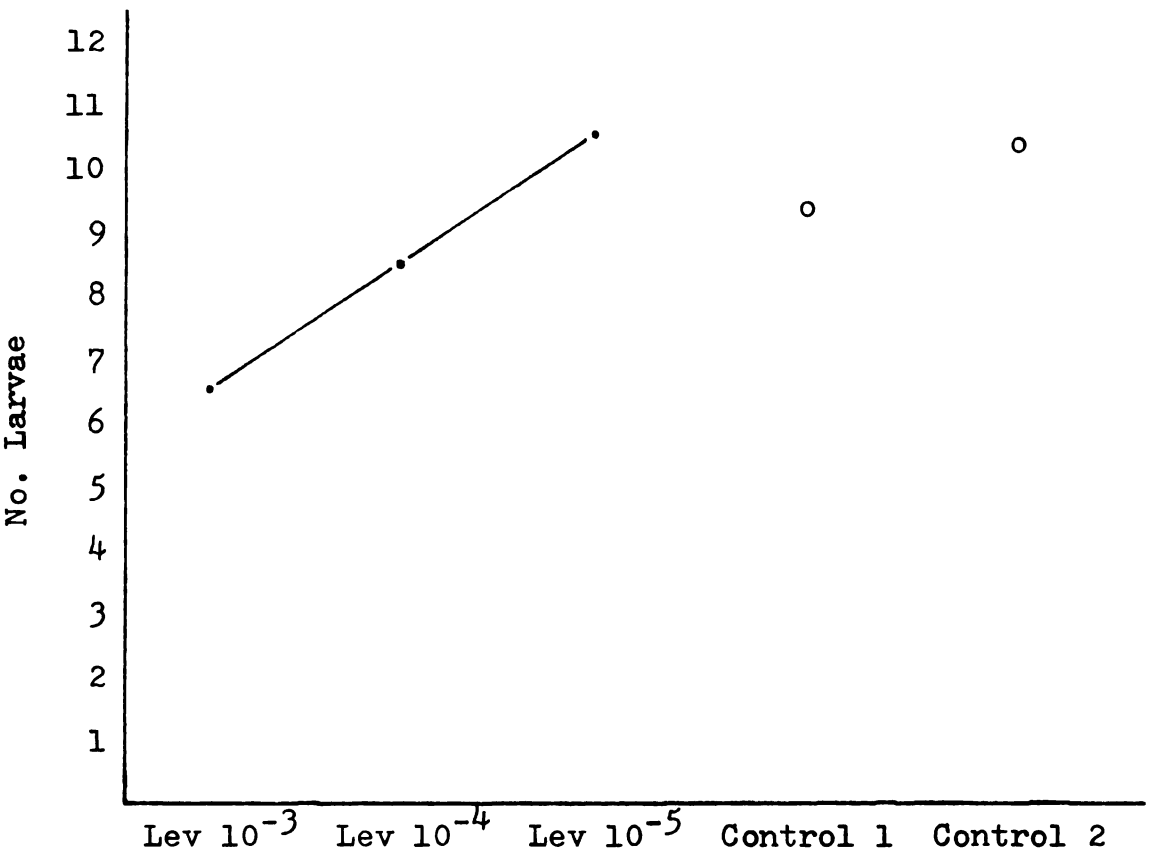

Figure 5. Overall means from Experiments 10, 11, 12, 14, and 15 of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) for levamisole treatment groups (%) and controls.

and orthogonal contrasts. The program package used was SAS (Statistical Analysis System) by Barr, Goodnight, Sall and Helwig of the SAS Institute in Raleigh, North Carolina, and the computer work was done at the Texas A&M University Computation Center.

To arrive at the analysis of variance (ANOVA) appropriate for my experimental design, shown in Table 20, two initial analyses of variance were performed using a general linear models procedure. The first ANOVA adjusted variation in treatment groups for weekly variation and adjusted interaction variation for both weekly and treatment group variation. The second ANOVA adjusted weekly variation for variation in treatment groups. These two analyses of variance

Table 20. Mathematical model for analysis of variance of a randomized block design experiment with replicates in subclasses (where σ^2 is estimated variance, K_1, ϕ_T^2, K_2 are constants, and W, T, WT, and E refer respectively to weeks, treatment, weeks*treatment, and error). (Graybill, 1976)

Source of Variation	Degrees of Freedom	Estimated Mean Square
Weeks (adjusted for treatment)	4	$\sigma_E^2 + K_1\sigma_{WT}^2 + K_2\sigma_W^2$
Treatment (adjusted for weeks)	7	$\sigma_E^2 + K_1\sigma_{WT}^2 + \phi_T^2$
Weeks*Treatment (adjusted for weeks and treatment, both)	27	$\sigma_E^2 + K_1\sigma_{WT}^2$
Error	433	σ_E^2

produced the appropriate ANOVA in which weeks were adjusted for treatment groups, treatment groups were adjusted for weeks, and the interaction between weeks and treatment groups was adjusted for both weeks and treatment groups. The final product is shown in Table 21. (See Appendix C.)

Table 21. Final analysis of variance of the results from Experiments 10, 11, 12, 14, and 15.

Source of Variation	df	Mean Square	F-value	Level of Significance
Weeks (adjusted for treatment)	4	187.7703	6.4837	0.01, $df_{4,27}$
Treatment (adjusted for weeks)	7	118.8106	4.1025	0.01, $df_{7,27}$
Weeks*Treatment (adjusted for both weeks and treatment)	27	28.9603	0.7953	NS
Error	433	36.4152	-	
TOTAL	471	-	-	

F-values for weeks and for treatment were computed using MS_{W*T} rather than $MS_{W*T} + MS_E$ as a divisor, to be conservative. F-values for both the weekly experiments and the treatment groups indicate significance at the 0.01 level. Significance for weeks can be overlooked, however, as significant variance among weeks was expected due to weekly variation in the microfilaremia count of Scotch's blood and other uncontrollable experimental conditions.

(2) Orthogonal analysis. To pinpoint the exact source of variation among the treatment groups, seven different orthogonal contrasts were performed comparing: (1) control group one to control group two; (2) all drug treatment groups together to both control groups combined; (3) all three concentrations of DEC together to all three concentrations of levamisole together; (4) the 10^{-3} percent and 10^{-4} percent concentrations of both drugs to the 10^{-5} percent concentrations of both drugs; (5) the 10^{-3} percent concentrations of both drugs to only the 10^{-4} percent concentrations of both drugs; (6) the 10^{-3} percent and 10^{-4} percent concentrations of DEC plus the 10^{-5} percent concentration of levamisole to the 10^{-5} percent concentration of DEC and the 10^{-3} percent and 10^{-4} percent concentrations of levamisole; and (7) the 10^{-3} percent concentration of DEC and the 10^{-4} percent concentration of levamisole to the 10^{-4} percent concentration of DEC and the 10^{-3} percent concentration of levamisole. The results of this orthogonal analysis are shown in Table 22.

The only contrasts which showed significance were contrasts 2 and 6 (both of which were significant at the 0.01 level), indicating that the results of the drug treatments differed significantly from those of both control groups, and that DEC and levamisole at the concentrations tested behaved quite dissimilarly. (See Appendix C.)

(3) Non-orthogonal analysis. To determine a more exact source for the treatment variation indicated by the

Table 22. Orthogonal analysis (seven contrasts) performed on the results of Experiments 10, 11, 12, 14, and 15.

Source of Variation	df	Mean Square	F-value	Level of Significance
Weeks (adjusted for treatment)	4	187.7703	-	
Contrast 1	1	2.0512	0.0708	NS
Contrast 2	1	286.5938	9.8961	0.01, $df_{1,27}$
Contrast 3	1	34.1332	1.1786	NS
Contrast 4	1	8.6453	0.2985	NS
Contrast 5	1	21.5740	0.7450	NS
Contrast 6	1	387.0572	13.3651	0.01, $df_{1,27}$
Contrast 7	1	92.0246	3.1776	NS
Weeks*Treatment (adjusted for both weeks and treatment)	27	28.9603	-	
Error	433	36.4152	-	
TOTAL	471	-	-	

results of the analysis of variance and orthogonal contrasts, eight non-orthogonal contrasts were performed, manually, using the formula

$$t^2 = \left(\Sigma c_{ij}\bar{y}_{ij}\right)^2 \left(\Sigma \frac{c_{ij}^2}{n_{ij}}\right)^{-1} \left(MS_{TW}\right)^{-1},$$

where (referring back to Table 18) i and j refer to columns and rows, respectively, $\bar{y}_{ij}$ = the mean value for each cell, n_{ij} = the number of observations represented by each cell, c_{ij} = a weighted value assigned to each contrast, and MS_{TW} = 28.96, the mean square for the interaction of weeks and treatment groups (from the analysis of variance). t^2 values were arrived at using the mean values for each treatment group from Experiments 10, 11, 12, 14, and 15 only, with 6.18 used as an estimated mean for the missing value in Experiment 10. A summary of these non-orthogonal contrasts appears in Table 23.

The t^2 results indicated that, when conservative levels of significance at $df_{7,27}$ of 8.08 at the 0.05 level and 12.35 at the 0.01 level were used, only those contrasts comparing DEC to Control group 1 and the 10^{-3} percent concentration of levamisole to Control group 1 were significant. (Such calculations used an n value of 1 for the missing Experiment 10 cell. If the harmonic mean (5.57) had been used instead, the t^2 values for the DEC vs Control 1 contrast and the DEC 10^{-5} percent vs Control 1 contrast would have been 10.09 and 14.07, respectively, indicating

Table 23. Results of non-orthogonal analysis (eight contrasts) performed on the results of Experiments 10, 11, 12, 14, and 15.

Contrasts	t^2	Level of Significance
DEC vs Control 1	8.55	0.05, $df_{7,27}$
Lev vs Control 1	2.25	NS
DEC 10^{-3}% vs Control 1	0.54	NS
DEC 10^{-4}% vs Control 1	5.51	NS
DEC 10^{-5}% vs Control 1	7.05	NS
Lev 10^{-3}% vs Control 1	8.75	0.05, $df_{7,27}$
Lev 10^{-4}% vs Control 1	1.16	NS
Lev 10^{-5}% vs Control 1	0.67	NS

significance at the 0.05 and 0.01 levels.) The t^2 computations for each of these contrasts appears in Appendix D.

Phase Two Analysis

(4) Frequency distributions. To determine whether or not the distribution of larvae within treatment groups fit the normal curve, frequency distributions were compiled based upon percentage development within that particular mosquito dissection. For example, if the results of three mosquito dissections revealed (0,2), (6,0), and (7,8) larvae per section of each mosquito, where x of (x,y) represents the number of larvae found in the abdominal region and y of (x,y) represents the number of larvae found in the head/thorax, then percentage development for each mosquito observation would be

$100(1 - \frac{x}{x + y})$, or 100, 0, and 53 percent, respectively. A frequency distribution of the treatment group from Experiments 10, 11, 12, 14, and 15 with the largest number of observations (n = 63) is shown in Figure 6.

As can be seen from Figure 6, over 50 percent of the observations fall into the 0 and 100 percent categories indicating a wide divergence in larval development within the same mosquito population. Similar findings are seen when one examines all treatment groups from the experimental trials, although, of course, such divergence can only be evident when most larvae are neither at the end or beginning of their developmental cycle (neither at the 0 percent or 100 percent end of the scale). The results from other treatment groups are shown in Table 24.

(5) Correlation coefficients and means. Because frequency distributions are limited in that they are based upon percentages rather than raw data, correlation coefficients (r), which use raw rather than converted data, were computed by the formula

$$r = \frac{s_{xy}}{s_x s_y}, \text{ where } s_{xy} = \frac{1}{n-1}\left(\sum x_i y_i - \frac{1}{n}\sum x_i \sum y_i\right),$$

$$s_x = \left(\frac{\sum x_i^2 - \left(\sum x_i\right)^2/n}{n - 1}\right)^{\frac{1}{2}} \text{ and}$$

$$s_y = \left(\frac{\sum y_i^2 - \left(\sum y_i\right)^2/n}{n - 1}\right)^{\frac{1}{2}}.$$

An r value to 0 to -1.0 indicates possible asynchrony of larval development and an r value of 0 to +1.0 indicates

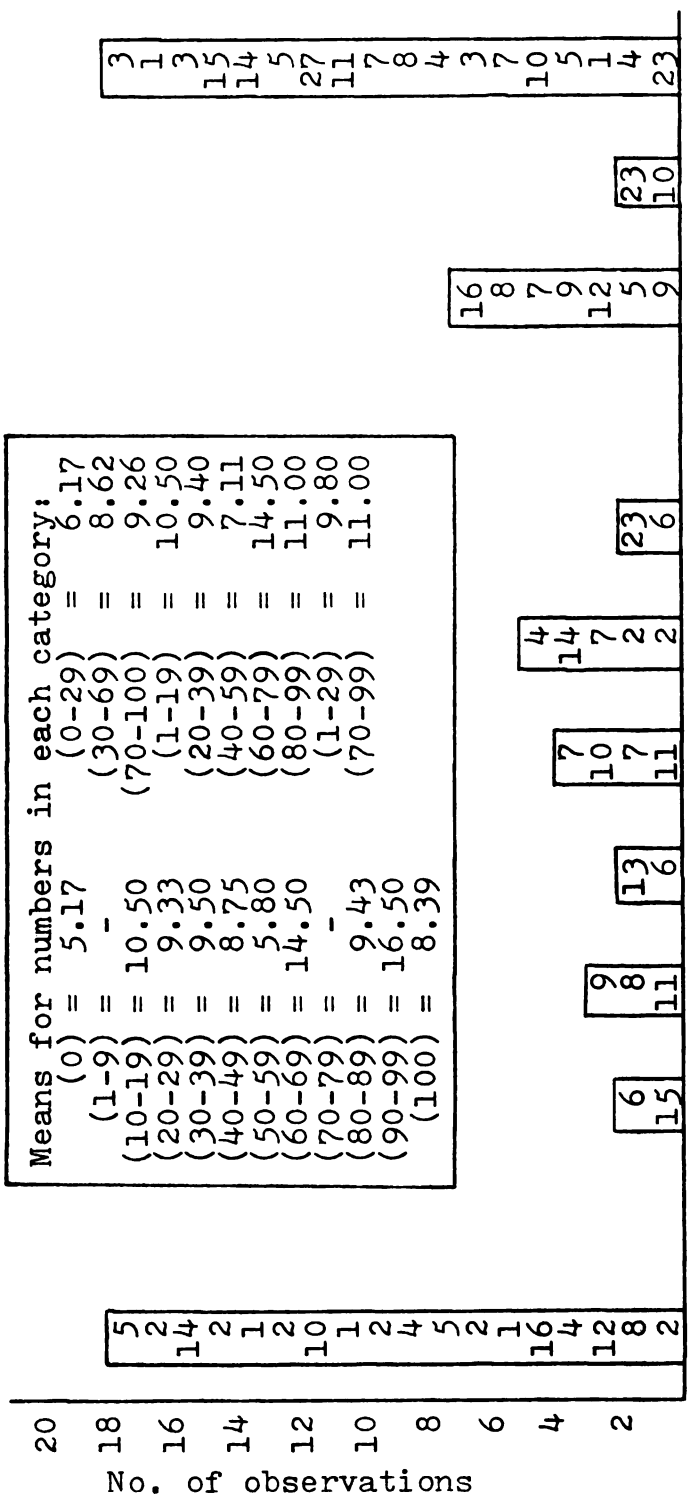

Figure 6. Observations from Experiment 11, Treatment Group Control 2, distributed according to percentage D. immitis development in A. triseriatus (Alabama strain). (The individual numbers forming each bar represent the total number of larvae from each mosquito dissected.)

Table 24. Number of observations for each treatment group; percentage of observations which were 0% and 100% developed (where (x,y) x = 0% and y = 100%; and total % development of that treatment group, determined by $100(1-(\Sigma x/\Sigma x+\Sigma y))$).

Treatment Group (%)	$DEC_{10^{-3}}$	$DEC_{10^{-4}}$	$DEC_{10^{-5}}$	$Lev_{10^{-3}}$	$Lev_{10^{-4}}$	$Lev_{10^{-5}}$	Control 1	Control 2
Experiment 10								
No. observs.	3	3	-	11	6	6	15	10
% at ends	(33,0)	(100,0)	-	(18,27)	(50,0)	(33,0)	(20,7)	(20,0)
Total %	29	0	-	70	12	18	25	59
Experiment 11								
No. observs.	12	18	9	4	7	9	30	63
% at ends	(75,0)	(83,0)	(89,0)	(25,0)	(71,0)	(22,11)	(60,3)	(29,29)
Total %	8	4	3	50	13	44	18	61
Experiment 12								
No. observs.	6	6	11	5	8	6	14	18
% at ends	(0,33)	(17,50)	(18,36)	(20,40)	(63,25)	(33,33)	(14,14)	(17,11)
Total %	74	89	65	76	61	54	45	62
Experiment 14								
No. observs.	5	7	10	7	6	5	17	14
% at ends	(40,20)	(57,0)	(60,10)	(71,0)	(33,33)	(80,0)	(76,0)	(50,14)
Total %	24	13	14	25	44	30	13	23
Experiment 15								
No. observs.	19	12	13	16	13	11	28	9
% at ends	(68,5)	(67,8)	(85,15)	(81,0)	(54,15)	(55,0)	(64,4)	(22,11)
Total %	17	20	6	6	19	35	35	44

in-phase larval development. The correlation coefficient is most reliable as an indicator of synchronization when the sample is large and the observations are taken at neither the beginning of larval movement out of the abdomen nor after most larvae have disappeared from the mosquito mouthparts.

A summary of weekly means and computed r values from Experiments 10, 11, 12, 14, and 15 is shown in Table 25. As can be seen in Table 25, 27 out of the 39 treatment groups had negative r values. The average r value for all treatment groups was -0.16. This indicated a slight negative correlation between the number of D. immitis larvae in the abdominal and head/thoracic regions of mosquitoes. The only r value which was significant at the 0.01 level was that for Experiment 11, Control Group 2 (-0.32 at df_{61}), but this may be due to the low number of observations within the treatment groups.

(6) "Developmental variances". Because the correlation coefficient (r) demonstrates correlation between head/thoracic and abdominal larval numbers but does not adequately differentiate between extremes in degree of larval development, a comparison was made of variance computed for the data obtained at the time of dissection and predicted variances at the beginning, end, and middle of larval development. (E.g., the correlation coefficient r = -1.0 for paired numbers (8,1) and (1,8), where x and y of (x,y) represent numbers of D. immitis larvae in the abdomen and head/thorax

Table 25. Summary of average number of late second and third stage D. immitis larvae found in total body, head/thorax, and abdomen dissections of infected A. triseriatus (Alabama strain) mosquitoes along with correlation coefficients (r) calculated from individual data. (See data sheets in Appendix B.)

Treatment Group (%)	Exper. 10	Exper. 11	Exper. 12	Exper. 14	Exper. 15
DEC 10^{-3}	9.33	8.25	10.33	9.80	6.32
Head/Thorax	6.67	7.58	2.67	7.40	5.21
Abdomen	2.67	0.67	7.67	2.40	1.11
Corr. coef.	-0.94	+0.42	+0.03	-0.65	-0.03
DEC 10^{-4}	2.33	7.67	6.17	11.14	6.83
Head/Thorax	2.33	7.39	0.67	9.71	5.50
Abdomen	0	0.28	5.50	1.43	1.33
Corr. coef.	-	+0.33	-0.62	-0.10	-0.24
DEC 10^{-5}	-	6.56	4.91	6.90	5.31
Head/Thorax	-	6.33	1.73	5.90	5.00
Abdomen	-	0.22	3.18	1.00	0.31
Corr. coef.	-	+0.81	+0.01	+0.68	-0.62
Lev 10^{-3}	6.45	6.50	7.60	7.43	4.19
Head/Thorax	1.91	3.25	1.80	5.57	3.94
Abdomen	4.55	3.25	5.80	1.86	0.25
Corr. coef.	-0.17	-0.21	-0.32	+0.02	-0.14
Lev 10^{-4}	10.67	9.86	7.63	8.33	6.08
Head/Thorax	9.33	8.57	3.00	4.67	4.92
Abdomen	1.33	1.29	4.63	3.67	1.15
Corr. coef.	-0.35	-0.25	-0.52	-0.68	-0.15
Lev 10^{-5}	12.83	9.33	14.83	8.60	7.00
Head/Thorax	10.50	5.22	6.83	6.00	4.55
Abdomen	2.33	4.11	8.00	2.60	2.45
Corr. coef.	-0.48	-0.14	-0.50	+0.12	-0.07
Control 1	9.27	7.77	12.79	10.82	7.54
Head/Thorax	6.93	6.33	7.07	9.41	4.93
Abdomen	2.33	1.43	5.71	1.41	2.61
Corr. coef.	+0.18	-0.17	-0.34	-0.32	+0.08
Control 2	11.60	8.00	10.11	12.79	8.89
Head/Thorax	4.70	3.14	3.83	9.79	5.00
Abdomen	6.90	4.86	6.28	3.00	3.89
Corr. coef.	-0.16	-0.32	+0.13	-0.45	-0.21

of individual mosquitoes. The first two pair of numbers, however, show a more "synchronous" development of larvae within that treatment group of two mosquitoes than the last two pair.) Although a comparison of "developmental variances" represents no valid statistical test, it is discussed here for illustrative purposes.

To compare existing larval synchrony levels within treatment groups to 100 percent synchrony, four types of "variance" were computed from v_1, w_1, v_2, w_2, . . . v_i, w_i, where i is the number of observations in that treatment group:

(a) "actual developmental variance" (ADV) or variance (s^2) where $v_i = s_i$ and $w_i = y_i$;

(b) "estimated variance" or variances (s^2) if all larvae were at the beginning of their cycle (EVB), where $v_i = -(x_i + y_i)$ and $w_i = 0$;

(c) "estimated variance" or variance (s^2) if all larvae were at the mid of half-way point of development (EVM), where $v_i = -\frac{1}{2}(x_i + y_i)$ and $w_i = \frac{1}{2}(x_i + y_i)$;

(d) "estimated developmental variance" (EDV) or variance (s^2) if all larvae at the present developmental level were 100 percent synchronized, where

$$EDV = \frac{|\Sigma x - \Sigma y|}{\Sigma x + \Sigma y} (EVB - EVM) + EVM \quad .$$

An index ADV/EDV was computed to compare existing developmental variance in a treatment group with variance expected if all observations within that treatment group were 100 percent synchronized at the existing stage of development.

Above computations performed for the results of Experiments 10, 11, 12, 14, and 15 are shown in Table 26. Although most treatment groups had ADV/EDV ratios over 1.0, ratios under 1.20 (or more conservatively, 1.25) were not considered significant. Seventeen of 39 treatment groups had ADV/EDV ratios greater than or equal to 1.20, ranging as high as 1.83, and the treatment group with the highest number of observations (n = 63) (Experiment 11, Control Group 2) had an ADV/EDV ratio of 1.53.

(7) Power curves. Data was fit to a variety of curves (power, exponential, logarithmic) to determine whether larval numbers fluctuated with stage of development. The three previous analyses of experimental data suggested development of D. immitis larvae within treatment groups was not synchronous, but provided little information about whether similar numbers of larvae were found in late, early, and middle stages of development. (Frequency distributions showed fluctuations in larval numbers as development progressed (Figure 6) but were based upon percentages rather than raw data and were affected by the magnitude of the numbers. Correlation coefficients indicated negative correlations in abdominal and head/thoracic larval numbers but fit the data to only a linear equation.)

Tryouts showed that the data fit the exponential curve ($y = ae^{bx}$) poorly, fit a linear regression and logarithmic curve equally well, and, in many cases, fit the power curve ($y = ax^{b}$) best. (Closeness of r or r^2 (coefficient of

Table 26. Comparisons of actual developmental variances (ADV) with estimated variances at the beginning (EVB) and middle (EVM) of D. immitis development in A. triseriatus (Alabama strain) and with estimated developmental variances (EDV).

Treatment Group (%)		DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Exper. 10	ADV	32.00	3.77	-	21.94	121.82	64.08	42.22	49.57
	EVB	26.67	3.77	-	20.37	111.52	55.36	44.65	47.96
	EVM	26.40	3.31	-	15.64	73.45	52.32	32.19	41.68
	EDV	26.52	3.77	-	17.57	102.02	60.91	38.38	42.87
	ADV/EDV	1.21	1.00	-	1.25	1.19	1.05	1.10	1.16
Exper. 11	ADV	28.00	24.43	25.35	18.86	49.17	41.32	38.96	41.10
	EVB	30.64	25.34	28.09	18.50	44.69	36.27	36.27	33.92
	EVM	25.24	20.23	19.74	15.29	35.42	25.81	25.81	24.98
	EDV	29.76	24.97	27.53	15.29	42.26	32.41	32.41	26.90
	ADV/EDV	0.94	0.98	0.92	1.23	1.16	1.20	1.20	1.53
Exper. 12	ADV	37.36	16.45	11.06	27.78	50.16	157.72	83.86	49.78
	EVB	37.61	14.81	11.12	25.07	45.02	108.63	70.99	52.23
	EVM	33.36	12.59	8.70	20.56	28.37	84.32	56.69	39.26
	EDV	35.42	14.33	9.41	22.93	31.93	86.24	58.21	42.40
	ADV/EDV	1.05	1.15	1.18	1.21	1.57	1.83	1.44	1.17
Exper. 14	ADV	56.55	41.73	21.81	26.42	32.49	83.86	45.70	88.36
	EVB	42.10	41.03	26.16	26.68	23.79	70.99	41.04	73.88
	EVM	34.39	37.23	19.34	20.77	21.36	56.69	35.61	58.13
	EDV	38.32	40.05	24.18	23.72	21.65	58.21	39.62	66.49
	ADV/EDV	1.48	1.04	0.90	1.11	1.50	1.44	1.15	1.33
Exper. 15	ADV	24.70	28.17	13.76	9.49	23.07	49.78	42.94	50.26
	EVB	24.41	25.04	12.16	9.25	21.96	52.23	44.91	44.61
	EVM	13.54	18.61	9.74	6.89	15.78	39.26	29.68	32.76
	EDV	20.59	22.54	11.88	8.97	19.62	42.40	34.37	34.24
	ADV/EDV	1.20	1.25	1.16	1.06	1.18	1.17	1.25	1.47

determination) to 1.0 determined "goodness of fit" to the curves.) Constants for the power curve equations which best fit treatment groups are shown in Table 27. Regression coefficients a and b were computed from the formulas

$$b = \frac{\sum(\ln x_i)(\ln y_i) - (\sum \ln x_i)(\sum \ln y_i)/n}{\sum(\ln x_i)^2 - (\sum \ln x_i)^2/n} \quad \text{and}$$

$$a = \exp\left[\frac{\sum \ln y_i}{n} - b\,\frac{\sum \ln x_i}{n}\right] .$$

Because zeros could not be used in the power curve computations, 0.1 values were substituted for 0 values in the data placed into the above equations producing minor but inconsequential flattening of the curves.

Power curve r values can be compared with correlation coefficients in Table 25. Table 27 shows that exponent b for most of the power curves was a negative number and that the curves produced "hug" the x and y axes. (The closer the b value is to zero, the flatter the curve.) Such curves confirm the frequency distribution results that the total number of larvae is greater when development is in either its earliest or latest stages than mid-stage; however, the power curve r values showed significance at the 0.01 level in only two cases (Experiment 11, Control Group 2, and Experiment 15, DEC 10^{-5} percent). Data from Experiment 11, Control Group 2 fitted to its power curve is shown in Figure 7.

Table 27. Constant values for the power curve ($y = ax^b$) for those treatment groups with r-values greater than the r-values for linear regression from Table 25.

Experiment	Treatment Group (%)	a	b	r^2	r	Correlation Coefficient
10	Lev 10^{-4}	1.11	-0.66	0.25	0.50	-0.35
10	Control 1	0.81	0.30	0.08	0.28	+0.18
11	Control 1	0.54	-0.26	0.03	0.17	-0.17
11	Control 2	1.69	-0.51	0.21	0.46	-0.32
12	DEC 10^{-3}	6.91	0.05	0.03	0.18	+0.03
12	DEC 10^{-4}	1.39	-0.77	0.39	0.63	-0.62
12	DEC 10^{-5}	1.65	-0.23	0.06	0.25	+0.01
12	Lev 10^{-4}	0.79	-0.97	0.49	0.70	-0.52
12	Lev 10^{-5}	2.96	-0.75	0.51	0.71	-0.50
14	DEC 10^{-3}	1.75	-0.72	0.54	0.73	-0.65
14	Lev 10^{-3}	0.21	0.22	0.01	0.09	+0.02
14	Lev 10^{-5}	0.12	0.57	0.08	0.27	+0.12
14	Control 2	1.75	-0.61	0.29	0.54	-0.45
15	DEC 10^{-3}	0.40	-0.28	0.04	0.19	-0.03
15	DEC 10^{-4}	0.48	-0.41	0.12	0.35	-0.24
15	DEC 10^{-5}	0.32	-0.68	0.90	0.95	-0.62
15	Lev 10^{-4}	0.56	-0.31	0.09	0.30	-0.15
15	Control 2	2.23	-0.35	0.09	0.30	-0.21

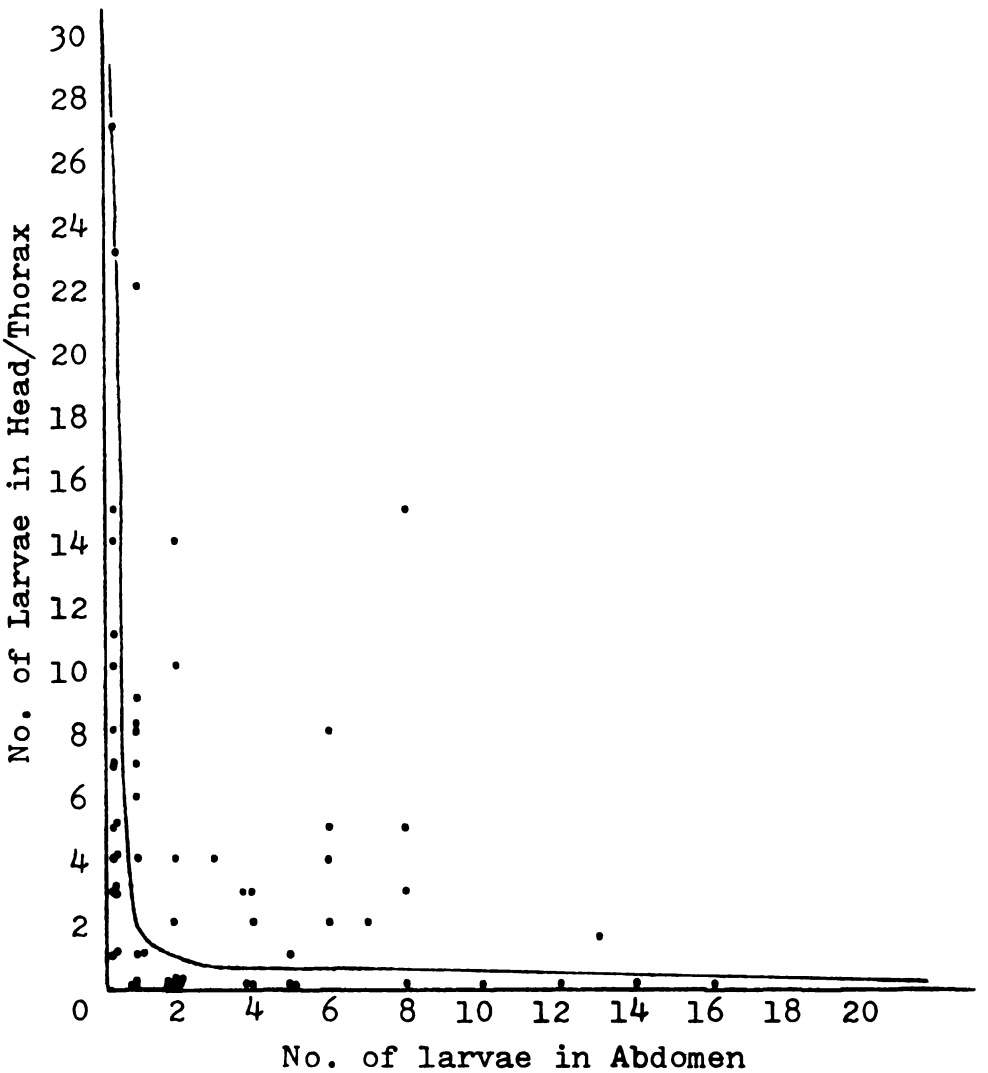

Figure 7. Experimental data from Experiment 11, Control Group 2 fitted to the equation $y = 1.69x^{-0.51}$ with 0-values from the data substituted with 0.1-values.

(8) Bimodality in mosquito dissections. The only mosquito dissections performed over time were those done to determine the optimum time for mosquito dissection. Tables 4, 9, and 10 show that a dip in total larval numbers occurred lasting one to two days after which larval numbers increased and thereafter tapered off slowly. Accompanying this drop in total larval numbers was a drop-off in abdominal larval numbers and a rise in head/thoracic larval numbers. All fluctuations occurred about the time when third stage larvae migrate from the Malpighian tubules into the thorax and head region and resulting graphs showed a distinctly bimodal distribution of larvae over time. A graph of data from Table 4 is shown in Figure 8.

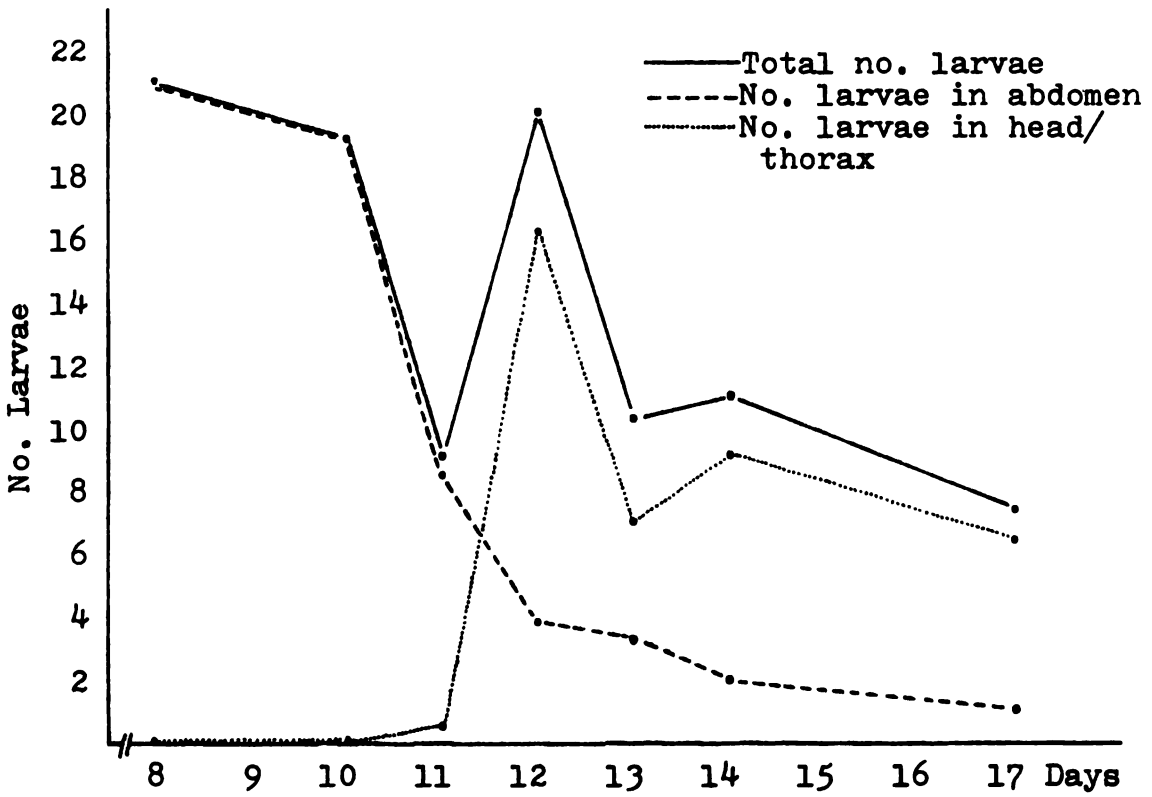

Figure 8. Average number of D. immitis larvae in head/ thorax, abdomen, and total body of infected A. triseriatus (Michigan strain) dissected over time. (Data taken from Table 4.)

DISCUSSION

Results of Background Information Experiments

Feeding Rates

The results of the membrane feeding experiments indicated that satisfactory numbers of mosquitoes could be infected with D. immitis using the artificial feeding device. Overall feeding rates for A. aegypti mosquitoes fed upon the gut membrane in Experiments 1 and 3 were 78.2 and 51.2 percent, respectively; feeding rates for A. triseriatus mosquitoes ranged from an overall rate of 50 percent in Experiment 3 before the introduction of the NaturaLamb membrane to approximately 75 to 80 percent after the introduction of the membrane in Experiment 8.

Mosquito Mortality after Infection

High mortality rates dropped after switching from the anesthetization method of sorting engorged mosquitoes to the aspiration method. The cumulative mortality rate at three days post-infection for Experiment 1 A. aegypti mosquitoes sorted by anesthetization after receiving a 1:5 concentration of Scotch's blood, was 42.3 percent. The cumulative mortality rate for A. aegypti infected in Experiment with a similar (1:4) concentration of blood but sorted by aspiration was only 3 percent three days after

the blood meal. As is shown in Table 2, mortality during the first day after infection in those mosquitoes sorted by anesthetization ranged from 21.5 to 41.7 percent for three different concentrations of blood, whereas after sorting by aspiration was introduced in Experiment 3, mortality rates during the first day after the blood meal were almost negligible.

Effect of Microfilaremia Level on Mosquito Survival

Before it was realized that the ROCK strain of A. aegypti would not support D. immitis development, a test of three concentrations of blood containing microfilariae was conducted over time to determine if, as reported by Duxbury et al. (1961) and as discussed by Travis (1947) and Weiner and Bradley (1970), high microfilaremia counts are frequently associated with high mortality rates of infected mosquitoes. Cumulative mortality rates among the three groups receiving the different concentrations of blood were very similar (73.0, 68.5, and 82.6 percent for 1:1, 1:5, and 1:11 concentrations, respectively, at six days after infection), but as the microfilariae were not developing in this strain of mosquito, these results are entirely inconclusive.

Susceptibility of Mosquito Strains to D. immitis

Reports of varying D. immitis susceptibilities in mosquitoes from different geographical sites are discussed by Intermill (1973) and other authors. Four different strains of A. triseriatus (Alabama, Walton, Ohio, and Michigan)

were tested in Experiment 9 for D. immitis development. Development in the four strains was very similar but the Alabama and Michigan strains showed development of most larvae (see Table 10). (These results, however, are based on very few mosquitoes and should not be considered conclusive.)

Results of Drug-Feeding Experiments

Preliminary Considerations

Before proceeding with interpretation of the experimental results, thought should be given to some potential drug-filariae cause-effect relationships. For example, as the research was conducted here, if a particular drug or substance were to kill developing D. immitis larvae, fewer numbers of larvae might be found in that treatment group receiving the highest concentration of that drug. If the drug were to slow down the rate of larval development, more larvae might be found in the Malpighian tubules than in the head/thorax (compared to the control groups), or higher total numbers of larvae because fewer had been lost from the head. Finally, if the drug were to act by speeding up the developmental rate of D. immitis, a greater proportion of larvae might be found in the head than in the abdominal region, if no larvae had escaped from the proboscis or in any other way been "lost" from the mosquito. To complicate interpretation and analysis, a drug might also have a compounding effect; that is, affect both the developmental rate and numbers of developing larvae, or affect just one

developmental stage. Although a drug might show greatest effect at its highest concentration, a leveling-off or even inverse effect might also be seen.

Experimental Results

The Phase One analyses performed on the results of Experiments 10, 11, 12, 14, and 15 indicated statistically significant differences in the effects of DEC compared to levamisole at the three concentrations tested as well as in certain concentrations of these drugs as compared to the control groups. Although the results of the non-orthogonal contrasts performed indicated that the only individual concentration which differed significantly from the control groups was the 10^{-3} percent concentration of levamisole, the DEC 10^{-5} percent concentration would have shown significance if the harmonic mean of 5.57 had been used as part of the calculations instead of 1.

Figures 4 and 5 show that the levamisole treatment groups results are ones which might be expected if levamisole were to cause inhibition or death of D. immitis larvae at its highest concentration. The results of the three DEC concentrations, however, indicating fewer numbers of larvae found at the lowest concentration of the drug, are surprising. As DEC has been considered relatively ineffective in vitro against D. immitis microfilariae, it might be expected to produce no effect or a very minimal effect on developing larvae. Levamisole might be expected to have statistically significant effects on developing larvae,

especially at higher concentrations, because it is active against filariae *in vitro* and in mosquito test systems. Additionally, as levamisole itself is a more potent drug than identical concentrations of DEC, as demonstrated by comparison studies involving dosed animals and *in vitro* studies, one might expect that levamisole would show significance at drug concentrations lower than DEC.

(NOTE: During this research it was realized that such a pattern for DEC was developing. To eliminate such possible factors as improperly prepared DEC concentrations, pipette contamination, or researcher fatigue during dissecting, certain preventative measures, previously discussed in this thesis, such as preparing new solutions of DEC (and levamisole) every two weeks, using new pipettes, and dissecting mosquitoes randomly, were taken. The DEC results did not change with the implementation of these measures.)

All of these expectations are based upon the assumption that the *D. immitis* developmental pattern for the experimental trials followed that found by other authors. As is shown in Figure 9, Ho et al. (1974) found that over time the total number of third stage larvae within an infected mosquito population gradually increased and then dropped off, accompanied by a drop in infective larval numbers found in the head. The numbers of infective larvae found in the abdomen increased after the second stage larval moult and then dropped substantially after larvae (supposedly) moved into the thorax and head regions. Although the developmental

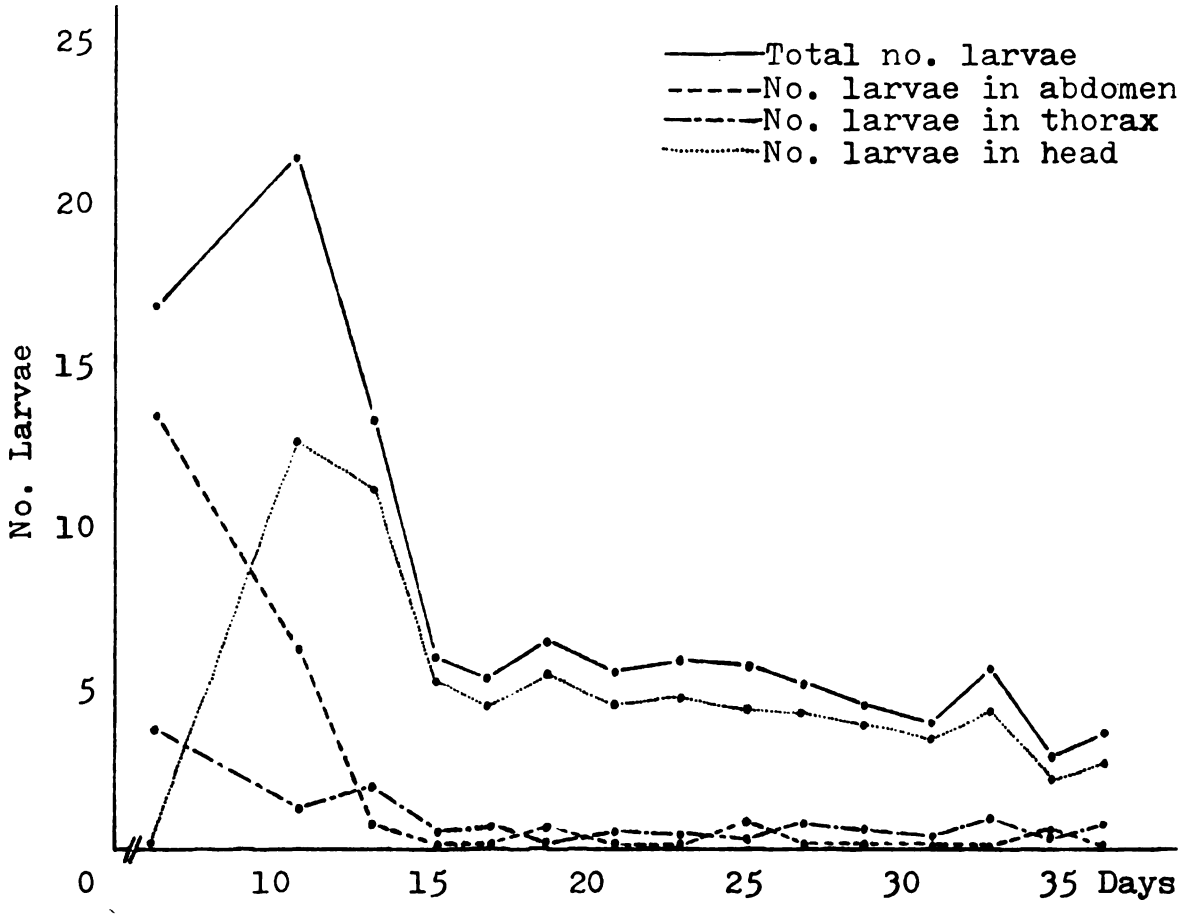

Figure 9. Average number of D. immitis infective larvae per individual mosquito. (Adapted from a figure on the mean number of D. immitis in infected A. togoi by Ho et al., 1974.)

rate of D. immitis may vary in different species of mosquitoes, and the ambient temperature at which they are maintained, the usual developmental pattern is approximately that shown in Figure 9. Any deviation from this pattern in laboratory tests would have a bearing on the interpretation of any results obtained.

Results of Statistical Analyses

The end product of the dissections over time in this research (Figure 8) was a distinctly bimodal distribution of D. immitis larvae, quite different from that shown in Figure 9. However, it does confirm the results of the other Phase Two analyses (4) through (7): total larval numbers in both individual mosquitoes and mosquito populations were lower mid-cycle than early or late in the developmental cycle and that "asynchrony" (real or apparent) in larval development may be a contributing factor.

Hypothesis A--Real asynchrony. As Figure 10 shows, asynchrony in larval development can occur when one portion of mosquitoes show early larval development and the other portion show late development. Such a pattern explains both demonstrated bimodality in larval numbers over time and the reduction in larval numbers in mosquito populations dissected at mid-cycle. Although each group of developing larvae (early and late) would themselves show distributions like that in Figure 9, lags in time needed for development and rapid loss of infective larvae within the first day after appearance in the head (35.1 percent

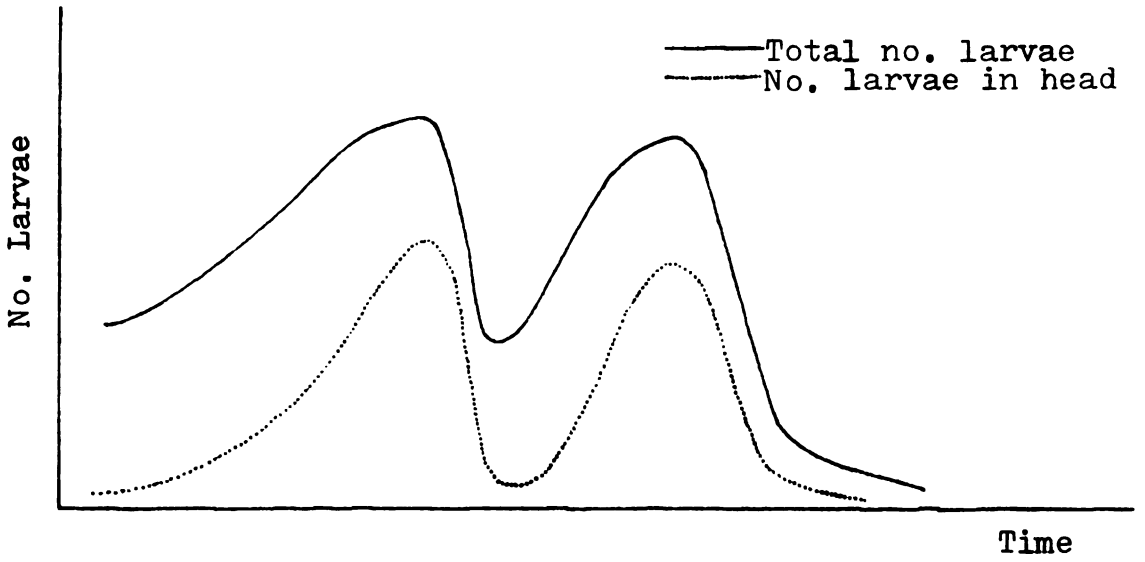

Figure 10. Hypothetical distribution of D. immitis larvae within a total mosquito population in which a large portion of the population is showing either early or late development of the larvae.

loss over a two-day period; Ho et al., 1974), would produce a dip in total larval numbers at the apparent mid-cycle point.

Hypothesis B--Apparent asynchrony. Although Hypothesis A does offer an explanation for much of the data obtained during this research, early and late larval development in an apparently uniform mosquito population is improbable. Another explanation for bimodality, and one which would also explain the reduction in total larval numbers within individual mosquitoes (rather than mosquito populations) at the mid-cycle point of development (Figure 9), is that asynchrony is apparent rather than real. According to this hypothesis, a two-peak distribution (like that in Figure 8) exists but represents larvae which had migrated to the thorax but were not discovered during dissection. (See Figure 11.) Infective larvae look similar to thoracic muscles and differential staining was not done during dissections so it is possible that a certain segment of the larval population was not counted. According to this hypothesis, the drop in the total larval numbers would be most dramatic if and when infective larvae made a massive migration into the thorax. Frequency distributions and developmental variances computed from such data would be identical to that obtained if Hypothesis A were true.

Explanation of experimental results. Whether or not either or both of the above-discussed hypotheses are true, a dip in the number of D. immitis larvae over time did occur here and numbers of larvae at mid-stage development

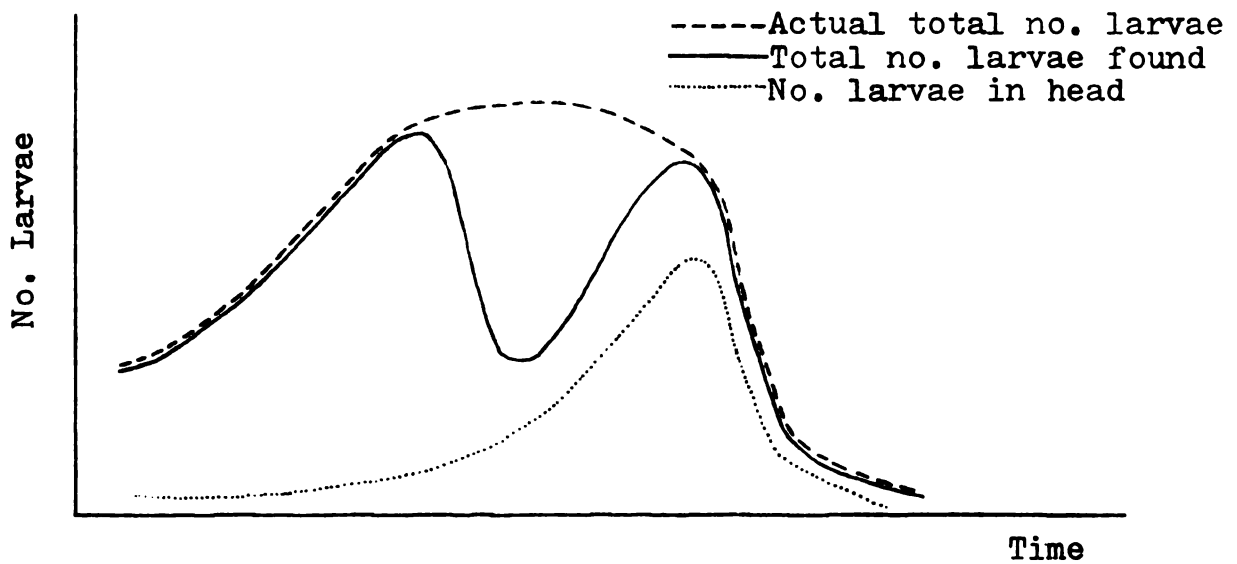

Figure 11. Hypothetical distribution of D. immitis larvae within a total mosquito population in which a portion of larvae present in the thorax is missing from observations.

were lower than at early or late-stage development. The inverse relationship between DEC concentrations and numbers of larvae might therefore be explained if DEC affects the rate of larval development, slowing them down most at the highest concentration of the drug. Such a slowdown would be proportional to the amount of drug given and could result in a distribution of larvae like that shown in Figure 12 in which the least concentrated dose group falls into the "valley" of the graph and the higher concentrations groups (and lesser developed larvae) time-wise precede the smallest concentration group.

If DEC does act in the above-described manner, then graphs of dissections performed early or late in the larval developmental cycle should be slightly different from that shown in Figure 12. Evidence supporting the above "slowdown" hypothesis comes from Experiments 4, 6, 13, and 16. Experiment 13 results represent early dissection of mosquitoes (day 14 after infection in mosquitoes incubated at $72\pm2^{o}F$) and show the DEC 10^{-3} percent concentration with lower numbers of larvae than the two other DEC concentrations (Figure 13). Experiments 4, 6, and 16 represent late mosquito dissections and show DEC concentration 10^{-4} percent with the lowest number of larvae of the three test groups (Figure 14). (Experiments 4 and 6 are considered late dissections because, even though dissections were done on post-infection day 12, the mosquitoes from those trials were incubated at a higher temperature and almost 80 percent of the

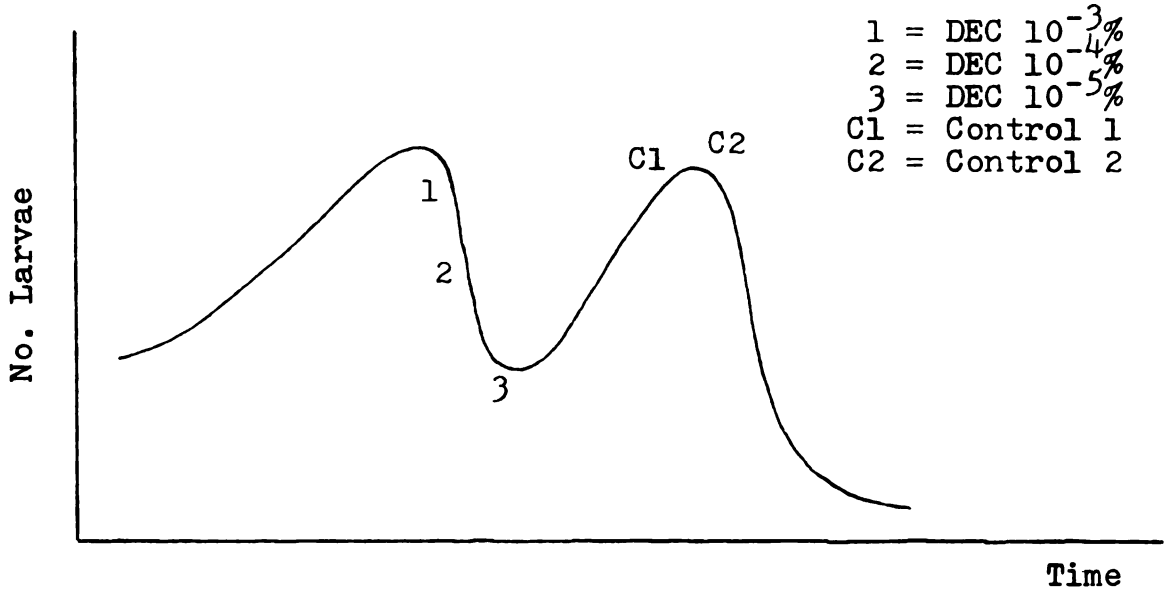

Figure 12. Hypothetical distribution of late second and third stage D. immitis larvae in A. triseriatus given that DEC slows down developmental rates of the larvae. (The information here comes from Tables 11, 12, 13, 15, and 16.)

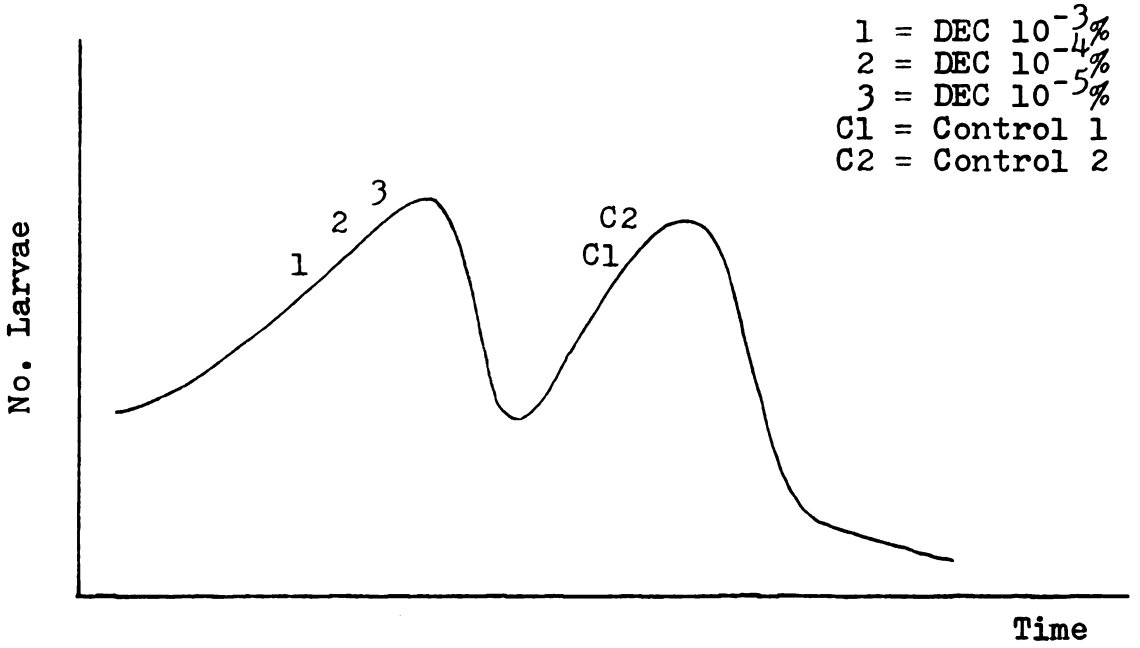

Figure 13. Hypothetical distribution of late second and third stage D. immitis larvae in A. triseriatus showing how early dissection affects the number of larvae from each DEC treatment group, given that DEC slows down larval development. (The information here comes from Table 14.)

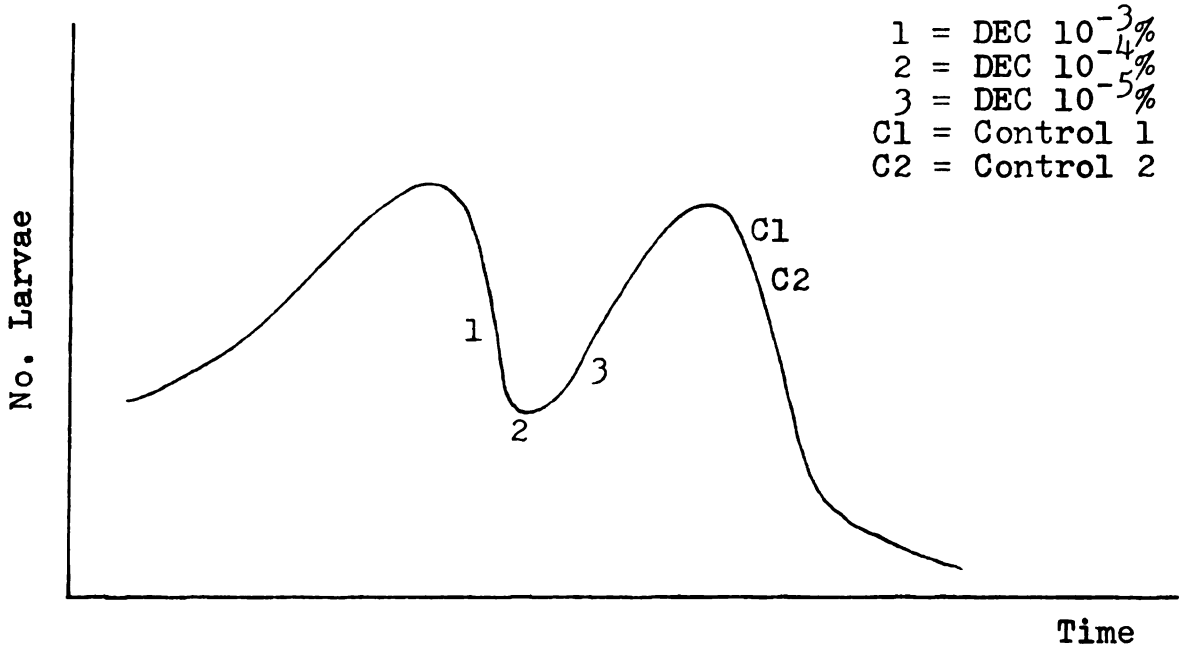

Figure 14. Hypothetical distribution of late second and third stage D. immitis larvae in A. triseriatus showing how late dissection affects the number of larvae from each DEC treatment group, given that DEC slows down larval development. (The information presented here comes from Tables 5, 7, and 17.)

larvae counted then were found in the head/thorax, indicating that development was well along the way.)

At this point one might feel that some indication of which asynchrony hypothesis is responsible for the above DEC pattern could be found by examining the proportion of head/thorax larvae to total body counts for each of the experimental test groups. (C.f. Figures 10 and 11.) If hypothesis A were correct, the proportion of head/thoracic larvae should be higher in mosquitoes dissected just prior to the "dip" and lower in those mosquitoes dissected just after the dip, compared to similar proportions for earlier-dissected mosquitoes. Numbers of larvae should drop in both abdominal and head/thoracic regions over this period of time. If hypothesis B were correct, the proportion of head/thoracic larvae should be higher compared to that of earlier dissections. Although numbers of larvae found in the Malpighian tubules might drop, the number of larvae in the head/thoracic region should increase over time. The results of such examinations, however, are generally inconclusive. (See Table 28.) The results of Experiments 11, 12, 14, and 15 support Hypothesis A, indicating that the percentage of head/thoracic larvae to total body larvae for the DEC 10^{-5} percent group was generally lower than for the 10^{-3} or 10^{-4} percent treatment groups and that the number of larvae in the head/thoracic and abdominal regions were reduced in the 10^{-5} percent DEC concentration. However, hypothesis B is also supported by

Table 28. Mean number and percentage of D. immitis larvae in abdominal and head/thoracic regions of A. triseriatus for Experiments 10, 11, 12, 14, and 15.

Treatment Group (%)	Experiment 10		Experiment 11		Experiment 12		Experiment 14		Experiment 15	
	Mean	Percent	Mean	Percent	Mean	Percent	Mean	Percent	Mean	Percent
DEC 10^{-3}										
Abdomen	6.67	71	7.58	92	2.67	26	7.40	76	5.21	83
H/Thorax	2.67	29	0.67	8	7.67	74	2.40	24	1.11	17
DEC 10^{-4}										
Abdomen	2.33	100	7.39	96	0.67	11	9.71	87	5.50	80
H/Thorax	0	0	0.28	4	5.50	89	1.43	13	1.33	20
DEC 10^{-5}										
Abdomen	-	-	6.33	97	1.73	35	5.90	86	5.00	94
H/Thorax	-	-	0.22	3	3.18	65	1.00	14	0.31	6
Lev 10^{-3}										
Abdomen	1.91	30	3.25	50	1.80	24	5.57	75	3.94	94
H/Thorax	4.55	70	3.25	50	5.80	76	1.86	25	0.25	6
Lev 10^{-4}										
Abdomen	9.33	88	8.57	87	3.00	39	4.67	56	4.92	81
H/Thorax	1.33	12	1.29	13	4.63	61	3.67	44	1.15	19
Lev 10^{-5}										
Abdomen	10.50	82	5.22	56	6.83	46	6.00	70	4.55	65
H/Thorax	2.33	18	4.11	44	8.00	54	2.60	30	2.45	35
Control 1										
Abdomen	6.93	75	6.33	82	7.07	55	9.41	87	4.93	65
H/Thorax	2.33	25	1.43	18	5.71	45	1.41	13	2.61	35
Control 2										
Abdomen	4.70	41	3.14	39	3.83	38	9.79	77	5.00	56
H/Thorax	6.90	59	4.86	61	6.28	62	3.00	23	3.89	44

the data in Tables 4, 9, and 10, indicating that no larvae were found in the head/thorax of dissected mosquitoes in the days just preceding the "dip."

The bimodal model was also examined to explain the levamisole results. Although levamisole behaved in a more "expected" manner than DEC and at first glance appears to act by killing or preventing development of certain numbers of larvae present in dosed mosquitoes, one cannot immediately rule out the possibility that levamisole also acts like DEC, by altering the rates of larval development, without first examining the experimental results in more detail. Early and late dissections, however, reveal that unlike DEC, levamisole behaved without regard to time; that is, both an early dissection (Experiment 13) and late dissections (Experiments 4, 6, and 16) show that the 10^{-3} percent levamisole concentration produced fewest larvae. Although it cannot conclusively be stated that levamisole does not affect the rate of development of larvae in dosed mosquitoes, these results suggest another mode of action, such as selective killing of those larvae at an early stage of development. Four out of five experiments shown in Table 28 support this hypothesis. At a given dissection day, fewest numbers of total larvae and abdominal larvae for all the levamisole treatment groups were found in the 10^{-3} percent group.

Alternate Modes of Drug Action

Although the experimental results discussed here point to DEC's causing a slowdown in D. immitis larval development

within the test mosquitoes, it is possible that the drug acts in another manner. Other possible explanations for this drug's "inverse" activity are that DEC (1) has a different pattern of activity at high vs. low concentrations; (2) affects the mosquito physiology at high concentrations in such a way that more D. immitis larvae develop; or (3) affects the nematode physiology at high concentrations in such a way that more larvae develop. (E.g., Coles and Jenkins reported in Rogers and Denham (1976) that reversible paralysis of an adult nematode was observed when the worm had prolonged contact with the test drug levamisole.)

SUMMARY AND CONCLUSIONS

Overall Statements

1. Mosquitoes could be infected with D. immitis with an artificial feeding apparatus using diluted blood containing microfilariae.

2. The ROCK strain of A. aegypti kept at the Pesticide Research Center of Michigan State University would not support the development of D. immitis.

3. The Alabama, Walton, Ohio, and Michigan strains of A. triseriatus all allowed complete development of D. immitis.

4. Mosquito feeding rates increased by using the NaturaLamb membrane in the artificial feeding apparatus rather than the laboratory-prepared cow gut membrane.

5. Mortality rates of mosquitoes infected with D. immitis remained quite low (one to two percent per day of infection) when mosquitoes were sorted by aspiration rather than by carbon dioxide anesthetization.

6. Daily dissections of infected mosquitoes indicated that a drop in the number of larvae observed occurred for a one to two day period shortly after the larvae migrated toward the head.

7. It was postulated that reasons for the above-described "dip" in larval numbers might have been either

asynchronization of larval development in the mosquito population as a whole or numbers of larvae present in the thorax not discovered during dissection.

8. The results of Experiments 10, 11, 12, 14, and 15 indicated that fewer number of D. immitis larvae were present in mosquitoes dosed with the lowest concentration of DEC (10^{-5} percent).

9. In view of the "dip" in larval numbers mentioned in (7), above, it was postulated that such results could have been caused by DEC's slowing down the rate of larval development.

10. Such "inverse" DEC results might also be possible, however, if DEC acted differently at high vs. low concentrations, or acted upon mosquito or nematode physiology, etc., in such a manner as to promote larval development.

11. The results of Experiments 10, 11, 12, 14, and 15 also indicated that more D. immitis larvae were present at the lowest concentration of levamisole (10^{-5} percent).

12. Such results might be possible if levamisole acted upon the nematode by killing or preventing development of the earlier larval stages.

13. No concentration of either drug completely eliminated D. immitis larvae in dosed mosquitoes.

14. No statistically significant difference was seen in numbers of D. immitis larvae which ultimately developed in the once-fed control group (Control 2) and the twice-fed group (Control 1).

15. Although the exact causes for reductions in numbers of D. immitis larvae developing in infected mosquitoes within treatment groups could not be conclusively determined here, the results indicated that both drugs in some way do affect either the rate of development or numbers of developing larvae.

Implications of Experimental Results

The results of these experimental trials raised certain questions which can only be answered by additional study and research. For example:

(1) Reductions in larval numbers by as much as 44 percent were observed in certain drug treatment groups although complete elimination of larvae was never seen. Ingestion of a single dose of a prophylactic drug possibly could have an effect on the ultimate heartworm transmission rate if mosquitoes contained a small number of infective stage larvae and the drug reduced the number to a subtransmission or threshold level, or selectively inhibited either the male or female nematode. A drug-induced delay in the development of larvae could also result in the mosquito obtaining a subsequent blood meal before the larvae had developed completely and could be transmitted to a new dog host.

(2) Although reports of resistance to either drug (DEC or levamisole) by D. immitis was not found in the literature, the question still can be raised whether long-term exposure to small amounts of these drugs may ultimately affect the

rate of resistance development. Several authors (Benz, 1973; Colglazier et al., 1973; Theodorides, 1974) have reported that resistance to chemotherapeutic agents by parasitic nematodes does occur. Such resistance generally has developed by exposure of the adult stages of the nematode rather than the immature stages, but drug resistance in D. immitis developing by exposure in the mosquito is not infeasible.

Suggestions for Further Study

Neither of the above questions can be answered without additional research and study but, before any such studies are undertaken, it should be kept in mind that such questions regarding reduction of transmission rates and development of drug resistance in D. immitis are only valid if it is known that (1) sufficient numbers of dogs will be taking prophylactic drugs and will be dosed at a time of day when biting mosquitoes will receive a sufficient dose of the drug, and (2) a number of infected mosquitoes will bite a dosed dog before development of the nematode is complete.

Although the following list of suggested topics for research is far from complete, it does offer a starting point:

(1) Repeat the research reported in this thesis, this time dissecting treated mosquitoes for all treatment groups over time to determine conclusively if rates of development are being affected by the drugs.

(2) Repeat the same research using blood from drug-dosed dogs rather than mixing the drug with the dog's blood.

(3) Determine the probability that an infected mosquito will bite a drug-dosed dog before ultimate transmission to a susceptible dog takes place.

(4) Determine the average nematode load of infected mosquitoes in nature, to ascertain whether ingestion of the drug(s) would cause a sufficient reduction in larval numbers as to directly prevent transmission of heartworm disease.

(5) Determine whether ingestion of the drug(s) by the mosquito in any way affects its later host-finding behavior.

APPENDIX A

Because it was virtually impossible to tell whether every mosquito which received the infective blood meal actually ingested one or more microfilariae, only those mosquitoes which were definitely infected (that is, ones in which D. immitis larvae could be seen) were used in computing mean numbers of larvae per mosquito group, or in the statistical analyses discussed in the main portion of of this thesis. Although such "zero" observations occurred infrequently during the course of the dissections, they are nevertheless listed along with all other individual observations in the following data sheets. Notation of the number of "zero" observations occurring per treatment group is made by placing a number indicating the frequency of the zero observations directly in front of a parenthetical "0". (E.g., 2(0) means that two mosquitoes were found in that particular treatment group in which no larvae could be seen.)

APPENDIX A-1

Number of late second and third stage D. immitis larvae in individual A. triseriatus (Michigan strain) from Experiment 4.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2	Control 2
Day Post-Infection	-	12	12	-	12	12	12	12	13
Individual Observations		3 7	30		3	6	42 7 1	17 22 12 47 32 37	21 23 27 17 15 13 7 13 6 21 35 35 2(0)
Total	-	10	30	-	3	6	50	167	233
N	-	2	1	-	1	1	3	6	12
$\bar{X}$	-	5.0	30.0	-	3.0	6.0	16.67	27.83	19.42
SD	-	2.83	-	-	-	-	22.14	13.20	9.55
SE	-	2.0	-	-	-	-	12.78	5.39	2.76
D(%)	-	40	-	-	-	-	77	19	14

APPENDIX A-2

Number of late second and third stage D. immitis larvae in individual A. triseriatus (Michigan strain) from Experiment 5.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	-	-	12	12	-	-	12	13
Individual Observations			19 1(0)	8			15	13 13 24 12 19 14 23 5 22 16 12 9 12 11

continued

APPENDIX A-2 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	-	-	12	12	-	-	12	13
Individual Observations								7 13 2(0)
Total	-	-	19	8	-	-	15	225
N	-	-	1	1	-	-	1	16
$\bar{X}$	-	-	19.0	8.0	-	-	15.0	14.06
SD	-	-	-	-	-	-	-	5.50
SE	-	-	-	-	-	-	-	1.37
D(%)	-	-	-	-	-	-	-	10

APPENDIX A-3

Number of late second and third stage *D. immitis* larvae in individual *A. triseriatus* (Michigan strain) from Experiment 6.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2	Control 2	Control 2
Day Post-Infection	12	12	12	12	12	12	12	13	14	15
Individual Observations	17	1	18	2	3	9	9	25	7	1
	8	6	9	9	3		14	15	9	2
		2(0)	5	3	7		14	20	7	6
				5	13		4	13	14	7
					16		14	7	25	18
					8		8	11	8	6
					20		9	8	6	8
					4		1(0)	11	19	17
					1(0)			9	1	8
								29	7	21
								12	18	15
								11	22	12
								3	15	18
								8	9	

continued

APPENDIX A-3 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2	Control 2	Control 2
Day Post-Infection	12	12	12	12	12	12	12	13	14	15
Individual Observations								59 11 16 3(0)	8 9 9 7 9 7 9 14 17 7 3 3 12 17 12 3(0)	
Total	25	7	32	19	74	9	72	269	294	139
N	2	2	3	4	8	1	7	17	27	13
$\bar{X}$	12.50	3.50	10.67	4.75	9.25	9.0	10.29	15.82	10.89	10.69
SD	6.36	3.54	6.66	3.10	6.41	-	3.86	12.91	5.91	6.55
SE	4.50	2.50	3.84	1.55	2.27	-	1.46	3.13	1.14	1.82
D	36	71	36	33	24	-	14	20	10	17

APPENDIX A-4

Number of late second and third stage D. immitis larvae in individual A. triseriatus (Michigan strain) from Experiment 7.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	-	-	-	-	-	-	-	14
Individual Observations								21 20 12 13 21 6 13 26 12 7 3 8 12 17

continued

APPENDIX A-4 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	-	-	-	-	-	-	-	14
Individual Observations								5 1 21 16 14 20 2(0)
Total	-	-	-	-	-	-	-	268
N	-	-	-	-	-	-	-	20
$\overline{X}$	-	-	-	-	-	-	-	13.40
SD	-	-	-	-	-	-	-	6.89
SE	-	-	-	-	-	-	-	1.54
D(%)	-	-	-	-	-	-	-	12

APPENDIX A-5

Number of late second and third stage *D. immitis* larvae in individual *A. triseriatus* (Alabama strain) from Experiment 10.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2	Control 2	Control 2
Day Post-Infection	16	16	-	16	16	16	16	16	17	18
Individual Observations	10	1	-	3	7	7	7	12	12	14
	10	1		1	11	16	5	14	9	2
	8	5		12	37	17	1	16	9	1
				1	3	18	18	4	2	7
				9	2	11	1	10	12	47
				5	4	8	9	15	21	11
				15			15	10	14	7
				7			23	5	9	16
				7			8	21	10	6
				3			6	9	18	1(0)
				8			3			
				1(0)			12			
							9			
							3			
							19			
Total	28	7	-	71	64	77	139	116	116	111
N	3	3	-	11	6	6	15	10	10	9
$\bar{X}$	9.33	2.33	-	6.45	10.67	12.83	9.27	11.60	11.60	12.33
SD	1.15	2.31	-	4.46	13.31	4.79	6.82	5.15	5.27	13.93
SE	0.67	1.33	-	1.34	5.43	1.96	1.72	1.63	1.67	4.64
D(%)	7	57	-	21	51	15	19	14	14	38

APPENDIX A-6

Number of late second and third stage *D. immitis* larvae in individual *A. triseriatus* (Alabama strain) from Experiment 11.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	17	16	17
Individual Observations	1	4	1	5	7	9	2	4
	1	2	20	12	21	18	8	5
	12	15	10	6	12	4	5	7
	10	4	1	3	9	9	12	2
	11	8	6	3(0)	6	4	9	3
	9	12	8		1	9	4	23
	18	4	4		13	1	8	10
	11	1	2		3(0)	14	4	7
	4	10	7			16	2	13
	10	5				11(0)	12	14
	2	3					5	2
	10	6					2	9
	1(0)	9					15	1
		9					4	2
		5					8	1
		14					3	16
		16					8	3
		11					6	15
		2(0)					4	8
							1	7
							13	11
							2	9

continued

APPENDIX A-6 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	17	16	17
Individual Observations							18	14
							14	10
							1	1
							24	14
							26	5
							3	11
							2	6
							8	7
							9(0)	27
								2
								23
								4
								11
								7
								2
								8
								6
								9
								8
								4
								3
								7
								5
								2
								10

continued

APPENDIX A-6 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	17	16	17
Individual Observations								12 1 10 16 5 6 1 5 4 15 4 12 8 23

continued

APPENDIX A-6 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	17	16	17
Individual Observations								2 2 14(0)
Total	99	138	59	26	69	84	233	504
N	12	18	9	4	7	9	30	63
$\bar{X}$	8.25	7.67	6.56	6.50	9.86	9.33	7.77	8.0
SD	5.19	4.59	5.96	3.87	6.34	5.79	6.53	5.99
SE	1.50	1.08	1.99	1.94	2.39	1.93	1.19	0.75
D(%)	18	14	30	30	24	21	15	9

APPENDIX A-7

Number of late second and third stage D. immitis larvae in individual A. triseriatus (Alabama strain) from Experiment 12.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Individual Observations	13	8	6	6	2	28	22	5
	8	11	3	13	5	7	7	2
	3	6	7	1	4	27	16	13
	14	2	4	8	3	14	16	12
	10	4	3	10	6	9	6	9
	14	6	6	4(0)	4	4	1	1
		1(0)	4		13		24	23
			1		24		6	11
			13		2(0)		26	4
			3				5	8
			4				11	10
			2(0)				18	20
							11	8

continued

APPENDIX A-7 (continued)

Treatment Group (%)	$DEC_{10^{-3}}$	$DEC_{10^{-4}}$	$DEC_{10^{-5}}$	$Lev_{10^{-3}}$	$Lev_{10^{-4}}$	$Lev_{10^{-5}}$	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Individual Observations							10 4(0)	29 6 8 8 5 2(0)
Total	62	37	54	38	61	89	179	182
N	6	6	11	5	8	6	14	18
$\bar{X}$	10.33	6.17	4.91	7.60	7.63	14.83	12.79	10.11
SD	4.32	3.13	3.18	4.51	7.42	10.34	7.71	7.31
SE	1.76	1.28	0.96	2.01	2.63	4.22	2.06	1.72
D(%)	17	21	20	27	34	28	16	17

APPENDIX A-8

Number of late second and third stage *D. immitis* larvae in individual *A. triseriatus* (Alabama strain) from Experiment 13.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	14	14	14	14	14	14	14	14
Individual Observations	19	12	9	9	7	5	8	3
	3	6	6	13	10	14	1	12
	5	10	5	4	5	5	6	9
	8	11	14	7	8	15	3	13
	7	8	9	9	2(0)	6	4	7
	8	8	10	3		11	5	11
	6	6	9			6	10	1
	3	4	9			7	5	13
	7	6	15			10	18	18
	6	8	5			10	9	19
		8	11				1	5
			3				9	5
			4				9	5

continued

APPENDIX A-8 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	14	14	14	14	14	14	14	14
Individual Observations			6 1(0)				4 8 9 8 3(0)	10 3 10 10 6 8 8 14 1(0)
Total	72	87	115	45	30	89	117	190
N	10	11	14	6	4	10	17	21
$\bar{X}$	7.20	7.91	8.21	7.50	7.50	8.90	6.88	9.05
SD	4.52	2.39	3.60	3.67	2.08	3.67	4.06	4.76
SE	1.43	0.72	0.96	1.64	1.04	1.16	0.98	1.04
D(%)	20	9	12	22	14	13	14	11

APPENDIX A-9

Number of late second and third stage D. immitis larvae in individual A. triseriatus (Alabama strain) from Experiment 14.

Treatment Group (%)	$DEC_{10^{-3}}$	$DEC_{10^{-4}}$	$DEC_{10^{-5}}$	$Lev_{10^{-3}}$	$Lev_{10^{-4}}$	$Lev_{10^{-5}}$	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Individual Observations	20	6	14	9	7	13	17	8
	8	10	9	17	12	20	14	5
	5	13	17	9	4	2	11	12
	9	13	1	6	9	7	8	4
	7	18	8	1	6	1	16	7
	2(0)	11	3	4	12	3(0)	13	27
		7	3	6			7	21
			1				10	14
			7				16	8
			6				6	2
			1(0)				3	19
							13	11
							6	14

continued

APPENDIX A-9 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Individual Observations							2 14 16 12 1(0)	27 2(0)
Total	49	78	69	52	50	43	184	179
N	5	7	10	7	6	5	17	14
$\bar{X}$	9.80	11.14	6.90	7.43	8.33	8.60	10.82	12.79
SD	5.89	4.06	5.36	5.06	3.27	7.96	4.73	8.09
SE	2.63	1.53	1.70	1.91	1.33	3.56	1.15	2.16
D(%)	27	14	25	26	16	41	11	17

APPENDIX A-10

Number of late second and third stage D. immitis larvae in individual A. triseriatus (Alabama strain) from Experiment 15.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Individual Observations	4	8	5	2	6	9	1	16
	17	2	13	4	12	7	8	2
	3	2	7	3	2	5	13	4
	6	8	3	3	4	4	1	8
	4	9	5	1	2	5	2	5
	2	17	2	4	9	1	1	18
	1	1	6	4	8	3	5	4
	10	12	2	1	19	13	9	3
	3	2	3	6	4	7	10	20
	3	10	2	12	7	12	7	
	3	10	8	1	2	11	3	
	4	1	8	2	3	1(0)	3	
	7	2(0)	5	7	1		6	
	4		1(0)	2	1(0)		16	
	4			6			7	

continued

APPENDIX A-10 (continued)

Treatment Group (%)	DEC_3 10	DEC_4 10	DEC_5 10	Lev_3 10	Lev_4 10	Lev_5 10	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Individual Observations	15 10 19 1			9 1(0)			7 1 5 42 1 4 8 4 6 13 10 7 11 5(0)	
Total	120	82	69	67	79	77	211	80
N	19	12	13	16	13	11	28	9
$\bar{X}$	6.32	6.83	5.31	4.19	6.08	7.0	7.54	8.89
SD	5.40	5.18	3.17	3.12	5.07	3.87	7.88	7.10
SE	1.24	1.50	0.88	0.78	1.41	1.17	1.49	2.37
D(%)	20	22	17	19	23	30	20	27

APPENDIX A-11

Number of late second and third stage D. immitis larvae in individual A. triseriatus (Alabama strain) from Experiment 16.

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	17	17	17	17	17	17	17	17
Individual Observations	1 9 15 3 4 4(0)	1 5 1 4 3(0)	2 3 2 8 1 1	1 2 2 2 9 1 1 1 2(0)	4 5 2 5 8 2 1 5 3(0)	2 1 6 3 3 7 2(0)	1 2 8 2 3 4 2 1 5(0)	9 6 4 5 8 7 3(0)
Total	32	11	17	19	32	22	23	39
N	5	4	6	8	8	6	8	6
$\bar{X}$	6.40	2.75	2.83	2.38	4.0	3.67	2.88	6.50
SD	5.64	2.06	2.64	2.72	2.27	2.34	2.30	1.87
SE	2.52	1.03	1.08	0.96	0.80	0.95	0.81	0.76
D(%)	39	37	38	41	20	26	28	12

APPENDIX B

APPENDIX B-1

Number of late second and third stage *D. immitis* larvae in *A. triseriatus* (Alabama strain) receiving only the infective blood meal (Control 2). Data is from Experiment 8 and is grouped according to location of dissection.

Day Post-Infection	12		13		15		16	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	5	0	3	0	0	2	0	7
	2	0	3	0	0	1	0	1
	1	0	0	12	0	8	0	2
	2	0	6	0	0	1	0	11
	1	0	7	0	1	1	0	1
	1	0	16	1	0	3	0	2
	2	0			0	2	0	1
	1	0			4	10	0	2
	2	0			3	10	3	2
	3	0			3	4	15	0
	2	0			0	2		
	3	0			1	0		
	2	0						
	2	0						
Total in Abd	29		44		12		18	
Total in H/T	0		13		44		29	
N	14		6		12		10	
$\bar{X}$ in Abdomen	2.07		7.33		1.00		1.80	
$\bar{X}$ in H/Thorax	0		2.17		3.67		2.90	
% in Abdomen	100		77		21		38	
% in H/Thorax	0		23		79		62	
Corr. coef.	-		+0.24		+0.67		-0.32	

APPENDIX B-2

Number of late second and third stage D. immitis larvae in A. triseriatus receiving only the infective blood meal. Data is from Experiment 9 and larvae are grouped according to location of dissection.

Mosquito Strain	Alabama		Alabama		Alabama		Alabama		Walton		Walton		Ohio		Ohio		Michigan	
Day Post-Infection	12		13		15		16		12		13		12		13		15	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	27	0	6	9	0	12	1	13	19	0	9	0	11	0	2	0	1	7
	29	0	4	7	0	1	2	6	13	0	13	0	6	0	1	0	0	20
	10	0	6	3	6	12	0	7	20	0	10	0	9	0	15	0	1	11
	19	0	8	7	1	28	0	21	6	2	12	0	10	0	13	0	0	22
			10	8	4	16	1	3			4	0	22	0			0	9
					3	3	1	18									5	8
					2	4	4	22									0	12
					0	14	0	13									10	9
					3	13	0	11										
					8	10	0	2										
							0	10										
							2	15										
							0	9										
							4	2										
							0	14										

continued

APPENDIX B-2 (continued)

Mosquito Strain	Alabama	Alabama	Alabama	Alabama	Walton	Walton	Ohio	Ohio	Michigan
Day Post-Infection	12	13	15	16	12	13	12	13	15
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations				0 4 0 19 0 10 1 18					
Total in Abd	85	34	27	16	58	48	58	31	17
Total in H/T	0	34	113	207	2	0	0	0	98
N	4	5	10	19	4	5	5	4	8
X in Abdomen	21.25	6.80	2.70	0.84	14.50	9.60	11.60	7.75	2.13
X in H/Thorax	0	6.80	11.30	10.89	0.50	0	0	0	12.25
% in Abdomen	100	50	19	7	97	100	100	100	15
% in H/Thorax	0	50	81	93	3	0	0	0	85
Corr. coef.	-	+0.23	-0.03	+0.05	-0.88	-	-	-	-0.42

APPENDIX B-3

Number of late second and third stage *D. immitis* larvae in *A. triseriatus* (Alabama strain) from Experiment 10, grouped according to location of dissection.

Treatment Group (%)	DEC_3 10^{-3}		DEC_4 10^{-4}		DEC_5 10^{-5}		Lev_3 10^{-3}		Lev_4 10^{-4}		Lev_5 10^{-5}		Control 1		Control 2		Control 2		Control 2	
Day Post-Infection	16		16		-		16		16		16		16		16		17		18	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	10	0	1	0			0	3	1	6	7	0	5	2	2	10	1	11	4	10
	6	4	1	0			1	0	10	1	6	10	3	2	6	8	1	8	1	1
	4	4	5	0			3	9	37	0	17	0	0	1	2	14	2	7	0	1
							0	1	2	1	17	1	10	8	4	0	2	0	0	7
							3	6	2	0	10	1	1	0	2	8	4	10	0	47
							0	5	4	0	6	2	6	3	8	7	5	7	0	11
							1	14					22	1	5	5	5	16	1	6
							1	6					7	1	5	0	0	9	3	13
							3	4					6	0	7	14	9	1	0	6
							1	2					1	2	6	3	16	2		
							8	0					5							

continued

APPENDIX B-3 (continued)

Treatment Group (%)	DEC 10^{-3}	DEC 10^{-4}	DEC 10^{-5}	Lev 10^{-3}	Lev 10^{-4}	Lev 10^{-5}	Control 1	Control 2	Control 2	Control 2
Day Post-Infection	16	16	-	16	16	16	16	16	17	18
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations							7 2 3 0 16 3 12 3			
Total in Abdomen	20	7	-	21	56	63	104	47	45	9
Total in H/Thorax	8	0	-	50	8	14	35	69	71	102
N	3	3	-	11	6	6	15	10	10	9
X in Abd	6.67	2.33	-	1.91	9.33	10.50	6.93	4.70	4.50	1.00
X in H/T	2.67	0	-	4.55	1.33	2.33	2.33	6.90	7.10	11.33
% in Abd	71	100	-	30	88	82	75	41	39	8
% in H/T	29	0	-	70	12	18	25	59	61	92
Corr. coef.	-0.94	-	-	-0.17	-0.35	-0.48	+0.18	-0.16	-0.42	-0.10

APPENDIX B-4

Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 11, grouped according to location of dissection.

Treatment Group (%)	DEC_3 10^{-3}		DEC_4 10^{-4}		DEC_5 10^{-5}		Lev_3 10^{-3}		Lev_4 10^{-4}		Lev_5 10^{-5}		Control 1		Control 2	
Day Post-Infection	16		16		16		16		16		17		16		17	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	1	0	4	0	1	0	3	2	7	0	9	0	2	0	2	2
	1	0	2	0	18	2	3	9	21	0	10	8	2	6	5	0
	12	0	15	0	10	0	4	2	12	0	2	2	5	0	4	3
	10	0	4	0	1	0	3	0	8	1	3	6	6	6	2	0
	10	1	8	0	6	0			6	0	4	0	6	3	0	3
	9	0	11	1	8	0			1	0	3	6	4	0	8	15
	13	5	4	0	4	0			5	8	0	1	8	0	6	4
	11	0	1	0	2	0					2	12	4	0	4	3
	4	0	10	0	7	0					14	2	2	0	8	5
	8	2	5	0									12	0	14	0
	2	0	3	0									3	2	2	0
	10	0	6	0									2	0	1	8
			9	0									4	11	1	0
			7	2									4	0	2	0
			5	0									8	0	0	1
			14	0									0	3	2	14
			14	2									5	3	0	3
			11	0									6	0	0	15

continued

APPENDIX B-4 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	17	16	17
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations							2 2 1 0 12 1 18 0 1 1 11 3 1 0 24 0 26 0 3 0 2 0 6 2	1 7 1 6 8 3 1 8 6 8 10 0 1 0 6 5 0 14 0 5 5 1 3 4 0 27 2 0 1 22 4 0 0 11 0 7

continued

APPENDIX B-4 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	17	16	17
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations								1 1 0 8 2 4 7 2 6 2 0 4 0 3 0 7 5 0 2 0 1 9 2 10 1 0 0 10 16 0 0 5 4 2 1 4 0 1 0 4

continued

APPENDIX B-4 (continued)

Treatment Group (%)	DEC_3 10^{-3}	DEC_4 10^{-4}	DEC_5 10^{-5}	Lev_3 10^{-3}	Lev_4 10^{-4}	Lev_5 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	17	16	17
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations								13 2 4 0 12 0 8 0 0 23 2 0 1 1
Total in Abd	91	133	57	13	60	47	190	198
Total in H/T	8	5	2	13	9	37	43	306
N	12	18	9	4	7	9	30	63
$\bar{X}$ in Abdomen	7.58	7.39	6.33	3.25	8.57	5.22	6.33	3.14
$\bar{X}$ in H/Thorax	0.67	0.28	0.22	3.25	1.29	4.11	1.43	4.86
% in Abdomen	92	96	97	50	87	56	82	39
% in H/Thorax	8	4	3	50	13	44	18	61
Corr. coef.	+0.42	+0.33	+0.81	-0.21	-0.25	-0.14	-0.17	-0.32

APPENDIX B-5

Number of late second and third stage *D. immitis* larvae in *A. triseriatus* (Alabama strain) from Experiment 12, grouped according to location of dissection.

Treatment Group (%)	DEC_3 10^{-3}		DEC_4 10^{-4}		DEC_5 10^{-5}		Lev_3 10^{-3}		Lev_4 10^{-4}		Lev_5 10^{-5}		Control 1		Control 2	
Day Post-Infection	16		16		16		16		16		16		16		16	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	0	13	0	8	2	4	0	6	0	2	0	28	22	0	0	5
	3	5	1	10	0	3	0	13	5	0	0	7	4	3	1	1
	0	3	0	6	2	5	1	0	4	0	27	0	1	15	2	11
	5	9	2	0	4	0	4	4	3	0	4	10	14	2	5	7
	2	8	1	3	3	0	4	6	6	0	6	3	0	6	2	7
	6	8	0	6	3	3			4	0	4	0	1	0	1	0
					2	2			0	13			20	4	2	21
					0	1			2	22			0	6	9	2
					3	10							16	10	0	4
					0	3							2	3	6	2
					0	4							4	7	6	14
													2	16	1	7
													10	1	10	19
													3	7	6	0

continued

APPENDIX B-5 (continued)

Treatment Group (%)	DEC 10^{-3}	DEC 10^{-4}	DEC 10^{-5}	Lev 10^{-3}	Lev 10^{-4}	Lev 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations								3 5 6 2 5 0 4 6
Total in Abd	16	4	19	9	24	41	99	69
Total in H/T	46	33	35	29	37	48	80	113
N	6	6	11	5	8	6	14	18
$\bar{X}$ in Abdomen	2.67	0.67	1.73	1.80	3.00	6.83	7.07	3.83
$\bar{X}$ in H/Thorax	7.67	5.50	3.18	5.80	4.63	8.00	5.71	6.28
% in Abdomen	26	11	35	24	39	46	55	38
% in H/Thorax	74	89	65	76	61	54	45	62
Corr. coef.	+0.03	-0.62	+0.01	-0.32	-0.52	-0.50	-0.34	+0.13

APPENDIX B-6

Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 13, grouped according to location of dissection.

Treatment Group (%)	DEC_3 10^{-3}		DEC_4 10^{-4}		DEC_5 10^{-5}		Lev_3 10^{-3}		Lev_4 10^{-4}		Lev_5 10^{-5}		Control 1		Control 2	
Day Post-Infection	14		14		14		14		14		14		14		14	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	19	0	12	0	9	0	9	0	7	0	5	0	8	0	3	0
	3	0	6	0	6	0	13	0	10	0	14	0	1	0	12	0
	5	0	10	0	5	0	4	0	5	0	5	0	6	0	9	0
	8	0	11	0	14	0	7	0	8	0	15	0	3	0	13	0
	7	0	8	0	9	0	9	0			6	0	4	0	7	0
	8	0	8	0	10	0	3	0			11	0	5	0	11	0
	6	0	6	0	9	0					6	0	10	0	1	0
	3	0	4	0	7	2					7	0	5	0	13	0
	7	0	6	0	15	0					10	0	18	0	18	0
	6	0	8	0	5	0					10	0	9	0	19	0
			8	0	11	0							1	0	5	0
					3								9	0	5	0
					4								9	0	5	0
					6								4	0	10	0
													8	0	3	0
													9	0	10	0
													8	0	10	0

continued

APPENDIX B-6 (continued)

Treatment Group (%)	DEC_{3} 10^{-3}	DEC_{4} 10^{-4}	DEC_{5} 10^{-5}	Lev_{3} 10^{-3}	Lev_{4} 10^{-4}	Lev_{5} 10^{-5}	Control 1	Control 2
Day Post-Infection	14	14	14	14	14	14	14	14
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations								6 0 8 0 8 0 14 0
Total in Abd	72	87	113	45	30	89	117	190
Total in H/T	0	0	2	0	0	9	0	0
N	10	11	14	6	4	10	17	21
$\bar{X}$ in Abdomen	7.20	7.91	8.07	7.50	7.50	8.90	6.88	9.05
$\bar{X}$ in H/Thorax	0	0	0.14	0	0	0	0	0
% in Abdomen	100	100	98	100	100	100	100	100
% in H/Thorax	0	0	2	0	0	0	0	0
Corr. coef.	-	-	+0.14	-	-	-	-	-

APPENDIX B-7

Number of late second and third stage *D. immitis* larvae in *A. triseriatus* (Alabama strain) from Experiment 14, grouped according to location of dissection.

Treatment Group (%)	DEC_3 10^{-3}		DEC_4 10^{-4}		DEC_5 10^{-5}		Lev_3 10^{-3}		Lev_4 10^{-4}		Lev_5 10^{-5}		Control 1		Control 2	
Day Post-Infection	16		16		16		16		16		16		16		16	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	20	0	6	0	10	4	9	0	0	7	13	0	10	7	0	8
	0	8	6	4	8	1	6	11	5	7	7	13	14	0	1	4
	5	0	13	0	13	4	9	0	0	4	2	0	9	2	0	12
	6	3	13	0	0	1	4	2	5	4	7	0	8	0	4	0
	6	1	15	3	8	0	1	0	6	0	1	0	16	0	2	5
			8	3	3	0	4	0	12	0			13	0	23	4
			7	0	3	0	6	0					4	3	10	4
					1	0							10	0	21	0
					7	0							16	0	8	0
					6	0							6	0	2	0
													3	0	14	5
													13	0	11	0
													6	0	14	0
													2	0	27	0

continued

APPENDIX B-7 (continued)

Treatment Group (%)	DEC 10^{-3}	DEC 10^{-4}	DEC 10^{-5}	Lev 10^{-3}	Lev 10^{-4}	Lev 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations							14 0 4 12 12 0	
Total in Abd	37	68	59	39	28	30	160	137
Total in H/T	12	10	10	13	22	13	24	42
N	5	7	10	7	6	5	17	14
$\bar{X}$ in Abdomen	7.40	9.71	5.90	5.57	4.67	6.00	9.41	9.79
$\bar{X}$ in H/Thorax	2.40	1.43	1.00	1.86	3.67	2.60	1.41	3.00
% in Abdomen	76	87	86	75	56	70	87	77
% in H/Thorax	24	13	14	25	44	30	13	23
Corr. coef.	-0.65	-0.10	+0.68	+0.02	-0.68	+0.12	-0.32	-0.45

APPENDIX B-8

Number of late second and third stage *D. immitis* larvae in *A. triseriatus* (Alabama strain) from Experiment 15, grouped according to location of dissection.

Treatment Group (%)	DEC_3 10^{-3}		DEC_4 10^{-4}		DEC_5 10^{-5}		Lev_3 10^{-3}		Lev_4 10^{-4}		Lev_5 10^{-5}		Control 1		Control 2	
Day Post-Infection	16		16		16		16		16		16		16		16	
Larvae Location	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T	Abd	H/T
Individual Observations	4	0	8	0	5	0	2	0	6	0	3	6	0	1	2	14
	17	0	0	2	13	0	2	2	9	3	3	4	8	0	2	0
	0	3	2	0	7	0	2	1	2	0	5	0	10	3	3	1
	6	0	8	0	3	0	3	0	4	0	4	0	1	0	2	6
	4	0	9	0	5	0	1	0	0	2	5	0	2	0	2	3
	2	0	17	0	2	0	4	0	8	1	1	0	5	0	18	0
	1	0	1	0	6	0	4	0	5	3	3	0	9	0	1	3
	5	5	3	9	0	2	1	0	19	0	5	8	2	8	0	3
	2	1	1	1	3	0	6	0	0	4	3	4	7	0	15	5
	3	0	10	0	0	2	1	0	5	2	7	5	3	0		
	3	0	6	4	8	0	12	0	2	0	11	0	3	3		
	3	1	1	0	8	0	2	0	3	0			13	3		
	7	0			5	0	6	1	1	0			7	0		
	4	0					2	0					3	0		
	4	0					6	0					1	0		
	10	5					9	0					5	0		
	19	0											7	35		
	4	6											1	0		
	1	0											4	0		

continued

APPENDIX B-8 (continued)

Treatment Group (%)	DEC 10^{-3}	DEC 10^{-4}	DEC 10^{-5}	Lev 10^{-3}	Lev 10^{-4}	Lev 10^{-5}	Control 1	Control 2
Day Post-Infection	16	16	16	16	16	16	16	16
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations							8 0 4 0 4 3 6 0 13 0 5 5 2 5 4 7 1 0	
Total in Abd	99	66	65	63	64	50	138	45
Total in H/T	21	16	4	4	15	27	73	35
N	19	12	13	16	13	11	28	9
$\bar{X}$ in Abdomen	5.21	5.50	5.00	3.94	4.92	4.55	4.93	5.00
$\bar{X}$ in H/Thorax	1.11	1.33	0.31	0.25	1.15	2.45	2.61	3.89
% in Abdomen	83	80	94	94	81	65	65	56
% in H/Thorax	17	20	6	6	19	35	35	44
Corr. coef.	-0.03	-0.24	-0.62	-0.14	-0.15	-0.07	+0.08	-0.21

APPENDIX B-9

Number of late second and third stage D. immitis larvae in A. triseriatus (Alabama strain) from Experiment 16, grouped according to location of dissection.

Treatment Group (%)	$DEC_{10^{-3}}$	$DEC_{10^{-4}}$	$DEC_{10^{-5}}$	$Lev_{10^{-3}}$	$Lev_{10^{-4}}$	$Lev_{10^{-5}}$	Control 1	Control 2
Day Post-Infection	17	17	17	17	17	17	17	17
Larvae Location	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T	Abd H/T
Individual Observations	0 1 4 5 1 14 3 0 4 0	0 1 1 4 1 0 0 4	2 0 3 0 2 0 8 0 1 0 1	1 0 0 2 2 0 2 0 9 0 0 1 1 0 1 0	5 0 0 2 5 0 2 6 4 0 0 2 0 1 5 0	2 0 1 0 2 4 3 0 0 3 6 1	1 0 2 0 3 5 2 0 3 0 4 0 2 0 1 0	3 6 0 6 4 0 0 5 2 6 5 2
Total in Abd	12	2	17	16	21	14	18	14
Total in H/T	20	9	0	3	11	8	5	25
N	5	4	6	8	8	6	8	6
X in Abdomen	2.40	0.50	2.83	2.00	2.63	2.33	2.25	2.33
X in H/Thorax	4.00	2.25	0	0.38	1.38	1.33	0.63	4.17
% in Abdomen	38	18	100	84	66	64	78	36
% in H/Thorax	62	82	0	16	34	36	22	64
Corr. coef.	-0.32	-0.14	-	-0.39	-0.49	-0.26	+0.29	-0.64

For the reasons presented in Appendix A, the statistical analyses discussed in the main body of this thesis were calculated using only non-zero observations. The results of the analysis of variance and orthogonal contrasts using all the data (including zeros) are presented in this appendix. (Results are essentially identical to those discussed in the text but show significance at a lower level.)

APPENDIX C

APPENDIX C-1

Analysis of variance of the results from Experiments 10, 11, 12, 14, and 15 using all the data (including zeros).

Source of Variation	df	Mean Square	F-value	Level of Significance
Weeks (adjusted for treatment)	4	255.18	5.76	0.01, $df_{4,27}$
Treatment (adjusted for weeks)	7	107.77	2.43	0.05, $df_{7.27}$
Weeks*Treatment (adjusted for both weeks and treatment)	27	44.30	1.14	NS
Error	512	39.02	-	
TOTAL	550	-	-	

APPENDIX C-2

Orthogonal analysis (seven contrasts) performed on the results of Experiments 10, 11, 12, 14, and 15 using all the data (including zeros).

Source of Variation	df	Mean Square	F-value	Level of Significance
Contrast 1	1	26.8898	0.61	NS
Contrast 2	1	347.2429	7.84	0.01, $df_{1,27}$
Contrast 3	1	13.8664	0.31	NS
Contrast 4	1	5.5103	0.12	NS
Contrast 5	1	36.4316	0.82	NS
Contrast 6	1	216.8264	4.89	0.05, $df_{1,27}$
Contrast 7	1	117.8109	2.66	NS

APPENDIX D

APPENDIX D-1

Calculations for the non-orthogonal analysis performed on the results of Experiments 10, 11, 12, 14, and 15, shown in Table 23.

Contrasts	Category Weights
C1 DEC vs. Control 1	1 1 1 0 0 0 -3 0
C2 Lev vs. Control 1	0 0 0 1 1 1 -3 0
C3 DEC 10^{-3}% vs. Control 1	1 0 0 0 0 0 -1 0
C4 DEC 10^{-4}% vs. Control 1	0 1 0 0 0 0 -1 0
C5 DEC 10^{-5}% vs. Control 1	0 0 1 0 0 0 -1 0
C6 Lev 10^{-3}% vs. Control 1	0 0 0 1 0 0 -1 0
C7 Lev 10^{-4}% vs. Control 1	0 0 0 0 1 0 -1 0
C8 Lev 10^{-5}% vs. Control 1	0 0 0 0 0 1 -1 0

$$C1 = \Big(44.03 + 34.14 + 29.86 - 3(48.19)\Big)^2 \Big((1/3 + 1/3 + 1/12 + 9/15 + 1/1 + 1/18 + 1/9 + 9/30 + 1/6 + 1/6 + 1/11 + 9/14 + 1/5 + 1/7 + 1/10 + 9/17 + 1/19 + 1/12 + 1/13 + 9/28)28.96\Big)^{-1} = 8.55$$

$$C2 = \Big(32.17 + 42.57 + 52.59 - 3(48.19)\Big)^2 \Big((1/11 + 1/6 + 1/6 + 9/15 + 1/4 + 1/7 + 1/9 + 9/30 + 1/5 + 1/8 + 1/6 + 9/14 + 1/7 + 1/6 + 1/5 + 9/17 + 1/16 + 1/13 + 1/11 + 9/28)28.96\Big)^{-1} = 2.25$$

$$C3 = \Big(44.03 - 48.19\Big)^2 \Big((1/3 + 1/15 + 1/12 + 1/30 + 1/6 + 1/14 + 1/5 + 1/17 + 1/19 + 1/28)28.96\Big)^{-1} = 0.54$$

$$C4 = \Big(34.14 - 48.19\Big)^2 \Big((1/3 + 1/18 + 1/6 + 1/7 + 1/12 + 1/15 + 1/30 + 1/14 + 1/17 + 1/28)28.96\Big)^{-1} = 5.51$$

$$C5 = \Big(29.86 - 48.19\Big)^2 \Big((1/1 + 1/9 + 1/11 + 1/10 + 1/13 + 1/15 + 1/30 + 1/14 + 1/17 + 1/28)28.96\Big)^{-1} = 7.05$$

continued

APPENDIX D-1 (continued)

$$C6 = \left(32.17 - 48.19\right)^2 \left((1/11 + 1/4 + 1/5 + 1/7 + 1/16 + 1/15 + 1/30 + 1/14 + 1/17 + 1/28)28.96\right)^{-1} = 8.75$$

$$C7 = \left(42.57 - 48.19\right)^2 \left((1/6 + 1/7 + 1/8 + 1/6 + 1/13 + 1/15 + 1/30 + 1/14 + 1/17 + 1/28)28.96\right)^{-1} = 1/16$$

$$C8 = \left(52.59 - 48.19\right)^2 \left((1/6 + 1/9 + 1/6 + 1/5 + 1/11 + 1/15 + 1/30 + 1/14 + 1/17 + 1/28)28.96\right)^{-1} = 0.67$$

LIST OF REFERENCES

LIST OF REFERENCES

Alls, M. E. and J. H. Greve. 1974. Canine dirofilariasis in Iowa. J. Am. Vet. Med. Assn. 165: 532-33.

Bangham, D. R. 1955a. Metabolism and excretion of ^{14}C-labelled diethylcarbamazine. Br. J. Pharmacol. 10: 397-405.

Bangham, D. R. 1955b. The mode of action of diethylcarbamazine investigated with ^{14}C-labelled drug. Br. J. Pharmacol. 10: 406-12.

Behin, R. 1967. Artificial feeding apparatus for mosquitoes. Mosq. News 27: 87-90.

Bemrick, W. J., B. L. Buchli, and H. J. Griffiths. 1965. Development of Dirofilaria immitis in Anopheles quadrimaculatus after exposure of the microfilariae to a freezing temperature. J. Parasitol. 51: 954-57.

Benz, G. W. 1973. Anthelmintic activities of cambendasole in calves: effect on nematode morphogenesis from ova to the infective larvae stage. J. Parasitol. 59: 166-68.

Bishop, A. and B. M. Gilchrist. 1944. A method for collecting sporozoites of Plasmodium gallinaceum by feeding infected Aedes aegypti through animal membrane. Nature 153: 713-14.

Bradley, R. E. 1971. The etiology of pathologic changes in canine heartworm disease. In Pathology of Parasitic Diseases. S. M. Gaafar (ed.). Purdue University Studies, Lafayette, Indiana, 161-71.

Bradley, R. E. 1976. Levamisole resinate as a Dirofilaria immitis microfilaricide in dogs. J. Am. Vet. Med. Assn. 169: 311-15.

Breinl, A. 1920. Preliminary note on the development of the larvae of Dirofilaria immitis in dog fleas Ctenocephalus felis. Ann. Trop. Med. Parasitol. 14: 389-92.

Brug, S. L. 1932. Chitinization of parasites in mosquitoes. Bull. Ent. Res. 23: 229-31.

Bryan, J. H. and B. A. Southgate. 1976. Some observations on filariasis in Western Samoa after mass administration of diethylcarbamazine. Trans. Roy. Soc. Trop. Med. Hyg. 70: 39-48.

Burkhart, R. L. and B. T. Alford. 1972. Pharmacology and mode of action of diethylcarbamazine. In Canine Heartworm Disease: The Current Knowledge. R. E. Bradley (ed.). University of Florida, Gainesville, Florida, 17-30.

Burton, G. J. 1963. Wuchereria bancrofti and Dirofilaria immitis larvae in palpi and antennae of mosquito vectors. J. Parasitol. 49: 771-72.

Center for Disease Control. 1974. Morbidity and Mortality Weekly Report 23: 374-77.

Coffin, D. L. 1944. A case of Dirofilaria immitis infection in a captive-bred timber wolf (Canis occidentalis, Richardson). N. Am. Vet. 25: 611-12.

Colglasier, M. L., K. C. Kates, and F. D. Enzie. 1974. Cambendazole-resistant Haemonchus contortus strain in sheep; further experimental developments. J. Parasitol. 60: 289-92.

Desowitz, R. S., C. C. Draper, and T. M. Phillips. 1975. The nature of the adverse reaction to diethylcarbamazine treatment in dogs infected with Dirofilaria immitis. Trans. Roy. Soc. Trop. Med. Hyg. 69: 430.

Duke, B. O. L. 1974. The effect of levamisole on Onchocerca volvulus. Trans. Roy. Soc. Trop. Med. Hyg. 68: 71.

Duxbury, R. E., A. P. Moon, and E. H. Sadun. 1961. Susceptibility and resistance on Anopheles quadrimaculatus to Dirofilaria immitis. J. Parasitol. 47: 687-91.

Erickson, A. B. 1944. Helminths of Minnesota Canidae in relation to food habits, and a host list and key to the species reported from North America. Am. Midland. Naturalist 32: 358-72.

Euzeby, J. and B. Laine. 1951. Sur la periodicite des microfilaires de Dirofilaria immitis. Rev. Ved. vet. Toulouse 102: 231-38.

Feldman, R. G. and M. J. Holden. 1974. Meningeal irritation, hemoptysis, and eosinophila--a case of human dirofilariasis? J. Am. Med. Assn. 228: 1018-19.

Foil, L. and T. C. Orihel. 1975. Dirofilaria immitis (Leidy, 1856) in the beaver Castor canadensis. J. Parasitol. 61: 433.

Gerberg, E. J. 1971. Evaluation of antimalarial compounds in mosquito test systems. Trans. Roy. Soc. Trop. Med. Hyg. 65: 358-63.

Gerberg, E. J., F. W. Kutz, and G. S. Tulloch. 1972. Preliminary evaluation of l-tetramisole as an antifilarial in a mosquito test system. In Canine Heartworm Disease: The Current Knowledge. R. E. Bradley (ed.). University of Florida, Gainesville, Florida, 105-107.

Gibson, D. W., D. H. Connor, H. L. Brown, H. Fuglsang, J. Anderson, B. O. Duke, and A. A. Buck. 1076. Onchocercal dermatitis: ultrastructural studies of microfilariae and host tissues, before and after treatment with diethylcarbamazine (Hetrazan). Am. J. Trop. Med. Hyg. 25: 74-87.

Graybill, F. A. 1976. Theory and Applications of the Linear Model. Duxbury Press, North Scituate, Mass.

Green, B. M. 1975. Microfilariae in a patient with systemic lupus erythematosis: case report. In Proceedings of the Heartworm Symposium '74. H. C. Morgan (ed.). School of Veterinary Medicine, Auburn University, Auburn, Alabama, 14-15.

Greenberg, J. 1949. A method for artificially feeding mosquitoes. Mosq. News 9: 48-50.

Grob, D. 1976. Use of drugs in myopathies. Ann. Rev. Pharmacol. Toxicol. 16: 215-28.

Harned, B. K., R. W. Cunningham, S. Halliday, R. E. Vessey, N. N. Yuda, M. C. Clark, C. H. Hine, R. Cosgrove, Y. SubbaRow. 1948. Studies on the chemotherapy of filariasis IV. Some pharmacological properties of 1-diethylcarbamyl-4-methylpiperazine hydrochloride, Hetrazan. J. Lab. Clin. Med. 33: 216-38.

Hawking, F. 1956. The periodicity of microfilariae IV. Stimuli affecting the migration of the microfilariae of Dirofilaria aethiops, D. immitis, D. repens, Dipetalonema blanci, and Litomosoides carinii. Trans. Roy. Soc. Trop. Med. Parasitol. 4: 397-417.

Hawking, F. 1967. The 24-hour periodicity of microfilariae: biological mechanisms responsible for its production and control. Roy. Soc. (London) Proc., Ser. B 169: 59-76.

Hawking, F., P. Sewell, and J. P. Thurston. 1950. The mode of action of Hetrazan on filarial worms. Brit. J. Pharmacol. 5: 217-38.

Hewitt, R. I., E. White, W. S. Wallace, H. W. Stewart, S. Kushner, Y. SubbaRow. 1947. Experimental chemotherapy of filariasis II. Effect of piperazine derivatives against naturally acquired filarial infections in cotton rats and dogs. J. Lab. Clin. Med. 32: 1304-13.

Ho, B. C., M. Singh, and E. H. Yap. 1974. The fate and migratory patterns of the infective larvae of Brugia malayi, Dirofilaria immitis, and Breinli sergenti in Aedes togoi denied access to a host. J. Med. Ent. 11: 622-28.

Intermill, R. W. 1973. Development of Dirofilaria immitis in Aedes triseriatus Say. Mosq. News 33: 176-81

Iyengar, M. O. T. 1957. Developmental stages of filariae in mosquitoes. So. Pac. Comm. Tech. Pap. 164: 1-11.

Jackson, R. F. 1972. Some new chemotherapeutic agents for dirofilariasis--a preliminary report. In Canine Heartworm Disease: The Current Knowledge. R. E. Bradley (ed.). University of Florida, Gainesville, Florida, 129-136.

Jackson, W. F. 1969a. Physical examination of the heartworm-infected patient. J. Am. Vet. Med. Assn. 54: 377-78.

Jackson, W. F. 1969b. Radiographic examination of the heartworm-infected patient. J. Am. Vet. Med. Assn. 54: 380-82.

Jackson, W. F. 1969c. Preventative therapy with diethylcarbamazine. J. Am. Vet. Med. Assn. 154: 396.

James, M. T. and R. F. Harwood, eds. 1969. Herms's Medical Entomology. MacMillan Publishing Co., Inc., New York. 484 pp.

Johnson, W. P., R. G. Eggert, G. P. Poeschel, and G. T. Wang. 1972. Levamisole as an anthelmintic for swine. J. Am. Vet. Med. Assn. 161: 1221-25.

Jones, J. C. 1967. Methods for dissection of mosquitoes. Mosq. News 27: 76-82.

Joon-Wah, M., V. Zaman, and S. Sivanandam. 1974. Antifilarial activity of levamisole hydrochloride against subperiodic Brugia malayi infection of domestic cats. Am. J. Trop. Med. Hyg. 23: 369-74.

Kartman, L. 1953a. An observation on the loss of microfilariae from the mosquito during its infective meal. J. Parasitol. 39: 571-72.

Kartman, L. 1953b. Factors influencing infection of the mosquito with Dirofilaria immitis (Leidy, 1856). Expl. Parasitol. 2: 27-78.

Kartman, L. 1956. Notes on the encapsulation of Dirofilaria immitis in the mosquito Aedes aegypti. Am. J. Vet. Res. 17: 810-12.

Keegan, H. L., C. M. Fitzgerald, T. L. McCrary, and M. D. Doyles. 1968. Studies on dog heartworm in South Texas. Tex. Rpts. Biol. Med. 26: 321-30.

Kocan, A. A. and H. E. Laubach. 1976. Dirofilaria immitis and Dipetalonema reconditum infections in Oklahoma dogs. J. Am. Vet. Med. Assn. 168: 419-20.

Kume, S. and S. Itagaki. 1955. On the life-cycle of Dirofilaria immitis in the dog as a final host. Br. Vet. J. 111: 16-24.

Kutz, F. and R. C. Dobson. 1974. Effects of temperature on the development of Dirofilaria immitis (Leidy) in Anopheles quadrimaculatus Say and on vector mortality resulting from this development. Ann. Ent. Soc. Am. 67: 325-31.

Lindsey, J. R. 1961. Diagnosis of filarial infections in dogs I. Microfilarial surveys. J. Parasitol. 47: 695-702.

McGreevy, P. B., J. H. Theis, M. M. J. Lavoipierre, and J. Clark. 1974. Studies on filariasis III. Dirofilaria immitis: emergence of infective larvae from the mouthparts of Aedes aegypti. J. Helmin. 48: 221-28.

Medway, W. and T. C. Wieland. 1975. Dirofilaria immitis infection in a harbor seal. J. Am. Vet. Med. Assn. 167: 549-50.

Merck and Company, Inc. 1967. The Merck Veterinary Index. Merck and Company, Rakway, New Jersey, 731-35.

Mills, J. N. and T. C. Amis. 1975. Levamisole as a microfilaricidal agent in the control of canine dirofilariasis. Austral. Vet. J. 51: 310-14.

Natarajan, P. N., V. Zaman, and T. S. Yeoh. 1974. In vitro activity of levamisole on the infective larvae, microfilariae, and adult worms of Breinlia sergenti. Internatl. J. Parasitol. 4: 207-10.

Navarrete, A. R. 1972. Pulmonary dirofilariasis. Chest 61: 51-55.

Newton, W. L. and W. H. Wright. 1956. The occurrence of a dog filariid other than Dirofilaria immitis in the United States. J. Parasitol. 42: 246-58.

O'Holohan, D. R. and V. Zaman. 1974. Treatment of Brugia malayi infection with levamisole. J. Trop. Med. Hyg. 77: 113-15.

Orihel, T. C. 1961. Morphology of the larval stages of Dirofilaria immitis in the dog. J. Parasitol. 47: 251-62.

Otto, G. F. 1969a. Geographical distribution, vectors, and life cycle of Dirofilaria immitis. J. Am. Vet. Med. Assn. 154: 370-73.

Otto, G. F. 1969b. Chemotherapeutic agents. J. Am. Vet. Med. Assn. 154: 387-90.

Otto, G. F. 1972. Epizootiology of canine heartworm disease. In Canine Heartworm Disease: The Current Knowledge. R. E. Bradley (ed.). University of Florida, Gainesville, Florida, 1-15.

PharmIndex. 1973. Skyline Publishers, Inc., Portland, Oregon, 130.

Ponnudurai, T., D. A. Denham, and G. S. Nelson. 1971. The use of a membrane feeding technique for infecting mosquitoes with filarial worms transported between laboratories. J. Helmin. 45: 415-18.

Robinson, M. J., M. Viamonte, and M. Viamonte. 1974. Parasites: diagnostic payoff in coin lesions. Modern Medicine 42: 75.

Rogers, R. and D. A. Denham. 1976. Studies with Brugia pahangi 12. The activity of levamisole. J. Helmin. 50: 21-28.

Rothstein, N., K. E. Kinnamon, M. L. Brown, and R. W. Carithers. 1961. Canine microfilariasis in the eastern United States. J. Parasitol. 47: 661-65

Rutledge, L. C., R. A. Ward, and D. J. Gould. 1964. Studies on the feeding response of mosquitoes to nutritive solutions in a new membrane feeder. Mosq. News 24: 407-19.

Schnelle, G. B. and R. M. Young. 1944. Clinical studies on microfilarial periodicity in war dogs. Bull. U. S. Army Med. Dept. 80: 52.

Schuermans, V. 1975. Levamisole in rheumatoid arthritis. Lancet 1: 111.

Sharp, J. J. 1974. Heartworms in a cat. J. Am. Vet. Med. Assn. 164: 519.

Snedecor, G. W. 1956. Statistical Methods Applied to Experiments in Agriculture and Biology. Iowa State College, Ames, Iowa. 534 pp.

Soulsby, E. J. L. 1965. Textbook of Veterinary Clinical Parasitology. F. A. Davis Co., Philadelphia, Pa., 100-113.

Tarshis, I. B. 1959. Feeding Aedes aegypti (L.) through animal derived membranes (Diptera: Culicidae). Ann. Ent. Soc. Am. 52: 681-87.

Taylor, A. E. R. 1960a. Studies on the microfilariae of Loa loa, Wuchereria bancrofti, Brugia malayi, Dirofilaria immitis, D. repens, and D. aethiops. J. Helmin. 34: 13-26.

Taylor, A. E. R. 1960b. The development of Dirofilaria immitis in Aedes aegypti. J. Helmin. 34: 27-38.

Terzian, L. A. 1947. A method for screening antimalarial compounds in the mosquito host. Science 106: 449-50.

Theodorides, V. J. 1974. Development of resistance by parasites to anthelmintics. Internatl. Congress Parasitol. Proc. 3: 1390-91.

Thienpoint, D., O. F. J. Vanparijs, A. H. M. Raeymaekers, J. Vandenberk, P. J. A. Demoen, F. T. N. Allewijn, R. P. H. Marsboom, C. J. E. Niemegeers, K. H. L. Schellekens, and P. A. J. Janssen. 1966. Tetramisole (R8299), a new, potent, broad-spectrum anthelmintic. Nature 209: 1084-86.

Travis, B. V. 1947. Relative efficiency of six species of mosquitoes from Guam, M. I., as developmental hosts for Dirofilaria immitis. J. Parasitol. 33: 142-45.

Tripodi, D., L. C. Parks, and J. Brugmans. 1973. Drug induced restoration of cutaneous delayed hypersensitivity in anergic patients with cancer. N. Eng. J. Med. 289: 354.

Tritch, V. R., H. A. James, and M. P. Ratner. 1973. Canine filariasis (Dirofilaria immitis and Dipetalonema reconditum): survey in southwestern Connecticut. Am. J. Vet. Res. 34: 1473-75.

Tulloch, G. S. and R. A. Anderson. 1971. Acceptability of daily l-tetramisole by pound dogs infected with Dirofilaria immitis. Nature 232: 644.

Tulloch, G. S. and R. A. Anderson. 1972. Tetramisole and canine dirofilariasis. In Canine Heartworm Disease: The Current Knowledge. R. E. Bradley (ed.). University of Florida, Gainesville, Florida, 101-104.

Tulloch, G. S., G. Pacheco, H. W. Casey, and R. A. Anderson. 1970. Efficacy of selected drugs as prophylactic agents against Dirofilaria immitis of dogs. Internatl. Congress Parasitol. Proc. 2: 348.

University of Notre Dame, Vector Biology Laboratory. 1974. Mosquito stock list (mimeographed).

Van den Bossche, H. and P. A. J. Janssen. 1967. Mode of action of levamisole on Ascaris. Life Science 6: 1781-82.

Wade, J. O. 1975. An improved method for the membrane feeding of mosquitoes. Trans. Roy. Soc. Trop. Med. Hyg. 69: 19.

Wallenstein, W. L. and B. J. Tibola. 1960. Survey of canine dirofilariasis in a Maryland area: incidence of Dirofilaria immitis and Dipetalonema. J. Am. Vet. Med. Assn. 137: 712-16.

Weiner, D. J. and R. E. Bradley. 1970. Ability of some mosquitoes to transmit Dirofilaria immitis in Florida. Mosq. News 30: 406-10.

Wills, W., D. F. Carroll, and G. E. Jones. 1974. A simple method for artificially feeding mosquitoes. Mosq. News 34: 119-120.

Woke, P. A. 1937. Comparative effects of the blood of different species of vertebrates on egg production of Aedes aegypti Linn. Am. J. Trop. Med. 17: 729-45.

Yoeli, M. 1938. Note on the experimental infection of *Anopheles elutus* with *Plasmodium faliciparum* by feeding through a prepared animal membrane. Riv. Malar. 17: 62-66.

Zaman, V. and M. Lal. 1973. Treatment of *Wuchereria bancrofti* with levamisole. Trans. Roy. Soc. Trop. Med. Hyg. 67: 610.

Zaman, V. and P. N. Natarajan. 1973. The antifilarial activity of levamisole on *Breinlia sergenti* compared with diethylcarbamazine. J. Trop. Med. Hyg. 76: 126-28.