ODETOYINBO, Joshua Adeniran. BIOLOGY OF SPLENDIDOFILARIA QUISCALI (VON LINSTOW, 1904) N. COMB. (NEMATODA: ONCHOCERCIDAE).

Iowa State University of Science, and Technology
Ph.D., 1960
Zoology
University Microfilms, Inc., Ann Arbor, Michigan

BIOLOGY OF *SPLENDIDOFILARIA QUISCALI* (VON LINSTOW, 1904) N. COMB. (NEMATODA: ONCHOCERCIDAE)

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Parasitology

Approved:

Signature was redacted for privacy.

In Charge of Major Work

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Ames, Iowa

1960

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INTRODUCTION

Nematodes belonging to the superfamily Filarioidea are known to parasitize every type of vertebrate except fishes. They infect animals in every clime, and have been recovered from nearly all organs of their hosts. A few of them are oviparous, but many of them produce immature forms (microfilariae) viviparously.

Although reports of microfilariae in blood of birds are numerous, they have never been reported heretofore from the blood of the bronzed grackle (Quiscalus quiscula versicolor Vieillot, 1819).

During 1957 to 1959, examination of the blood of bronzed grackles trapped mainly on the campus of Iowa State University and adjacent areas in Ames, Iowa, revealed that these birds are hosts for microfilariae and adult onchocercid worms of at least two species. The first type Splendidofilaria quiscali (von Linstow, 1904) n. comb. is remarkably localized in the cerebral hemispheres of its host, and produces sheathed microfilariae which are blunt anteriorly and posteriorly. Adults of the second type, producing microfilariae that are pointed posteriorly, were not recovered during the present investigation. Their microfilariae (as yet unnamed), herein referred to as Microfilaria "X", have a striated cuticle enclosed in a sheath slightly longer than the actual microfilaria.

The incidence of filarial infections in bronzed grackles is surprisingly high. Of the 112 grackles examined between 1957 and 1959, only 42 birds (38%) showed no microfilariae in their blood. Forty-five (40%) were infected with only Splendidofilaria quiscali, nineteen (17%) had both Microfilaria quiscali and the unnamed microfilaria referred to above

as Microfilaria "X", and a total of six birds (5%) had only Microfilaria "X". It is interesting to note that ten of the 42 uninfected birds were nestlings.

Until recently, intensive studies of microfilarial periodicity have been hampered by the fact that there are few suitable experimental definitive hosts which may be used in the laboratory to harbour adult filarial worms whose microfilariae are characterized by well-marked periodicity such as that exhibited by Wuchereria bancrofti. Dirofilaria repens of dogs for example, shows only incomplete periodicity, while Litomosoides carinii of cotton rats, used extensively in laboratory studies since 1944, shows no periodicity at all. Hawking's (1956) discovery of Dirofilaria aethiops of monkeys provided the first worms whose juveniles demonstrated well-marked nocturnal periodicity which could be studied in experimental animals.

Splendidofilaria quiscali, too, exhibits a very marked nocturnal periodicity, and this species holds promise of many possibilities for laboratory studies on avian filariasis. Grackles, the definitive hosts of Splendidofilaria quiscali, are very abundant in nature. They are easy to trap and to maintain in the laboratory, and have remarkable ability to endure the rigours of many experiment conditions.

The purposes of this investigation were as follows: to redescribe the adults and microfilariae of this species completely, to determine its incidence in nature, to study the periodicity of its microfilariae, and to elucidate features of its life-cycle.

In conjunction with studies on the microfilariae, many successful

transfusions of microfilaremic blood of grackles into other grackles, laboratory-reared chickens, and into European corn borer larvae were performed, and an intensive study of microfilarial periodicity was conducted. Experiments were performed on the normal periodicity and on reversal of periodicity. Furthermore, exploratory studies were made on the effects of continuous light and continuous darkness on microfilarial periodicity, and experimental studies on the longevity of microfilariae were also undertaken.

HISTORICAL REVIEW

A chronological historical review of the superfamily Filarioidea at the present time is somewhat difficult. Many nematodes long regarded as filarial worms are constantly being removed from the group. Even within the last two years, the subfamily Cardionematinae was transferred to the order Strongylata by Anderson (1958). Moreover, descriptions of worms by early investigators lack diagnostic details, and until recently, specimens were rarely preserved. Among the earliest records of filarial worms are those dealing with Loa loa. According to Chatterjee (1952), Pigafetta gave an account of Loa loa in a book "Travels on the Congo" as early as 1589.

The lack of a universally accepted scheme of classification has hindered systematic study on filarial worms. The classification of Yorke and Mapleston (1926), based on external cuticular characteristics, is no longer accepted. Those of Skrjabin and Schikhobalova (1936) and Lopez-Neyra (1947) have been revised by Skrjabin and Schikhobalova (1948). However, authorities regret that this 1948 work, published in Russian is difficult to obtain, even in Europe. Furthermore, as pointed out by Chabaud and Choquet (1953) the genera were discussed in alphabetical order, and "les tableaux ..... sont basés sur des caractères qui semblent trop artificiels" (p. 191).

The classification proposed by Wehr (1935), although incomplete, has been acclaimed by many nematologists, and is used as a basis for all modern dichotomous keys. It is based on morphological elements such as the pattern of cephalic papillae, the labial structures, and the characters

of the first-stage juveniles--characters which have phylogenetic validity. Unfortunately, Wehr's work includes but a few keys, and many genera have been omitted. Three modern dichotomous keys based on Wehr's work have been presented by Chabaud and Choquet (1953), Anderson (1958), and Chabaud and Anderson (1959). These 1959 authors recognized five families, 16 subfamilies and 76 genera of filarial worms. They regarded 45 other genera as synonyms for some of the recognized ones. Furthermore, they suggested that the length of the tail should be regarded as an important element in classification, since the length is, in general, the same in both males and females of the same species.

Wehr (1935) created the family Dipetalonematidae which he divided into two subfamilies. Chabaud and Choquet (1953) retained the family name Dipetalonematidae but divided it into six subfamilies among which was Splendidofilariinae, Chabaud and Choquet 1953.

Chabaud and Anderson (1959) suggested that the family name Dipetalonematidae Wehr 1935, should be replaced by Onchocercidae Leiper 1911, in compliance with paragraphs 45 and 46 of the 1953 Copenhagen Decisions on Zoological Nomenclature regarding adherence to the "Law of Priority." These authors recognize the subfamily Splendidofilariinae.

Anderson and Chabaud (1959) published the latest key to this subfamily. Further details on the taxonomic characteristics of the family, subfamily, and genera are found in a later section of this study. One of the genera included in this subfamily is Splendidofilaria Skrjabin 1923 to which the species Splendidofilaria quiscali belongs.

Brief preliminary reports dealing with microfilariae, adults, and microfilarial periodicity of Splendidofilaria quiscali were published by Odetoyinbo and Ullmer (1959, 1960).

MATERIALS AND METHODS

Source of Experimental Birds

All birds used in these experiments, namely 112 grackles (Quiscalus quiscula), 16 blue jays (Cyanocitta cristata), 26 sparrows (Passer domesticus), 4 robins (Turdus migratorius), 3 woodpeckers (Centurus carolinus zebra), and 2 cardinals (Richmondena cardinalis cardinalis) were trapped in the vicinity of Ames, Iowa. Approximately 70% were captured on the campus of Iowa State University.

Three types of traps were employed for this purpose. The first, a funnel-type described fully by Moorman (1956), proved most efficient for capturing the 26 sparrows examined during the investigation. The second type was a rectangular cage, three feet by two feet and two feet high, made of a two by one-inch welded wire-netting. It was divided into two compartments, each with a trap-door. This type proved very effective for ensuring against the escape of trapped birds, but allowed the capture of only a single bird each time. The third type, of simplest construction, was a cage three feet by three feet by two feet high, made of the same material as the second type. It was divided into two compartments, each with an entrance six inches by four inches cut from the sides and flush with the ground. From the bottom of the cage at the site of the entrance, a piece of wire netting six inches by four inches was removed. In order to prevent trapped birds from escaping, a tunnel-like device with converging finger-like projections was constructed from hardware cloth and placed at each entrance. This type of cage was used most frequently because it proved very efficient for capturing grackles, blue jays,

robins, cardinals, and woodpeckers. Furthermore, it allowed trapping many birds at a time. Sometimes as many as eight birds were caught in a single trap of this type. Traps were baited with cracked corn mixed with sorghum and oats. Chickens used in the transfusion experiments were obtained when only one or two weeks old, from the Iowa State University Poultry Farms. They were maintained in the laboratory until required for experimental purposes. The leghorn roosters used for rearing mites were also supplied by the University Poultry Farms.

Rearing of Birds in the Laboratory

Trapped grackles and other birds were removed to the laboratory and kept in small animal cages, at most two in a cage. In the laboratory, birds thrive very well on commercial pigeon feed, although any well-balanced chicken feed may be substituted. Birds were supplied regularly with ample water and food since they sometimes tended toward cannibalism. In many instances, when two were kept in one cage, the stronger killed and devoured the weaker, leaving only the feathers, beaks, wings, and legs. With proper care, however, it was possible to maintain the birds in captivity for more than two years.

Owing to the vicious nature of the grackles, especially when first captured, a pair of work gloves were worn when handling them.

Methods of Blood Examination

There has been much controversy among parasitologists on methods of blood examination for microfilarial periodicity, especially with regard to the site of blood withdrawal, the quantity required for each examination, and the actual methods of withdrawal and examination.

Yorke and Blacklock (1917) in their studies on Wuchereria bancrofti showed that microfilarial periodicity is essentially the same in blood taken from the basilic vein of the arm as in blood removed from cutaneous vessels. These workers, however, contended that at the peak period, there were twice as many microfilariae in cutaneous vessels as in basilic veins. They therefore concluded that periodicity is more marked in the skin because microfilariae are "held up in their passage through cutaneous vessels."

Fülleborn (1929) and many other investigators have reported that, in the case of Wuchereria bancrofti and Loa loa blood withdrawn from the veins contained almost as many microfilariae as did blood obtained by skin-puncture, especially at the peak of microfilarial content in the peripheral blood.

Augustine, Field and Drinker (1936) confirmed the findings of Yorke and Blacklock and suggested that microfilariae may cause temporary blockage of those vessels too narrow to be traversed. They injected microfilariae of Dirofilaria immitis intravenously into brown bats and observed the microfilariae afterwards in the capillaries, veins, and arteries of the wings. They maintained that microfilariae formed temporary plugs whenever they reached vessels with narrow lumens, and that such microfilariae turned round and travelled against the current of blood-flow.

Lane (1948) believed that when skin-pricked blood is used, periodicity may be detected with accuracy. He contended, however, that cutaneous blood is not a quantitative measure of microfilarial content of the freely-circulating blood because it magnifies the total number of microfilariae.

Hawking and Thurston (1951) believed that the conclusion of Yorke and Blacklock (1917) were probably due to statistical error. They observed that in the case of monkeys, the curve for microfilariae in venous blood is closely similar to that in cutaneous blood, and discovered that the nocturnal microfilarial count was as high in the arteries as in the capillaries and arterioles of the tail. They also agreed with the description given by Augustine and his colleagues (1936) concerning the movements of microfilarie of Dirofilaria immitis introduced into the circulation of bats, and of Vagrifilaria columbigallinae introduced into the circulation of frogs (Augustine, 1937). Hawking and Thurston observed the behaviour of living microfilariae in the mesenteries and intestines of cotton-rats infected with Litomosoides, and in the mesenteries and omenta of dogs infected with Dirofilaria repens and discovered that microfilariae pass through the capillaries rapidly and smoothly. Microfilariae were never observed to go against the blood current, neither were they observed to accumulate in the small vessels except as a terminal event before death of the host. They believed that the observation of Augustine and his colleagues (1936) might have been due to their use of unnatural hosts, or to the fact that the experimental animals were probably almost dead at the time of observation.

Investigators working with humans and large mammals generally withdraw blood from the basilic vein of the pectoral appendage, from the ear, or sometimes from the tail and fingers. Avian hosts, however, have presented investigators with many problems. Wehr and Herman (1956) made four-hourly heart punctures of California quails but reported that experimental birds died from loss of blood. Boughton et al. (1938) were

able to withdraw 0.2 cc of blood from the common crow only four times a day, while O'Connor and Beatty (1938) obtained blood from brachial veins of ground doves.

There is general agreement today that the site from which blood is withdrawn is immaterial because periodicity can be detected in blood obtained from any part of the host's body. In the present investigation, known volumes of blood were always withdrawn from the metatarsal veins of infected birds.

With regard to the actual volume of blood withdrawn from infected animals at each examination, it was the common practice among early investigators working with large mammals, to examine large volumes of blood. However, since Galliard and Ngu (1947) demonstrated that microfilarial count in 10 cm^3 of blood was no more accurate than in 5 cm^3 or 1 cm^3, many investigators have reduced considerably the volume of blood withdrawn. Moreover, the size of many birds and some mammals does not permit easy withdrawal of large volumes from veins at short intervals over a period of two to four days. Thus, Knott (1935) withdrew 10 cc of human blood, Schnelle and Young (1944) 2 cc of canine blood, Boughton et al. (1938) 0.2 cc of avian blood, and Hanson et al. (1956) working with geese relied entirely on single unmeasured drops obtained from venipuncture. However, it is generally accepted that examination of a large volume of blood gives a more accurate quantitative estimation of total microfilarial content than does examination of drops obtained from skin-pricked blood.

Regarding the actual method of blood examination, various investigators have suggested a great variety of methods, all of which, in

essence are devices for diluting withdrawn blood, haemolysing it with acetic acid or formaldehyde, and concentrating the microfilariae by centrifugation or filtering (Gordon and Webber 1955). All these methods facilitate easy examination of live microfilariae in large quantities of blood, but are impractical for periodicity studies especially in small animals and birds. Moreover, the many procedural steps involve increased possibility of mathematical error in the estimation of microfilarial content of the blood.

For periodicity studies, the method employed by McCarthy (1956) is the one used by many modern investigators. A 20 c.mm. (0.02 ml) sample of blood is withdrawn from the host, smeared on a slide over an area about one inch by half an inch, and air dried. It is 'dehaemoglobinized' in 0.5% acetic acid, dried again, fixed in methanol, and stained with Giemsa.

The method employed for blood examination in these experiments consisted essentially in the withdrawal of 0.0125 ml of blood from the metatarsal veins of infected grackles every two hours throughout the two or three days of examination.

The materials employed consisted of a 2 or 4% sodium citrate solution in saline (or a blood-diluting fluid made up of 2 grams sodium citrate, 0.75 grams sodium chloride, 0.20 grams glucose and 100 ml distilled water), a half spear point needle (or a sharply pointed scalpel), a graduated blood diluting pipette, piece of gauze and 70% alcohol.

Blood films were obtained and preserved in the following manner:

A small incision of the metatarsal vein was made with a half spear point needle or a fine scalpel, and with the aid of a graduated

blood-diluting pipette (thoroughly rinsed dry with the citrate solution), approximately 0.0125 ml blood was transferred to a clean slide. A film of this was then made, air-dried, fixed in absolute methyl alcohol for at least three minutes and stained with Giemsa. The toe of the birds and the scalpel were always wiped dry with a piece of gauze soaked in 70% alcohol. All the microfilariae on each slide were carefully counted using the low power of a compound microscope. The size of the microfilariae made the use of higher magnifications unnecessary except for detailed morphological studies.

This method was found to be efficient for detecting microfilarial periodicity. With minimum practice, the whole operation takes less than a minute. The method allows blood examinations of birds at frequent intervals without causing much discomfort to them, and the small volume of blood used eliminates the necessity for haemolysing or centrifuging the blood. It also eliminates the mathematical errors often associated with blood dilution and the concentrating of microfilariae through centrifugation.

Histological Techniques

Blood films examined in this study were fixed in absolute methyl alcohol for at least three minutes, dried, and stained in Giemsa. A dilution of one part stain to 50 parts of distilled water was used, and films were stained for 20 to 25 minutes. This stain was found particularly satisfactory especially when thin blood films were prepared.

Adult worms were fixed either in hot 70% ethyl alcohol or more frequently in glycerine alcohol (10 parts glycerine in 90 parts 70% ethyl

alcohol). They were preserved in glycerine alcohol until required for mounting. The fixed and preserved worms were transferred into a petri-dish with glycerine alcohol and covered with a filter paper; the alcohol was allowed to evaporate very slowly leaving the worms in pure glycerine. Permanent mounts of adults were made either in glycerine-jelly, or in pure glycerine. A synthetic resin was used for ringing the coverslip. Such preparations have remained in very satisfactory condition for more than two years.

For temporary preparations demonstrating internal organs of adult worms, especially in specimens where the vulva and the anus are hidden by the digestive system or the ovaries, the addition of a drop of very dilute Giemsa stain proved helpful. When added to a preserved specimen on a slide, this dye differentiates very clearly all the various organs including microfilariae and eggs. The reproductive system takes on the stain more rapidly than does the digestive tract.

The heart, lungs, liver, and spleen of infected birds were fixed in AFA, embedded in paraffin, sectioned, stained in Delafield's haematoxylin and counterstained with either eosin, erythrosin, or fast green. Some sections were stained with Mallory's triple connective-tissue stain or Heidenhain's azan triple stain in order to demonstrate any signs of fibrosis due to the presence of worms in the tissues. A double-infiltration method employing celloidin and paraffin was employed in some instances especially in the case of lungs and brain.

Brains of birds with the worm <u>in situ</u> were fixed in 5% formalin for 24 hours, embedded in paraffin, sectioned and stained. Some were also doubly infiltrated.

To observe various stages of microfilarial development, gravid females were dissected, and smears of representative portions of the body at various levels were prepared. Such smears, stained in Giemsa, were useful in following development of juvenile worms from the one-celled stage to the fully developed microfilariae.

All drawings were made with the aid of the camera lucida and micro-projector.

THE GENUS SPLENDIDOFILARIA

According to Chabaud and Anderson (1959), members of the family Onchocercidae Chabaud and Anderson 1959 (Dipetalonematidae Wehr 1935), parasites of amphibians, reptiles, birds, and mammals, are characterized by the possession of eggs with very thin shells. The microfilariae have no spines and they accumulate in the tissues of the host. The anterior end of the adult worms is smooth and rounded, and the esophagus is sometimes divided into anterior muscular and posterior glandular portions. The vulva is anterior and the life cycle when known, involves haematophagous insects.

The subfamily Splendidofilariinae, as suggested by Anderson and Chabaud (1959) and by Chabaud and Anderson (1959), includes avian filarial worms, the females of which possess an anal opening situated neither terminally nor subterminally, but some distance from the posterior end of the body. Other characteristics of the subfamily include four pairs of oral papillae surrounding the mouth, an esophagus which may be variable in shape, and the lack of caudal alae. Reptiles, birds, and mammals serve as definitive hosts for nematodes of this subfamily.

Anderson and Chabaud (1959) published the most recent key to the genera of Splendidofilariinae. Six genera were recognized by these authors, namely: Lerouxinema Singh 1949, Splendidofilaria Skrjabin 1923, Paraprocta Maplestone 1931, Aproctella Cram 1931, Ornithofilaria Gonnert 1937 and Pseudaproctella Anderson 1956.

Members of the genus Splendidofilaria Skrjabin 1923 are distinguished from those of the other genera by the absence of anterior lateral alae

and by the possession of a long[1] tail (portion between the anus and the posterior extremity). Former genera now regarded as synonymous with Splendidofilaria include Chandlerella Yorke and Maplestone 1926, Vagrifilaria Augustine 1937, Ularofilaria Lubimov 1946, Skrjabinocta Tschertkowa 1946, and Parachandlerella Caballero 1948. The type species is Splendidofilaria pawloskyi Skrjabin 1923. Thirteen species, all avian nematodes, have been described heretofore. These include: S. pawlowskyi Skrjabin 1923, S. Bosei (Chandler 1924), S. gedoelsti Travassos 1925, S. sinensis (Li 1933), S. columbigallinae (Augustine 1937), S. papillocerca (Lubimov, 1946) Anderson and Chabaud 1959, S. petrovi (Tschertkowa 1946) Anderson and Chabaud 1959, S. periarterialis (Caballero 1948), S. verrucosa Oschmarin 1959, S. shaldybini Goubanov 1954, S. singhi (Ali 1956), S. braziliensis (Yeh 1957) and S. gretillati Chabaud, Anderson, and Brygoo 1959. The original descriptions of all these species have been carefully studied and compared with the species Splendidofilaria quiscali under consideration.

Splendidofilaria quiscali differs from all the above-named species in several respects. S. periarterialis, S. braziliensis, S. columbigallinae, S. sinensis, and S. singhi all have the esophagus definitely divided into an anterior muscular portion and a posterior glandular portion. In Splendidofilaria quiscali the esophagus is undivided. S. verrucosa and S. pawlowskyi all have ornate or striated cuticle, while the cuticle of Splendidofilaria quiscali is smooth and unstriated.

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[1]The tail is considered long when it is greater than twice the diameter of the body at the region of the anus.

The unvaried location of S. quiscali in the brain of grackles and its complete absence from any other region of the host set it apart from all previously-described species of the genus. More than 500 specimens of S. quiscali were recovered during the course of this investigation and all were taken from the cranial region. Other species of this genus, however, have been reported from the heart, lungs, air sacs, between the proventriculus and gizzard, subcutaneous tissue of the breast bone, connective tissues of pulmonary arteries, and body cavities of their hosts.

S. petrowi (which was recovered by Tschertkowa in 1946 from the eye Streptopelia orientalis meena), is a stout worm considerably less than half the length of Splendidofilaria quiscali. Its spicules are considerably larger and are unequal. According to Tschertkowa, the worms are remarkably localized in the hosts, the number recovered from the 14 infected birds he examined varied from one to eleven.

The only species approaching S. quiscali in length is S. braziliensis which inhabits the air sacs. This species however has a striated cuticle and its esophagus is divided into an anterior muscular portion and a posterior glandular portion. Moreover, it is at least twice as stout as Splendidofilaria quiscali.

Splendidofilaria quiscali is very probably the same species which Shipley (1905) found in the cerebral hemispheres of Quiscalus quiscula versicolor in 1904. von Linstow (1904), to whom Shipley sent the worms for identification, recognized that they were filarial worms, but remarked that he was unable to name them accurately because they were fragments of females without anterior ends. However, von Linstow named

the species "Filaria Quiscali." The only description of the worms, according to Shipley (1905, page 253) reads as follows:

> "The tail is rounded. The breadth 0.21 mm. The body is uncommonly soft. The cuticle is smooth and not rigid. The eggs are 0.029 mm. long and 0.021 mm. broad."

The present species is designated Splendidofilaria quiscali (von Linstow, 1904) n. comb.

SPLENDIDOFILARIA QUISCALI

Location in Host

Reports on the occurrence of nematodes in the brain of avian hosts are quite uncommon. A thorough search of the literature indicates that filarial worms have been recovered from the cranial regions of only three species of birds.

Molin (1858) recovered worms from the surface of the cerebellum of the snakebird, Anhinga anhinga. Wyman (1868), Leidy (1882, 1886) and Walton (1927) reported recovery of filarial worms from the same species of birds but gave the worms different names. Wehr and Hwang (1957) studied these nominal species and concluded that they all belong to the same genus and species, namely Wymania helicina (Molin, 1858). A new subfamily Anhingofilariinae was created by Wehr and Hwang (1957) to include them. Anderson (1958), however, believed that Wymania helicina belongs to the subfamily Onchocercinae.

Kazubski (1958), while working at Gorki University, U.S.S.R., recovered from the "cranium" of one of three nightjars, (Caprimulgus europaeus L.), four filarial worms (one male and three females) which he classed as a new genus and species Cerebrofilaria caprimulgi. Chabaud and Anderson (1959) believed that these worms belong to the genus Aprocta.

Although Shipley (1905) reported the first filarial worms from the brain of the bronzed grackles, Quiscalus quiscula versicolor, it is surprising that no one has confirmed this report. Shipley noted that the infected bird fell down from its perch "in a fit." Neither Shipley (1905) nor von Linstow (1904) described the worms fully, nor did they

treat them adequately from a taxonomic standpoint because the specimens recovered consisted only of fragments of female worms.

During the present investigation, it was discovered that adults of Splendidofilaria quiscali are remarkably localized in the avian host. They invariably inhabit the cerebral hemispheres. Adult worms vary in number (from one to 61 in the 64 infected grackles examined). Heavily-infected birds were kept alive in the laboratory for over two years. In birds with light infections, adult worms were recovered only from the lateral ventricles of the brain. This was accomplished by separating the anterior portions of the cerebral hemispheres along the sagittal fissure and carefully removing brain tissue covering the lateral ventricles. However, in heavily-infected birds, worms were easily discovered by examining the posterior border of the cerebral hemispheres, where the parasites frequently lie immediately beneath the pia mater. (Figs. 1 and 2)

The exact route of migration of adult worms to the brain has not been determined, but the restriction of worms to the lateral ventricles, and their complete absence from other regions of the ventricular system suggest that it is very unlikely that they reach their final location by way of the spinal cord.

Examination of adult female worms in situ never showed that they lie in close proximity to any blood vessels, neither did the hundreds of cross, frontal, and sagittal serial sections of brains of infected birds show blood vessels in close proximity to any worm. Furthermore, microfilariae were remarkably absent from sections of cranial blood vessels, and from the brain tissues. Adult worms were found in greater concentration in the main portion of the lateral ventricles, while the greatest concentra-

tion of microfilariae was found in the ventral horns of the lateral ventricles far removed from adult worms. It is therefore surmized that microfilariae are released into the lateral ventricles and pass out into the subarachnoid space. From here they can gain easy access to the lymphatic system of the host without passing through a vascular membrane. Once the microfilariae have reached the lymphatic circulation they are either carried directly into the thoracic duct (from whence they eventually reach the anterior vena cava), or they may gain access to the blood circulation via the right lymph duct which empties itself at the juncture of the right subclavian vein and the right internal jugular vein.

The ratio of males to females in the brains of infected grackles examined throughout this investigation reveals that, in general, females predominate. In five of the birds examined, only female worms were recovered. Microfilariae were absent from the worms as well as from the blood of the hosts. Thus the absence of microfilariae in the blood of grackles is not always a sure indication that birds are free from adult worms. The following are the ratios of males to females in 25 of the infected birds examined: (0:4), (0:3), (0:2), (0:1), (0:1), (1:1), (1:1), (1:1), (2:1), (2:1), (2:2), (1:2), (2:3), (2:4), (3:5), (3:6), (4:6), (4:6), (4:7), (4:8), (7:8), (10:13), (16:16), (23:26), and (26:25). In the case of birds harbouring only one female and one male worm, the infection could be easily overlooked since the number of microfilariae in the blood circulation of the host may be too few to detect in only one or two blood films. However, a series of blood films made between 10 P.M. and 4 A.M. invariably reveal the presence of microfilariae, however light

the infection may be.

Pathology

Although heavily-infected grackles maintained in the laboratory for over two years showed no noticeable external effects, attributable to the presence of worms in their cranial cavities, careful microscopic studies of serial sections of infected brains were made in order to detect any pathological lesions. Whole brains were also examined macroscopically.

In all of the brain sections examined, there was no evidence of necrosis (encephalomalacia). There was no liquefaction of brain tissues, and the cells appear healthy and normal. There was no hyperemia, neither was there any signs of oedema (external or internal hydrocephalus). Haemorrhage of the brain was completely absent, and thrombosis and embolism were never observed.

Infected birds were free from any form of meningitis, pachymeningitis, or leptominingitis. Encephalitis and myelitis were never discovered, and neither was there any sign of encephalomyelitis. Since the worms inhabit the cranial cavities, special efforts were made to discover any signs of ependymitis, but there was no inflammation of the ependyma despite occasional pressure exerted by worms on the ependyma.

Apart from the usual eosinophilia, microfilariae produced no pathological lesions on cardiac or lung tissues although they occur in large quantities in these tissues. (Figs. 17 and 18)

It is thus evident that neither adult worms nor microfilariae produce any appreciable pathological lesions.

Description of Adults

Adult worms are whitish yellow, delicate, filiform and attenuated anteriorly and posteriorly. The cuticle is smooth and non-striated. There are no lips or lateral anterior alae and both males and females are rounded at both extremities. The oesophagus is not divided into anterior muscular and posterior glandular portions. The posterior portion of the digestive tract is not atrophied as in some members of the family, and the anus is conspicuous in both sexes. There are no caudal alae, and the tail is long. Although there is great variation in the total lengths of worms, and pro rata, in other diagnostic features, it is believed that all the hundreds of worms recovered during the present investigation belong to the same species.

Males

The male is about two-fifths the size of the female in length. It is coiled posteriorly and has three pairs of small post-anal papillae which are not always conspicuous in whole mounts. The spicules are almost equal in length in all fifteen specimens measured. The testis is invariably coiled round the digestive tract at the juncture of the oesophagus and the gut. The following measurements are based on the study of 15 mature worms, fixed in warm glycerine-70% alcohol. Maximum and minimum measurements are indicated in parentheses. Length of body, 24.46 mm (20 mm-28 mm); greatest diameter of body, .132 mm (.0945 mm-.1505 mm); width of head, .03805 mm (.0332 mm-.045 mm); diameter of body at the nerve ring, .06241 mm (.0525 mm-.0735 mm); body width at the anus, .0756 mm (.105 mm-.175 mm); length of the oesophagus, .7414 mm (.6450 mm-.8250 mm);

maximum width of oesophagus, .0429 mm (.035 mm-.0490 mm); maximum width of intestine, .0851 mm (.070 mm-.0875 mm). The spicules are more or less equal in length and similar in form in each worm.

Photomicrographs and line drawings of male worms are shown in Figs. 7, 8, 9, 10, and 15.

Females

Females of this species are, on the average, about two and one-half times as long as males. They taper somewhat anteriorly but more markedly posteriorly. The cuticle is so delicate and transparent that all the internal organs can be clearly seen. The different stages of development of microfilariae are easily observed especially when females are stained in a very weak solution of Giemsa. The female reproductive system is didelphic. The uteri of gravid worms are always packed with microfilariae which can be observed leaving the worms through the vulva. The vulva is anterior, and is located in the region of the juncture of the oesophagus and the gut. The relative position of the ovaries with regard to the anus is of interest. In the case of gravid females, the ovaries coil or loop around the intestine and extend to the region of the anus, and frequently even into the tail, reaching almost the posterior end of the body (Fig. 14). However, in the case of females containing only a few microfilariae or only eggs (as in the case of unfertilized females) there is a considerable distance between the anus and the ovaries (Fig. 16). The tail is long, attaining a length of 0.19 mm in some cases.

The following measurements are based on the study of 15 mature worms, fixed in warm glycerine-alcohol. Maximum and minimum measurements are

indicated in parentheses. Total length of body, 65.47 mm (50.5 mm-84 mm); maximum diameter of body, .1619 mm (.1120 mm-.1890 mm); width of the head .319 mm (.0280 mm-.0350 mm); body width at the nerve ring, 0.655 mm (.0490 mm-.0840 mm); and the body width at the anus, .0745 mm (.0525 mm-.1085). The oesophagus in the region of the mouth is .849 mm (.7500 mm-1.0350 mm) long, and .0275 mm (.0245 mm-.0315 mm) wide, and at its juncture with the gut, it is .0339 mm (.0210 mm-.0525 mm) wide. The nerve ring is .1477 mm (.1190 mm-.1925 mm) from the oral opening, and the vulva .873 mm (.5850 mm-.1.140 mm) from the anterior extremity. The tail (distance from anus to the posterior end of the body) is .1477 mm (.1190 mm-.1925 mm) long. Photomicrographs and line drawings of female worms are shown in Figs. 11, 12, 13, 14 and 16.

Longevity of Adults

Longevity of adult filarial worms *in vitro* has been a matter of conjecture. Many investigators have attempted to culture these worms without success. The first successful attempt was made by Taylor (1960) who maintained adult *Litomosoides carinii* and *Dirofilaria immitis in vitro* for 23 and eight days, respectively. Taylor observed that the former produced microfilariae during the first two days only. Taylor employed sterile techniques and maintained the cultures at 37°C. The different culture media contained sera of rats, cotton-rats, dogs, or horses, and various salt solutions containing glucose, chick embryo extracts, raw-liver extracts and various vitamins.

In the present investigation adult worms could be kept alive *in vitro* (at room temperature) for a maximum period of only two and one-half

days in saline solution containing some glucose. However, it was discovered that adult worms could remain alive for as long as 16 days in the brain of sacrificed birds kept under refrigeration at approximately 5°C. The severance of the head from the body before refrigeration apparently has no effect on the longevity of worms in the brain. No attempt was made to determine the maximum length of time worms could remain alive in the brain of dead birds kept under refrigeration. However, five refrigerated severed heads of sacrificed infected grackles contained living worms 9, 9, 10, 13, and 16 days respectively.

For examination purposes, a severed refrigerated head was soaked in saline solution for a few hours and the brain was dissected in saline under a dissecting microscope. In one of the examined birds, all 49 worms (26 females and 23 males) recovered from the brain 13 days after the death of the host were actively moving in saline solution.

These observations indicate that adult worms may remain alive for more than two weeks following decapitation of the avian host.

Morphology of Microfilariae

Microfilaria quiscali is a serpentine immature filarial worm enclosed in a delicate transparent sheath which in some cases is about one and one-half times as long as the microfilaria itself. It consists of 3 or 4 columns of round nuclei demarcated from the sheath by a very thin cuticle. When observed under a phase microscope, the cuticle of a living specimen shows very fine striations which are infrequently visible in specimens stained with Giemsa.

The thin transparent sheath does not stain readily with Giemsa, but

is easily observed in living specimens. The microfilaria moves forward and backward within the sheath, which could be observed trailing behind an actively moving microfilaria.

Microfilaria quiscali is round and blunt at both extremities. The anterior end is bounded by a well-developed cephalic space demarcated from the columns of nuclei by a single anterior nucleus. The "Mundgebilde," however, was never observed within the cephalic space. Posteriorly, the microfilaria tapers gently to a blunt tail, and the columns of nuclei behind the anal pore diminish gradually from three to a single row arranged irregularly to the tip of the tail. No "Schwanzgebilde" was ever observed at the posterior extremity.

When filaremic blood films obtained directly from infected birds are stained in Giemsa, the columns of nuclei in the microfilaria tend to run together, but the shape and location of individual nuclei show up clearly in films made from partially clotted blood from the heart of dead infected birds kept under refrigeration for a few days. In histological sections of cardiac muscles stained in Delafield's haematoxylin, hundreds of sheathed microfilariae containing 3 or 4 columns of round, distinct nuclei (varying in number from 163-216 in 20 specimens) could be observed lying parallel to the muscle fibres. (Figs. 17 and 19)

Specimens stained in Giemsa depicted the nerve ring, excretory pore, inner body ("Innen Korper of Fulleborn, 1913) and the anal pore as clear, unstained, morphological landmarks along the length of the body, but the excretory cell and the rectal cell 1 were hardly recognisable. However, the excretory cell and rectal cell 1 were clearly differentiated from the rows of nuclei in stained preparations of partially clotted blood obtained

from the heart of sacrificed birds preserved by refrigeration. They also showed up clearly in many of the microfilariae transfused into chickens. Rectal cells 2-4 were seldom seen.

The size of Microfilaria quiscali varies very greatly. Measurements of hundreds of microfilariae obtained from various hosts at different times of the day suggest that neither the time of day nor the host reaction has any significant effect on the size of microfilaria. However, stained specimens are comparatively shorter than live ones. The lengths of 25 living specimens vary from .154 mm to .208 mm with an average length of .187 mm, while 100 stained specimens varied in length from .098 mm to .196 mm with an average of .154 mm.

Measurements of 100 Microfilaria quiscali obtained from five infected grackles at various times of the day are shown in Table 1 and daily measurements of microfilariae preserved in vitro at 5°C. for 17 days are shown in Table 2. (Ranges are shown in parentheses below the average lengths in both tables) The detailed morphology is shown in Figs. 19 and 20.

Table 1. Measurements[a] of stained Microfilaria quiscali obtained from five birds at various hours of day

Hosts	Grackle A	Grackle B	Grackle C	Grackle D	Grackle E	Av. of all mf.
Number of Mf. measured	20	20	20	20	20	100
Average length	.148 mm. (.098-.196)	0.161 mm. (.119-.185)	.153 (.106-.189)	.159 mm. (.123-.186)	.153 mm. (.123-.171)	.154 mm. (.098-.196)
Width	.0053 mm. (.005-.006)	.0049 mm. (.003-.006)	.0054 mm. (.005-.006)	.0049 mm. (.005-.006)	.0055 mm. (.005-.006)	.0052 mm. (.003-.006)
Cephalic space	.0026 mm. (.002-.003)	.0031 mm. (.003-.005)	.003 mm. (.002-.005)	.0038 mm. (.002-.006)	.0034 mm. (.002-.005)	.0032 mm. (.002-.006)
Nerve ring from anterior end	.0386 mm. (.025-.049)	.0424 mm. (.032-.05)	.0403 mm. (.028-.049)	.0403 mm. (.031-.058)	.0431 mm. (.031-.045)	.0398 mm. (.025-.058)
Excretory pore from anterior end	.0555 mm. (.037-.077)	.0612 mm. (.046-.072)	.060 mm. (.043-.086)	.0611 mm. (.045-.077)	.0572 mm. (.046-.065)	.0590 mm. (.037-.086)
Inner body from anterior end	.0969 mm. (.068-.139)	.1054 mm. (.0816-.123)	.1014 mm. (.069-.136)	.1042 mm. (.079-.126)	.0980 mm. (.08-.112)	.1012 mm. (.068-.139)
Anal pore from anterior end	.1310 mm. (.086-.177)	.1434 mm. (.106-.166)	.1364 mm. (.092-.171)	.1419 mm. (.108-.168)	.1358 mm. (.109-.153)	.1377 mm. (.086-.177)
Anal pore from post. end (tail)	.0161 mm. (.012-.019)	.0172 mm. (.012-.02)	.0159 mm. (.012-.019)	.0169 mm. (.104-.019)	.0169 mm. (.014-.019)	.0166 mm. (.012-.02)

[a]Average distances of the excretory Pore and rectal Cell 1 from the anterior end in 15 stained microfilariae were .0704 mm. (.0585-.0878 mm.) and .1209 mm. (.1063-.1371 mm.) respectively.

Table 2. Daily measurements of microfilariae in blood kept at 3°C.

	Days of refrigeration										
	1	2	3	4	5	6	7	8	13	17	Average
Number of Mf. measured	15	15	15	15	15	15	15	15	15		135
Average length in mm.	.1317	.1687	.1381	.1245	.1409	.1241	.1167	.1639	.1585		.1392 (.1001-.1802)
Width in mm.	.0059	.0058	.0051	.0047	.0049	.0046	.0046	.006	.0053		.0052 (.0039-.0062)
Cephalic space in mm.	.0033	.0031	.0029	.0027	.0029	.0024	.0028	.0045	.0046		.0031 (.0007-.0062)
Nerve ring from ant. end in mm.	.0360	.0452	.0385	.0341	.0389	.0323	.0316	.0429	.0427		.0427 (.0262-.0477)
Excretory pore from ant. end in mm.	.0525	.0666	.0534	.0493	.0544	.0474	.0458	.0628	.0612		.0540 (.0385-.07084)
Inner body from ant. end in mm.	.0895	.1126	.0913	.0841	.0958	.0829	.0784	.1075	.1064		.0927 (.0678-.1201)
Anal pore from ant. end in mm.	.1195	.1528	.1234	.112	.1271	.1106	.1043	.1475	.1429		.1249 (0909-.1648)
Anal pore from post. end (tail) in mm.	.0125	0159	.0148	.0134	.0139	.0136	.0124	.0163	.0157		.0142 (.00920-.0200)

Longevity of Microfilariae

Longevity of microfilariae in vitro

Many investigators have observed that microfilariae may survive for considerable periods outside their hosts. Some of these observations were incidental, others were the result of experiments undertaken to provide additional evidence discrediting the belief that new broods of microfilariae are produced by adult female worms every 24 hours.

Fulleborn (1912) claimed that microfilariae of Dirofilaria repens lived in the blood of a parasitized dog for four to seven weeks when the blood was maintained at a temperature of approximately 0°C. He also observed that the microfilariae remained alive for 10 minutes at the temperature produced by a mixture of ice and sodium chloride.

Ochoterena (1930) working with the microfilariae of Onchocerca volvulus, was able to maintain these microfilariae alive for three days at room temperature in a mixture of saline solution, Ringer's solution and a drop of human serum.

Rao, as reported by Hinman, Faust and DeBakey (1934), kept microfilariae of Wuchereria bancrofti alive in vitro at 4°C. under aseptic conditions for four to six weeks.

Joyeux and Sautet (1937) observed that microfilariae of Dirofilaria immitis lived for 12 days in the blood of a parasitized dog, when the blood was preserved in a "cool place."

Schnelle and Young (1944) reported that Microfilaria immitis survived in the serum of an infected dog for eight days when the room temperature varied between 40°F. (4°C.) at night to 65°F. (18°C).

Gonzalez, according to her major professor, Mazzotti (1953), observed that microfilariae of Onchocerca volvulus lived for three days in either Ringer's solution or Locke's solution, and for four days in either human serum or the plasma of sheep. All the solutions were maintained at approximately 20°C. Vargas (1952), on the other hand, found that the microfilariae of O. volvulus remained alive for six days in a mixture of human serum and dextrose at a temperature of 4°C.

Mazzotti (1953) observed that microfilariae of D. immitis and Onchocerca verticulata could live for 18 and 10 days respectively at a temperature of 25°C below zero.

Hanson et al. (1956) while working on the filariae of Canada geese made incidental observations on the survival of microfilariae in serum of infected birds. They discovered that at a temperature of approximately 50°C., microfilariae of Sarconema eurycerca survived for 38 days, and those of Ornithofilaria sp. for 25 and 34 days respectively in two tests. At room temperature, microfilariae of Ornithofilaria lived for seven days.

In the present investigation, two experiments were performed to determine the longevity of microfilariae of Splendidofilaria quiscali in vitro. In the first experiment, which was not conducted under aseptic conditions, microfilaremic blood was maintained at a temperature of approximately -5°C., whereas in a second experiment it was maintained under aseptic condition at approximately 3°C. In both experiments some blood-diluting fluid (described in a previous section) was added to the blood before refrigeration. Blood used in each case was obtained from the heart, lungs, and adjoining blood vessels of recently-killed, naturally infected grackles.

Experiment 1 The blood of the grackle used for this experiment contained not only microfilariae of Splendidofilaria quiscali, but also Microfilariae "X" whose adults have not yet been located in the host. The diluted blood was divided into 10 vials on October 14, 1959 and placed in a refrigerator maintained at -5°C. Each day, for 10 consecutive days, a new vial was examined along with those previously examined. Thus, over a period of 10 days, Vial No. 1 was examined 10 times, while Vial No. 10 was examined only once.

For examination purposes, the vials containing the frozen blood were left at room temperature until warm enough to induce activity of microfilariae. A drop or two of the warmed blood was put on a slide, covered with a cover slip and examined under low power of a compound microscope. Between 100 and 362 microfilariae were counted at each examination and the numbers observed alive were expressed as percentages of the total number counted. Each vial was returned to the refrigerator after examination.

Table 3 indicates the results obtained on the longevity of Microfilaria quiscali, and Table 4 the results on that on Microfilaria "X". Data obtained from this experiment indicate that Microfilaria quiscali lived for at least nine days, and Microfilaria "X" lived for 11 days.

Experiment 2 Examinations of samples in Experiment 1 revealed the necessity for repeating the experiment under aseptic conditions, because most of the vials became contaminated with bacteria and other micro-organisms after the eighth day. All the equipment (including the blood-diluting fluid) was sterilized in an autoclave at 254°C. and 16 lbs. pressure for 25 minutes.

Table 3. Longevity[a] of *Microfilaria quiscali*[b] in vitro[c]

1959	Vial 1 % alive	2 % alive	3 % alive	4 % alive	5 % alive	6 % alive	7 % alive	8 % alive	9 % alive	10 % alive
Oct. 15	100	—[d]	—	—	—	—	—	—	—	—
" 16	87	—	—	—	—	—	—	—	—	—
" 17	92	47	—	—	—	—	—	—	—	—
" 18	93	73.5	80.6	—	—	—	—	—	—	—
" 19	86.6	84.7	69.8	80	—	—	—	—	—	—
" 20	97.4	75.9	81	90	92.5	—	—	—	—	—
" 21	80	89	*[e]	73	81.6	47.4	—	—	—	—
" 23	*	*	*	—	*	*	1	—	—	—
" 25	0[f]	0	0	—	0	0	0	0	—	—
" 26	0	0	0	—	0	0	0	0	0	0

[a]Bird was sacrificed on October 14, 1959.

[b]Microfilariae were maintained at approximately -5°C.

[c]Experiment was not conducted under aseptic conditions. Vials became contaminated with microorganisms after a few days.

[d]— denotes that vial was not checked.

[e]* denotes that some microfilariae were alive but % not determined.

[f]0 denotes that all microfilariae were dead.

Table 4. Longevity[a] of Microfilaria "X"[b] in vitro[c]

1959	Vial 1 % alive	2 % alive	3 % alive	4 % alive	5 % alive	6 % alive	7 % alive	8 % alive	9 % alive	10 % alive
Oct. 15-18	—[d]	—	—	—	—	—	—	—	—	—
Oct. 19	78	75	15.40	70	—	—	—	—	—	—
Oct. 20	60	72.7	14.3	41.6	36.8	—	—	—	—	—
Oct. 21	34.8	8.3	*[e]	15.3	17.3	0[f]				
Oct. 23	*	*	*	—	*	*	30	—	—	—
Oct. 25	0	*	0	—	0	0	0	0	—	—
Oct. 26	0	0	0	0	0	0	0	0	0	0

[a]Bird was sacrificed on October 14, 1959.

[b]Microfilariae were maintained at approximately -5°C.

[c]Experiment was not conducted under aseptic conditions. Vials became contaminated with microorganisms after a few days.

[d]— denotes that vial was not checked.

[e]* denotes that some microfilariae were alive but % not determined.

[f]0 denotes that all microfilariae were dead.

This experiment was performed in essentially the same way as Experiment 1 except that the blood contained only Microfilaria quiscali and the vials were maintained at a temperature of approximately 3°C. Thus, from November 10, 1959 (when the bird was killed) to November 28, 1959 (when the experiment was discontinued) the blood never froze.

Data obtained from this experiment are presented on Table 5. This experiment indicated that Microfilaria quiscali can live for at least 17 days outside the host at approximately 3°C. Vial No. 1 was not returned to the refrigerator on November 11, 1959 but maintained at room temperatures (approximately 26°C.). All the microfilariae were dead on November 12, 1959. Furthermore, daily examination of microfilariae under higher magnifications showed that many of them had shed their sheaths.

Longevity of Microfilaria quiscali in dead host

On many occasions during the present investigation, it was necessary to keep dead or sacrificed infected birds under refrigeration for a few days until they could be necropsied. It was discovered that at a temperature of approximately 5°C., such dead birds remained for two weeks in a satisfactory condition.

During the necropsy of such birds kept under refrigeration, it was discovered that when a drop of saline solution was added to a small portion of the partially clotted blood from the heart, microfilariae became active after a few minutes. Although no definite experiments were conducted in an effort to determine how long microfilariae could remain alive in the heart of dead birds kept under refrigeration, careful records were kept of the number of days dead birds were refrigerated before

Table 5. Longevity[a] of Microfilaria quiscali maintained at approximately 3°C. under aseptic condition

1959	Vial 1[b] % alive	2 % alive	3 % alive	4 % alive	5 % alive	6 % alive	7 % alive	8 % alive	9 % alive	10 % alive
Nov. 11	100	--[c]	--	--	--	--	--	--	--	--
Nov. 12	0[d]	98	--	--	--	--	--	--	--	--
Nov. 13	--	100	100	--	--	--	--	--	--	--
Nov. 14	--	99	99	99	--	--	--	--	--	--
Nov. 16	--	100	98	99	98	--	--	--	--	--
Nov. 18	--	34	60	99	99	100	100	--	--	--
Nov. 20	--	--	--	--	--	95	83	--	--	--
Nov. 23	--	0	0	0	0	0	92	0	--	--
Nov. 27	--	0	0	0	0	0	0	0	23	--
Nov. 28	--	0	0	0	0	0	0	0	0	0

[a]Bird was sacrificed on November 10, 1959.

[b]Vial No. 1 was kept at room temperature .

[c]-- denotes that the vial was not checked.

[d]0 denotes that all microfilariae were dead.

necropsy.

The data accumulated from these observations (Table 6) reveal that microfilariae of Splendidofilaria quiscali can remain alive for as long as 18 days, in the heart of dead, infected birds maintained at a temperature of approximately 5°C.

Table 6. Observations on the longevity of microfilariae in dead grackles kept under refrigeration[a]

Number of days dead grackles were kept under refrigeration	% of mf. alive
4 days	99
9 days	43
9 days	39
13 days	34
18 days	6

[a]Sacrificed hosts were kept under refrigeration at approximately 5°C.

Periodicity of Microfilariae

Normal periodicity

The periodic variation of microfilarial concentrations in the peripheral blood of infected animals has interested parasitologists ever since Manson (1878) observed nocturnal periodicity of *Wuchereria bancrofti* microfilariae. Attempts to explain the mechanism of the phenomenon, however, were complicated by later investigations, many of which involved other species of filarial worms. Manson-Bahr (1912) and O'Connor (1923) showed that the Pacific form of *Wuchereria bancrofti* exhibits a diurnal periodicity. *Loa loa* also shows diurnal periodicity as indicated the work of Gordon *et al.* (1950). On the other hand, *Litomosiodes carinii* according to Bell and Brown (1945), and *Lophortofilaria californiensis* according to Wehr and Herman (1956), show no periodicity at all. A complete review of the numerous theories proposed as explanations for periodicity was presented by Lane (1948), and a shorter review by Hawking and Thurston (1951). Despite these many theories, no entirely satisfactory explanation has been widely accepted. Briefly, the major features of these various theories are indicated below.

Myers' Theory (1881)

> Myers (1881, 1886) maintained that parturition by female worms is continuous. The young remain in the lymphatics 12 to 24 hours, but re-enter the circulating blood because of a "selective ability" on the part of microfilariae for the favourable conditions in the blood. Microfilariae that escape the night-biting mosquitoes are "dissolved" when day comes.

Manson's Theory (1882)

Manson believed in continuous parturition and asserted that "during the diurnal temporary absence from the cutaneous circulation, microfilariae retire principally to the larger arteries and to the lungs where during the day they are found in enormous numbers."

Lane's Theory (1929)

Lane held the view that periodicity must be due to simultaneously-timed parturitions of the female worms once in every 24 hours.

Harley (1932) accepted Lane's theory and suggested that the stimulus producing periodicity is the saliva of the normal vector. His suggestion is supported by the facts that microfilariae with diurnal periodicity are transmitted by day-biting insects, those with nocturnal periodicity, by night-biting insects, while those with no periodicity are transmitted by insects that bite at any time of day. However, since Harley believed in the theory of synchronized parturition, he assumed that the injected saliva or perhaps something the host produced from it induces parturition.

Poynton and Hodgkin's Theory (1938)

Poynton and Hodgkin maintained that "the periodic migrations of the larvae from the deep vessels and lungs to the surface ..... is linked with the biting habits of the vector mosquitoes." From the answers that they obtained to questions

asked 334 patients, they contended that the attacks of lymphangitis "recur at regular intervals," and concluded that it was "probable that the attacks are associated with the parturition of adult females ..... Enormous number of embryos are delivered at regular intervals ....." Chemotaxis induced by the vectors' bites also affects microfilarial migration.

Khalil's Theory (1938)

Khalil postulated that the site of the adult worm, the feeding hours and the posture of the host, all affect periodicity. With the adults in the upper limbs, the lymph flow carries microfilariae into the great veins; with the worms in the lower limbs, they are carried into the receptaculum chyli. While the host is upright they will stagnate there, and when he lies down at night, gravity will cease to 'precipitate' them. He also postulated that 12 or 13 hours after the principal meal (assumed to be taken at midday), microfilarial migration is helped by the after-food flush of the chyle. Later, however, (1938b) he questioned the use of a time interval of 12 or 13 hours after a meal as the time of greatest chyle flow.

Yokogawa's Theory (1939)

Yokogawa believed that the periodicity of microfilariae is chiefly controlled by the diurnal and nocturnal alterations of the physiological function of the reticulo-endothelial system which normally destroys parasites.

Lane (1948), after reviewing the various theories, reaffirmed his earlier theory on the basis of his examination of 800 slides. He remarked inter alia: "the nightly rise in the microfilarial blood tide is due primarily to synchronized parturitions by female worms ..... The fall ..... is due to the destruction of the nightly brood in the liver, spleen and adrenals, probably in lymph nodes and possible elsewhere ..... The absence of the tide is explicable either by lack of synchronization of worm parturitions or by blockage of the host's macrophage system."

A markedly different proposal as to the cause of periodicity was suggested by Hawking and Thurston (1951) who believed that periodicity is independent of the presence of the adult worms in the host animal. These authors concluded from a series of experiments with dogs and monkeys that the appearance and disappearance of microfilariae (of two species of Dirofilaria) in the blood is due to their periodic liberation from and accumulation in the small vessels of the lungs. They admitted an ignorance of the mechanism by which the liberation and accumulation is brought about, but suggested that it "apparently involves an active response of the microfilariae to some unknown periodic change in the blood of the host which habitually preceded sleeping and waking." They postulated that periodicity of microfilariae is a mechanism by which "a compromise is arranged between two requirements of the microfilariae (optimum survival and transmission)."

Further evidence of the validity of Hawking and Thurston's (1951) explanation was provided by Hawking (1953) in a transfusion experiment involving Microfilaria repens. In this study he showed that periodicity

of microfilariae in the recipient dog was essentially the same as that in the donor.

Busa (1955) attempted to relate periodicity to certain environmental changes of the host. He found that such factors as temperature and atmospheric pressure apparently affect periodicity, and that injection of adrenalin increases the number of microfilariae. The effect of the latter, however, had previously been shown by Schnelle and Young (1944).

More recently, Hawking (1956) attempted to explain the mechanism whereby microfilariae are accumulated in the lungs during the day and are released from them at night. He tried to show a relationship between periodicity and certain internal environmental conditions of the host such as increase and decrease of oxygen pressure, hyperventilation, exercise, and changes resulting from the infusion of certain chemicals. However, the marked difference in response to these changes as shown by the various species of microfilariae he employed, prevented him from reaching any definite conclusions. Later in 1956, McFadzean and Hawking, studying additional species of microfilariae, again concluded that accumulation of microfilariae in the lungs is in response to "some still unidentified stimulus provided by the 24-hour physiological rhythm of the host."

Edeson, Hawking and Symes (1957) suggest the possibility of a close correlation between microfilarial periodicity and the oxygen pressure of the host.

From the foregoing, it is apparent that there is not, as yet, unanimity of opinion regarding the reasons for microfilarial periodicity.

Several experiments concerning normal periodicity of microfilariae

of Splendidofilaria quiscali were undertaken. These experiments all involved removal of blood from the metatarsal veins of infected grackles. All the films were stained with Giemsa's stain and accurate records of the total number of microfilariae recovered were kept. Details of the techniques involved in these experiments are indicated in a previous section on Materials and Methods.

Experiment 1 The purpose of this experiment was to discover whether Microfilaria quiscali shows any kind of periodicity.

The experiment involved the maintenance of the host under laboratory conditions but substituting artificial light in place of daylight. A single, naturally-infected grackle (A) was placed in a dark room and supplied with artificial light daily from 700 to 1700 (7 A.M. to 5 P.M.). Stained blood films were prepared every two hours for 88 consecutive hours by removing each time one drop of blood from the metatarsal vein. Although the quantity of blood withdrawn each time was not accurately measured, it was approximately the same. All microfilariae on each slide were counted and recorded. The results of this experiment, shown on Table 7 indicate a pronounced increase in microfilarial number during hours of darkness.

Experiment 2 A second experiment was undertaken to determine if this same nocturnal periodicity could be observed under normal conditions of natural daylight and darkness.

Six naturally infected grackles (B, C, D, E, F, and G) were put in a room with large glass windows so as to ensure an ample supply of daylight throughout the two days during which the experiment was conducted. Blood smears were made, as explained above, every two hours for 48 con-

Table 7. Exploratory experiment on periodicity using artificial light

Date of examination	Time of day	Experimental condition	Number of mf./ drop of blood
March 7, 1958	900	Artificial light	1
"	1100	"	1
"	1300	"	0
"	1500	"	0
"	1700	"	1
"	1900	Darkness	6
"	2100	"	4
March 8, 1958	2300	"	8
"	100	"	5
"	300	"	9
"	500	"	3
"	700	Artificial light	0
"	900	"	1
"	1100	"	0
"	1300	"	3
"	1500	"	1
"	1700	"	0
"	1900	Darkness	12
March 9, 1958	2200	"	8
"	2330	"	12
"	100	"	39
"	300	"	Prep. not made
"	500	"	"
"	700	Artificial light	3
"	900	"	2
"	1100	"	0
"	1330	"	3
"	1500	"	0
"	1700	"	3
"	1945	Darkness	17
"	2100	"	15
March 10, 1958	2300	"	20
"	130	"	19
"	300	"	21
"	500	"	22
"	700	Artificial light	1
"	900	"	2
"	1100	"	1
"	1300	"	1
"	1500	"	0
"	1700	"	0
"	1930	Darkness	10
"	2200	"	32
"	2400	"	9

secutive hours. The results of this experiment are shown on Table 8. More microfilariae appeared in the peripheral blood circulation during the hours of darkness especially between 2000 and 600 (i.e., 8 P.M. and 6 A.M.).

Experiment 3 Since the two experiments noted above involved unmeasured quantities of blood from infected birds, a more carefully-conducted experiment was undertaken in which the same volume of blood (0.0125 ml.) was removed from each of five experimental birds every two hours for 72 consecutive hours. Other conditions of the experiments were the same as in experiment 2.

Data obtained from four of these experimental birds are presented graphically on Table 9 and in Graphs 1 and 2. Infection of the one remaining bird used in these experiments was so light that results obtained could not be indicated graphically.

Reversal of Periodicity

That reversal of microfilarial periodicity can be accomplished has been known for many years. Mackenzie (1882), working with Wuchereria bancrofti, showed that periodicity can be reversed by altering the patient's sleeping and waking hours. This was confirmed by Manson (1882), Yorke and Blacklock (1917) and by many others as indicated by Fülleborn (1929). However, because the 'microfilarial tide' begins to rise in late afternoon (many hours before patients actually sleep), and because a period of some days is required after the reversal of patients' sleeping habits before there is a reversal of the microfilarial tide, it is apparent that periodicity is influenced by well-established sleeping and

Table 8. Exploratory experiment on periodicity[a] using natural daylight and darkness

Date	Time	Grackle number					
		B	C	D	E	F	G
October 7, 1958	1000	0	2	0	3	2	0
"	1200	9	10	0	0	5	9
"	1400	7	6	0	11	4	15
"	1600	3	5	1	1	0	21
"	1800	0	1	0	5	2	5
"	2000	27	0	38	4	12	123
"	2200	33	53	2	19	17	24
"	2400	26	47	1	2	5	15
October 8, 1958	200	43	90	3	6	9	45
"	400	18	83	0	8	9	80
"	600	80	33	1	2	31	36
"	800	3	1	0	0	34	4
"	1000	1	0	1	1	0	11
"	1200	0	1	12	17	0	4
"	1400	4	1	0		2	9
"	1600	12	5	0		1	5
"	1800	6	2	0		5	21
"	2000	311	49	0		13	99
"	2200	45	101	1		115	30
"	2400	511	21	3		157	145
October 9, 1958	200	443	35	6		36	71
"	400	313	244	4		20	92
"	600	1	39	0		8	11
"	800	6	2	0		2	9

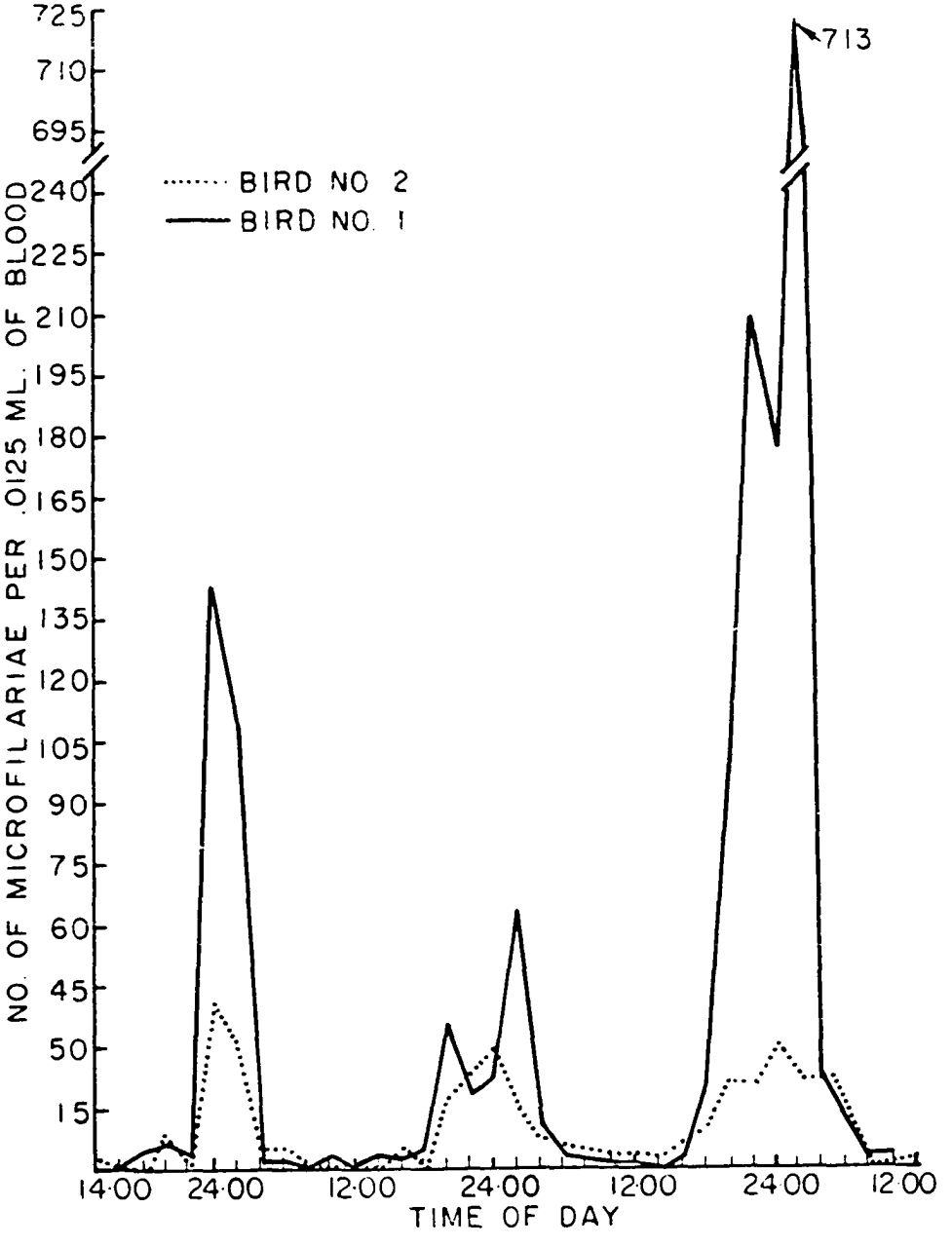
[a]The drop of blood withdrawn from the metatarsal vein of each grackle every two hours was not accurately measured.

Table 9. Normal periodicity[a] of *Microfilaria quiscali* using natural daylight and darkness

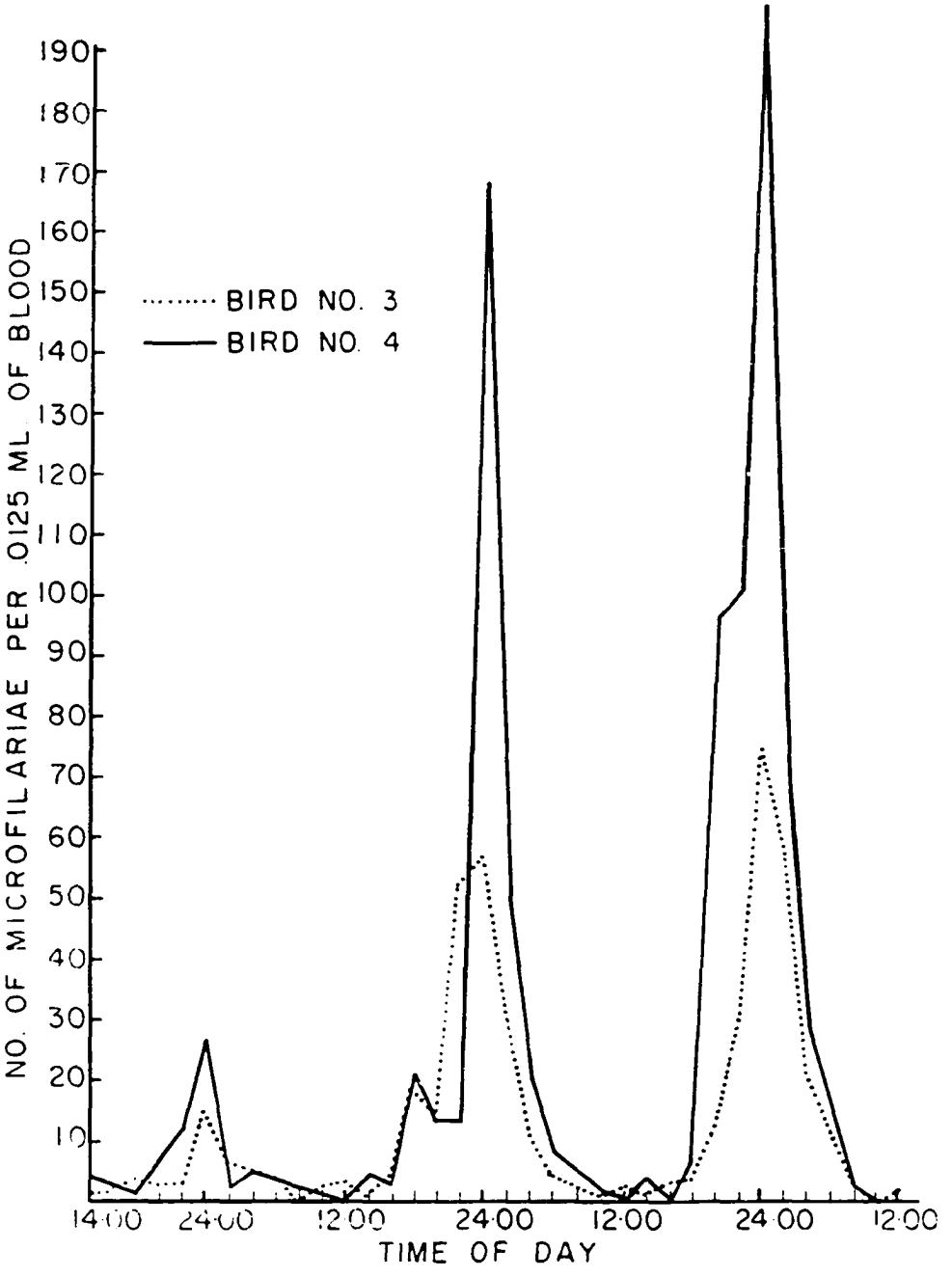
Date	Time	Grackle number				
		1	2	3	4	x
December 2, 1958	1400	0	3	1	4	0
"	1600	0	0	2	3	0
"	1800	4	0	4	1	0
"	2000	6	7	3	7	0
"	2200	3	2	3	12	0
"	2400	142	42	15	3	3
December 3, 1958	200	110	31	7	2	0
"	400	1	6	5	5	0
"	600	2	6	4	4	0
"	800	1	1	0	2	0
"	1000	3	1	2	1	0
"	1200	1	0	3	0	0
"	1400	3	2	1	4	0
"	1600	3	4	4	3	0
"	1800	4	0	19	21	2
"	2000	36	17	13	12	1
"	2200	17	*	52	13	3
"	2400	22	29	57	168	2
December 4, 1958	200	64	15	31	50	1
"	400	11	9	11	20	0
"	600	5	7	5	8	0
"	800	Preparation not made				
"	1000	2	3	0	1	2
"	1200	2	5	2	0	0
"	1400	0	4	1	3	0
"	1600	2	6	3	0	0
"	1800	21	9	4	6	0
"	2000	97	20	12	96	9
"	2200	208	19	30	101	4
"	2400	117	29	75	198	6
December 5, 1958	200	713	22	57	69	21
"	400	24	22	21	28	2
"	600	Preparation not made				
"	800	3	1	4	3	0
"	1000	3	2	0	0	0
"	1200	0	2	1	0	0

[a]Approximately 0.0125 ml. blood was withdrawn from the matatarsal vein of each bird every two hours.

Graph 1. Normal periodicity of Microfilaria quiscali using natural daylight and darkness


725
710
695
240
225
210
195
180
165
150
135
120
105
90
75
60
45
50
15
713
BIRD NO 2
BIRD NO. 1
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
14·00
24·00
12·00
24·00
12·00
24·00
12·00
TIME OF DAY

Graph 2. Normal periodicity of *Microfilaria quiscali* using natural daylight and darkness


190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
NO. OF MICROFILARIAE PER .0125 ML OF BLOOD
BIRD NO. 3
BIRD NO. 4
14·00
24·00
12·00
24·00
12·00
24·00
12·00
TIME OF DAY

waking habits rather than by any sudden change in such habits. It was thus established that periodicity is more closely related to the activity of the host than it is to periods of daylight and darkness.

Boughton, Byrd and Lund (1938), working with an unnamed filaria of the crow (Corvus brachyrhynchos brachyrhynchos), showed that nocturnal periodicity of these microfilariae could be easily reversed and that presence or absence of the microfilariae in quantity was closely related to the activity of the host. They further observed that a period of two days is sufficient to effect of a complete reversal of periodicity, contrary to the reports of experiments with Wuchereria bancrofti in man, as presented by Mackenzie (1882) and by Low and Manson-Bahr (1934), and with Dirofilaria immitis of dogs according to Hinman (1936) who indicates the necessity of a longer period before reversal can be effected.

Robinson (1955 b) was able to reverse the nocturnal periodicity in a common crow, and in a fish crow (Corvus ossifragus). He remarked that the periodicity was disturbed between 20 and 44 hours, and that reversal was accomplished within 72 hours.

Because of the experimental results of previous workers, it was decided to determine whether or not reversal of periodicity of S. quiscali could be accomplished.

Six naturally-infected grackles were placed in a dark windowless room on February 1, 1960 midnight. Artificial light was supplied by a 200-watt bulb turned on every day from 2000 to 800 (8 P.M. to 8 A.M.) of the following day. Thus, the birds were left in darkness daily between 8 A.M. and 8 P.M. Since the examination of blood films from these birds

began at 6 A.M. on February 3, 1960, there was an interval of 2½ days before the taking of blood was initiated. Films were then made every two hours for 72 succeeding hours.

The birds were supplied with ample food and water throughout the five experimental days, and the thermometer in the room always registered approximately 25°C. Methods of blood examination were the same as those employed for normal periodicity studies. Withdrawal of blood was done in the adjoining room so that the birds were exposed to a minimum of light during the darkness period.

Results of these experiments, presented in Graphs 3, 4, and 5, and on Table 10, show that periodicity of Microfilaria quiscali was reversed in all six experimental birds. Since room temperatures remained constant throughout the course of the experiment, and since adequate food and water were available at all times, these factors were not involved.

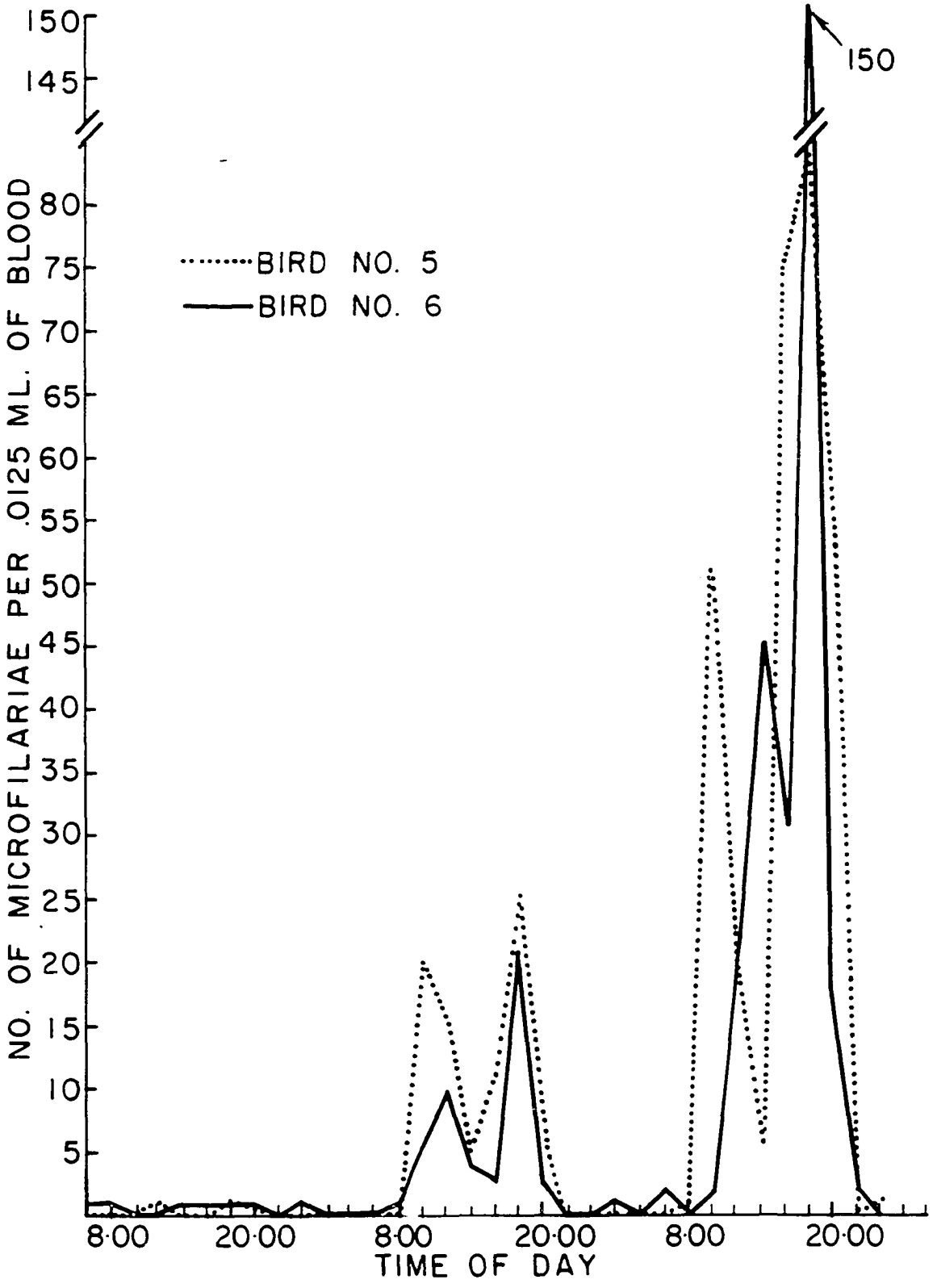
Effect of continuous light on periodicity

In all the preceding experiments involving normal periodicity and reversal of periodicity, microfilariae were almost negligible in number in the peripheral blood during those periods when birds were in the light. The following experiments were undertaken in order to determine what effect five days of continuous exposure to artificial light might have on periodicity.

The same birds employed in the preceding experiment on reversal were used in these investigations, and the same dark room was used. Food and water were ample every time and the temperature remained approximately at 25°C. throughout the experimental days.

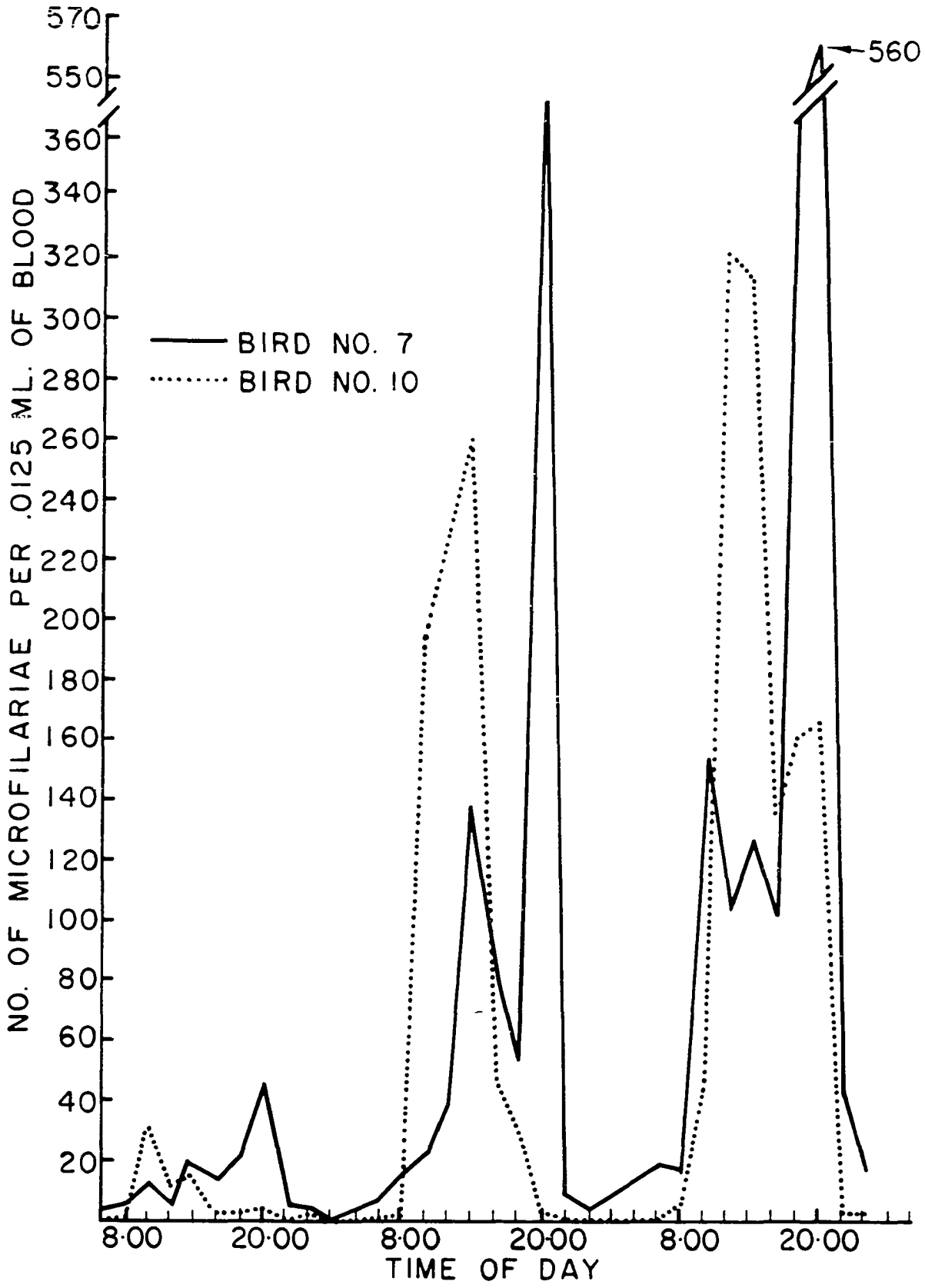
Graph 3. Reversal of periodicity of Microfilaria quiscali

(Birds exposed to artificial light from 8 P.M. to 8 A.M.)


150
145
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
150
BIRD NO. 5
BIRD NO. 6
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
8·00
20·00
8·00
20·00
8·00
20·00
TIME OF DAY

Graph 4. Reversal of periodicity of *Microfilaria quiscali*

(Birds exposed to artificial light from 8 P.M. to 8 A.M.)


570
550
360
340
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
560
BIRD NO. 7
BIRD NO. 10
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
8·00
20·00
8·00
20·00
8·00
20·00
TIME OF DAY

Graph 5. Reversal of periodicity of Microfilaria quiscali

(Birds exposed to artificial light from 8 P.M. to 8 A.M.)

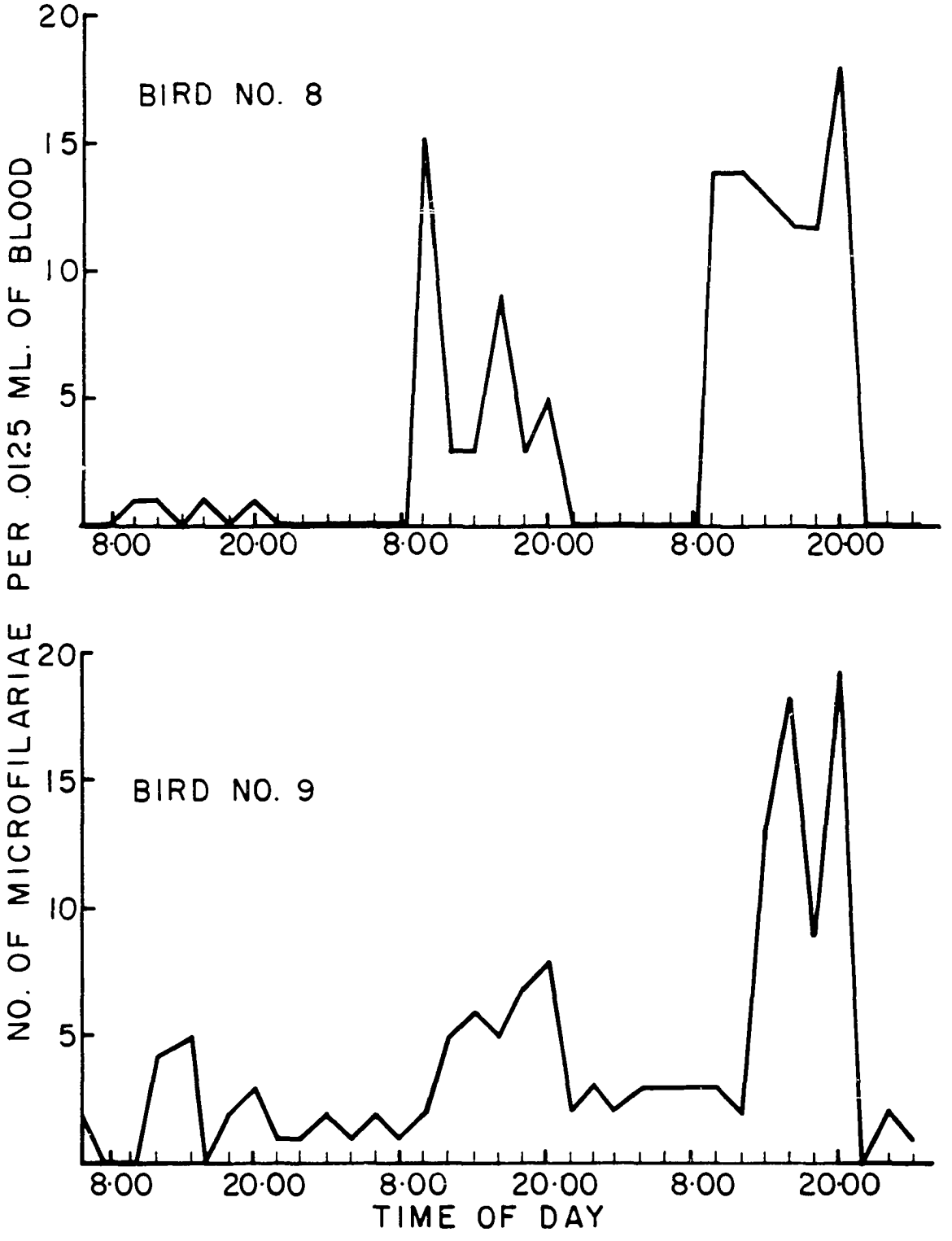

BIRD NO. 8
BIRD NO. 9
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
20
15
10
5
8·00
20·00
TIME OF DAY

Table 10. Reversal[a] of periodicity[b] of *Microfilaria quiscali*

Date	Time	Room condition	Grackle number 5	6	7	8	9	10
Feb. 3, 1960	600	Light	0	1	2	0	2	1
"	800	"	0	1	6	0	0	2
"	1000	Darkness	0	0	12	1	0	31
"	1200	"	1	0	5	1	4	13
"	1400	"	0	1	17	0	5	15
"	1600	"	0	1	14	1	0	3
"	1800	"	1	1	21	0	2	3
"	2000	"	0	1	45	1	3	4
"	2200	Light	0	0	5	0	1	1
"	2400	"	0	1	4	0	1	2
Feb. 4, 1960	200	"	0	0	0	0	2	0
"	400	"	0	0	5	0	1	0
"	600	"	0	0	6	0	2	0
"	800	"	0	1	15	0	1	2
"	1000	Darkness	20	5	22	15	2	19
"	1200	"	15	10	39	2	5	*
"	1400	"	5	4	138	3	6	258
"	1600	"	11	3	80	8	5	48
"	1800	"	25	21	56	3	7	30
"	2000	"	6	3	375	5	8	3
"	2200	Light	0	0	7	0	2	1
"	2400	"	0	0	3	0	3	0
Feb. 5, 1960	200	"	0	1	7	0	2	2
"	400	"	0	0	12	0	3	0
"	600	"	0	2	18	0	3	0
"	800	"	1	0	17	0	3	7
"	1000	Darkness	51	2	153	14	3	47
"	1200	"	20	21	102	14	2	322
"	1400	"	6	45	127	13	13	314
"	1600	"	75	31	103	12	18	136
"	1800	"	84	150	370	12	9	160
"	2000	"	54	18	560	18	19	166
"	2200	Light	0	2	42	0	0	2
"	2400	"	1	0	17	0	2	2
Feb. 6, 1960	200	"	0	0	18	0	1	1

[a]Lights were turned on at 2000 (8 P.M.) and turned off at 800 (8A.M.).

[b]Approximately 0.0125 ml. blood withdrawn was from metatarsal vein of each bird every two hours.

The six experimental birds (Nos. 5, 6, 7, 8, 9, and 10) were exposed to normal periods of darkness and light for four days (February 6 through 9) following the experiments on reversal. They were then exposed to continuous light supplied by a 200 watt bulb from 8 A.M. on February 10, 1960, to 8 P.M. on February 15, 1960. Blood examinations were made from 10 A.M. on February 13, to 8 P.M. on February 15, 1960. The methods of examination employed were the same as those used in Experiment 3 (above) and in studies on reversal of periodicity.

The results of these experiments are presented in Graphs 6, 7, 8, and 9 and Table 11. These results indicate clearly that the regular pattern of periodicity was disrupted and that in many instances the maximum numbers of microfilariae present at certain times far exceeded those in the earlier experiments dealing with normal and reversed periodicity. In the case of No. 10, it is interesting to note that an apparently regular increase in microfilarial number occurred about 6 P.M. No other bird, however, showed this tendency.

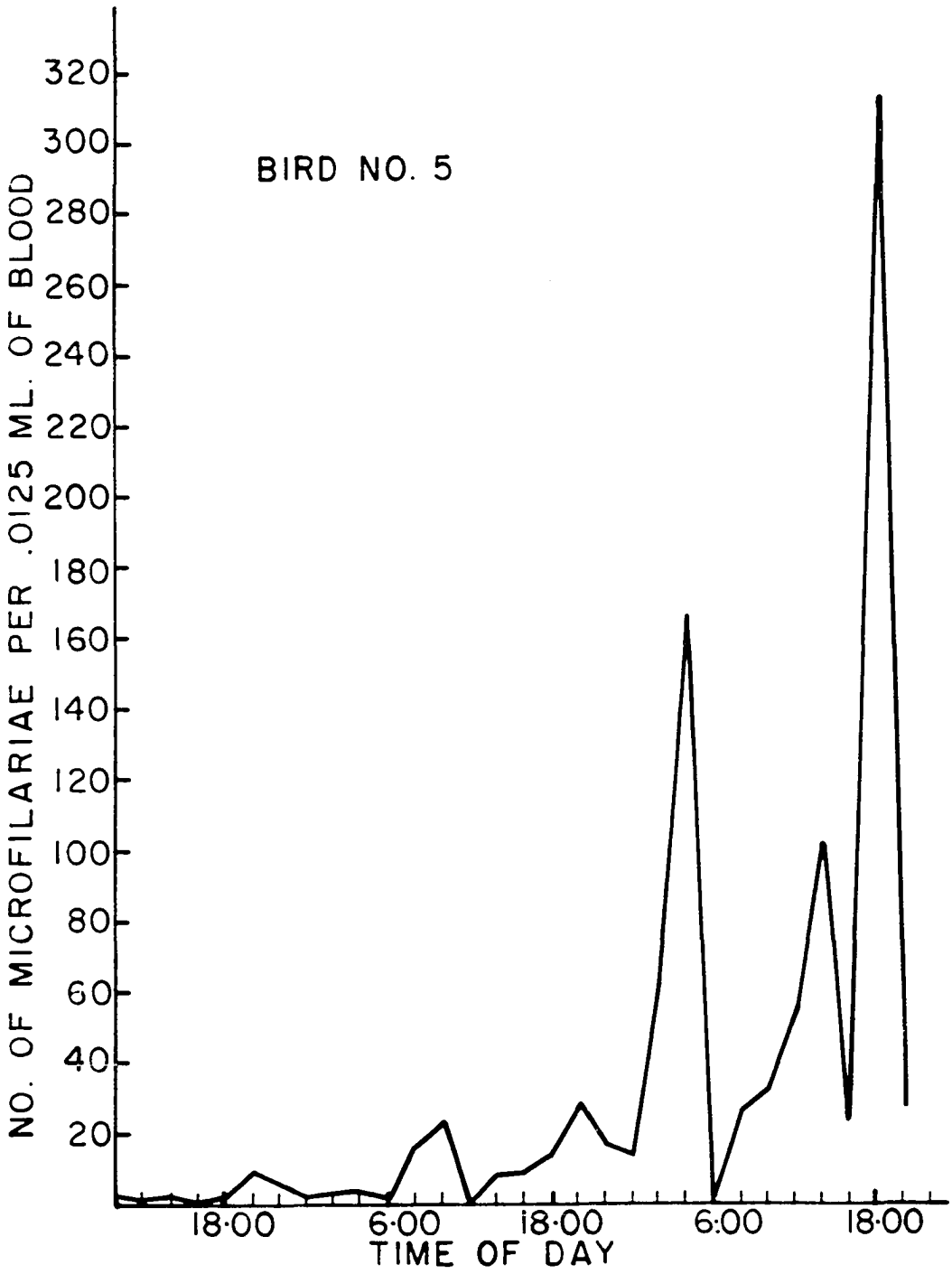
Re-establishment of normal periodicity after reversal

Only three of the infected experimental birds used in previous experiments were employed in this study. Birds Nos. 5 and 7 died on February 20, 1960, and bird No. 10 died on March 5, 1960. One of the birds, (No. 5) was examined immediately after death and no unusual pathological changes were observed.

Because of the unusually large number of microfilariae observed in the peripheral blood circulation of some of the birds exposed to five days of continuous light, and the apparent disruption of periodicity, an

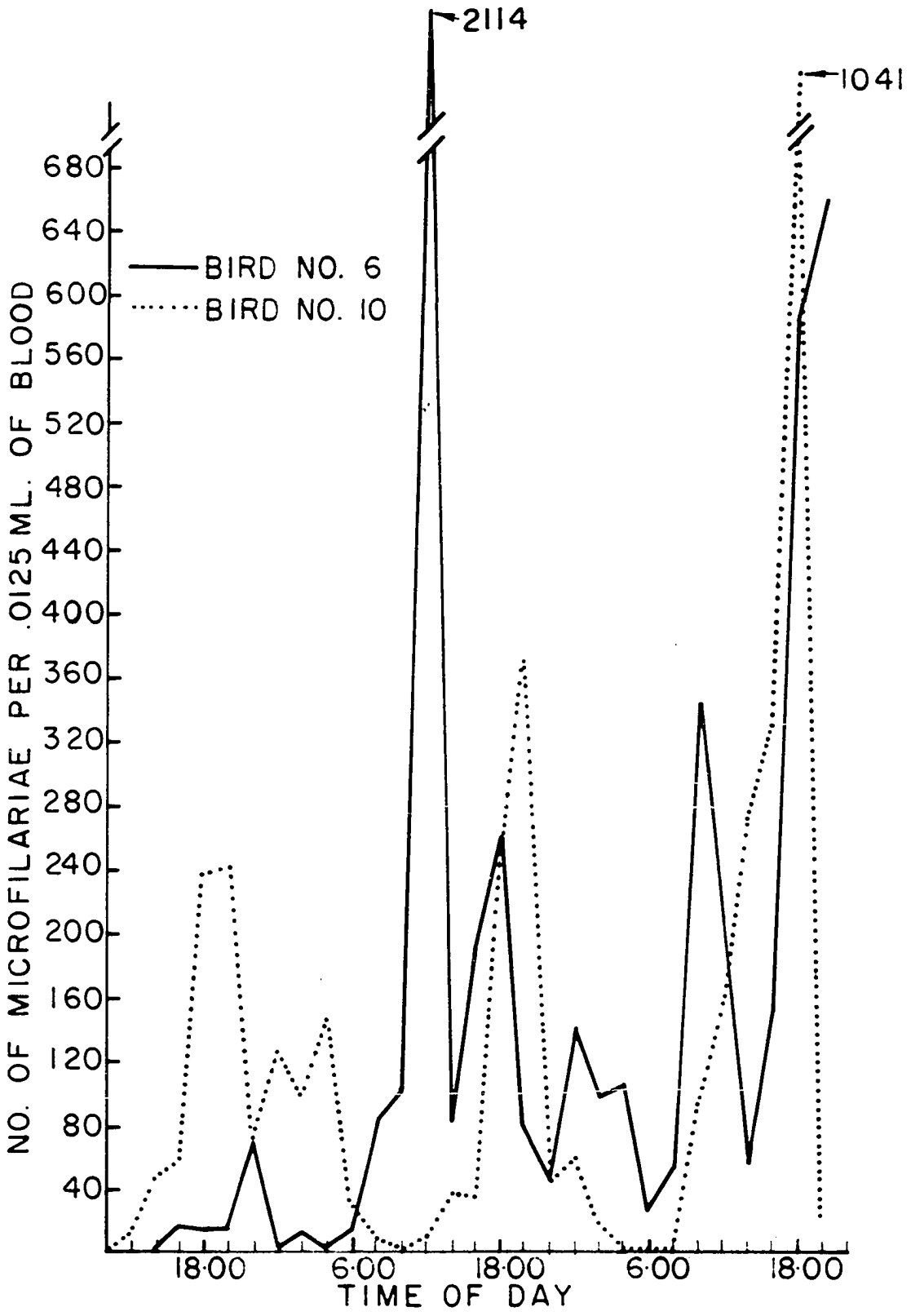
Graph 6. Effect of continuous light on periodicity of *Microfilaria quiscali*

(All birds remained in light from 800 on February 10, 1960 to 2000 on February 15, 1960.)


BIRD NO. 5
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
320
300
280
260
240
220
200
180
160
140
120
100
80
60
40
20
18·00
6·00
18·00
6·00
18·00
TIME OF DAY

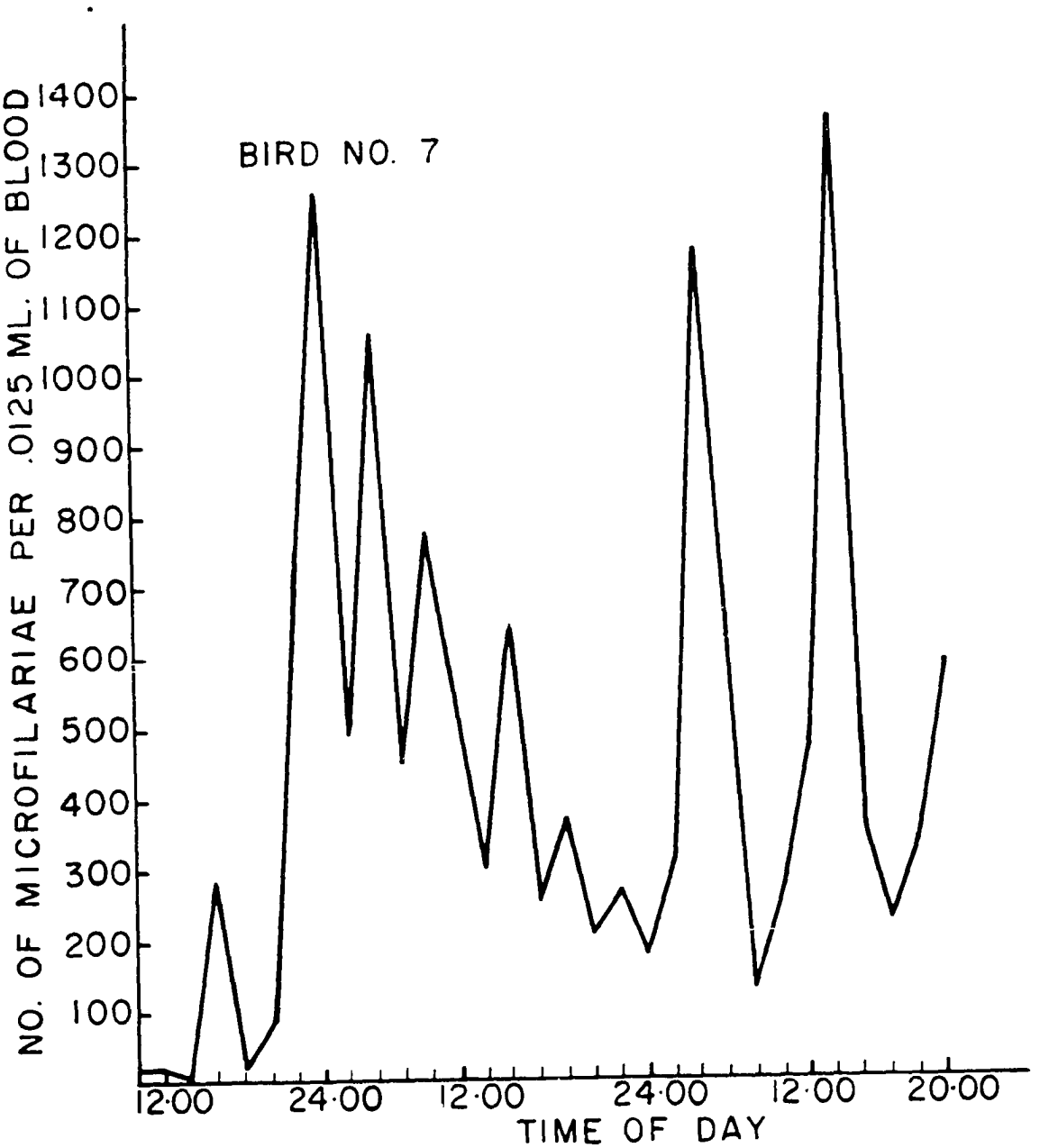
Graph 7. Effect of continuous light on periodicity of *Microfilaria quiscali*

(All birds remained in light from 800 on February 10, 1960 to 2000 February 15, 1960.)


2114
1041
680
640
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
BIRD NO. 6
BIRD NO. 10
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
18·00
6·00
18·00
6·00
18·00
TIME OF DAY

Graph 8. Effect of continuous light on periodicity of *Microfilaria quiscali*

(All birds remained in light from 800 on February 10, 1960 to 2000 February 15, 1960.)


BIRD NO. 7
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
12·00
24·00
12·00
24·00
12·00
20·00
TIME OF DAY

Graph 9. Effect of continuous light on periodicity of Microfilaria quiscali

(All birds remained in light from 800 on February 10, 1960 to 2000 February 15, 1960.)

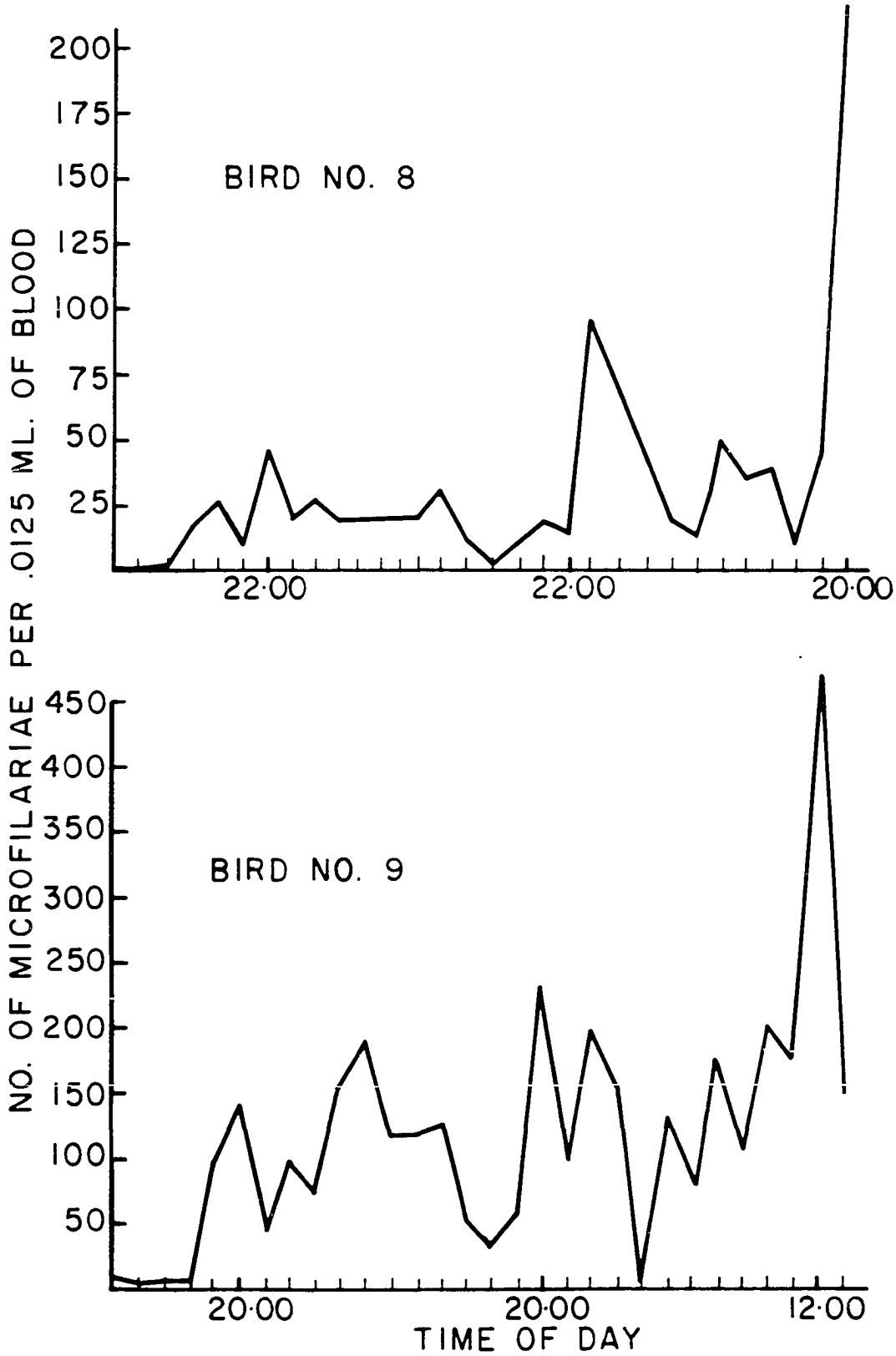

BIRD NO. 8
200
175
150
125
100
75
50
25
22·00
22·00
20·00
BIRD NO. 9
450
400
350
300
250
200
150
100
50
20·00
20·00
12·00
TIME OF DAY
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD

Table 11. Effect of continuous light[a] on periodicity[b] of *Microfilaria quiscali*

Date	Time	Grackle number 5	6	7	8	9	10
February 13, 1960	1000	2	1	22	0	10	0
"	1200	1	1	21	0	5	13
"	1400	2	4	12	2	7	44
"	1600	0	18	282	17	7	58
"	1800	2	15	21	25	96	238
"	2000	9	16	86	11	137	240
"	2200	7	69	724	44	46	63
"	2400	2	3	1257	21	87	127
February 14, 1960	200	4	12	495	26	77	99
"	400	4	4	1062	21	103	148
"	600	2	18	451	21	188	36
"	800	16	83	782	20	122	10
"	1000	23	103	299	21	121	3
"	1200	0	2114	637	28	126	9
"	1400	8	82	256	11	52	37
"	1600	9	188	372	3	34	35
"	1800	14	262	199	11	57	251
"	2000	28	80	265	17	229	369
"	2200	17	46	178	14	98	45
"	2400	14	142	302	94	195	61
February 15, 1960	200	83	99	1174	65	155	21
"	400	166	106	265	44	18	2
"	600	2	27	132	18	131	3
"	800	26	54	274	13	82	4
"	1000	31	346	472	50	173	91
"	1200	53	209	1362	30	108	162
"	1400	101	60	366	35	200	273
"	1600	23	152	224	9	179	330
"	1800	313	583	330	46	469	1041
"	2000	27	660	690	204	148	23

[a]All birds remained in light from 800 on February 10, 1960, to 2000 on February 15, 1960.

[b]Approximately 0.0125 ml. blood was withdrawn from metarsal vein of bird every two hours.

attempt was made to discover if the normal (nocturnal) periodicity could be re-established under conditions of regularly alternating periods of darkness and artificial light. The birds now received artificial light from 6 A.M. to 6 P.M. every day. Blood examinations were made in the manner previously described every two hours for 72 consecutive hours between 2 P.M., March 7, 1960, and 12 noon, March 10, 1960.

The results of this investigation, as shown on Graph 10 and Table 12 indicate that the normal nocturnal periodicity had been re-established.

Effect of continuous darkness on periodicity

Birds Nos. 6, 8, and 9 were put in a dark room at 6 P.M. on March 30, 1960, and remained in darkness until 4 A.M. on April 4, 1960 when the experiment was terminated. Blood examination, however, was begun at midnight on April 2, 1960. Birds were not exposed to light throughout the duration of the experiment except for a maximum period of about one minute every two hours when blood was withdrawn in an adjoining room. A spot light was used at this time so as to minimize exposure of the birds to light.

All techniques employed were the same as those already described for previous experiments, and results are shown on Graphs 11 and 12 and in Table 13. Despite the exclusion of light from March 30 to April 4, the microfilariae apparently maintained their normal nocturnal periodicity which had been re-established in previous experiments.

Graph 10. Re-establishment of normal periodicity of *Microfilaria quiscali* after reversal and 5 days of continuous exposure to light

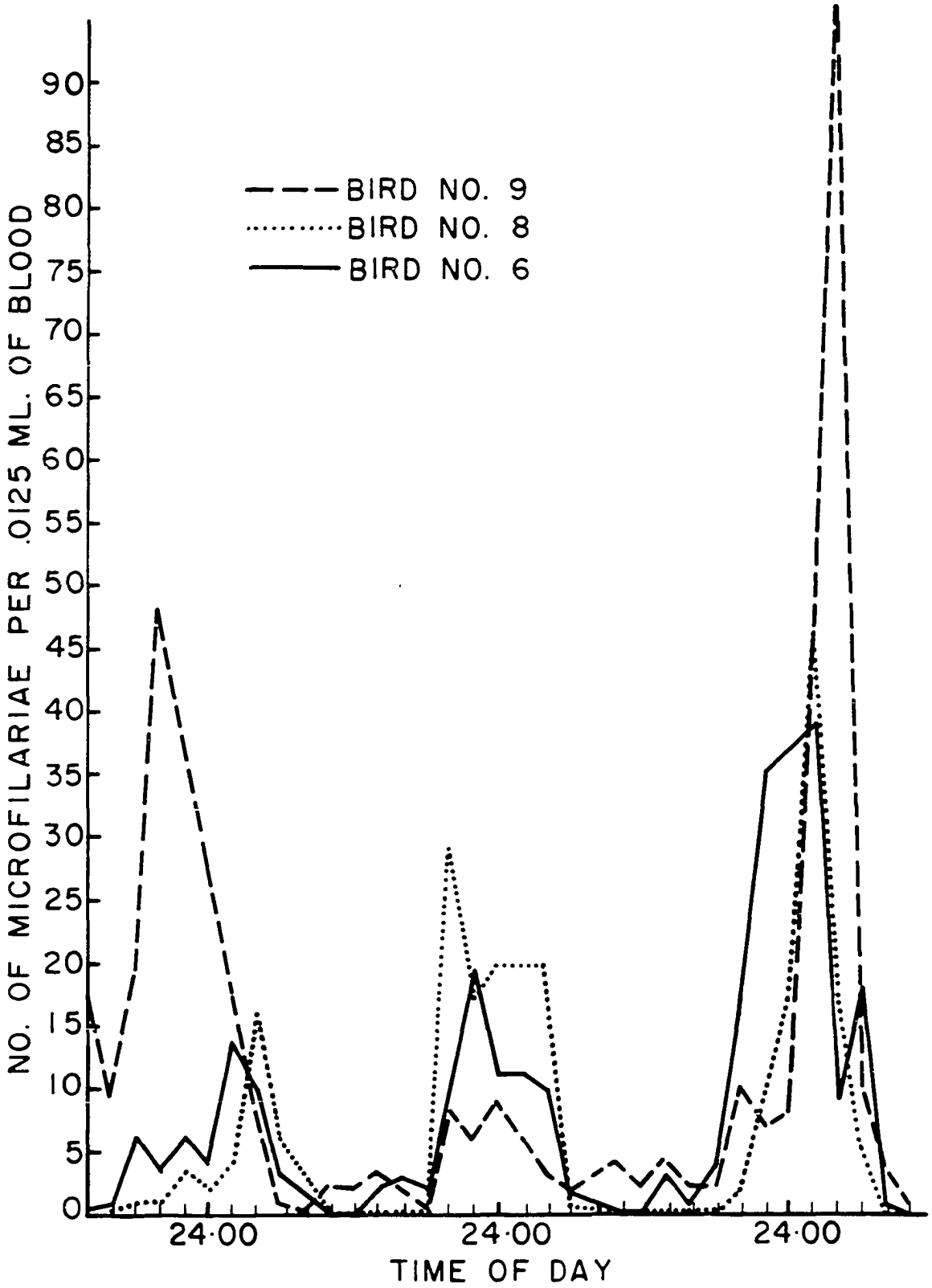

BIRD NO. 9
BIRD NO. 8
BIRD NO. 6
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
0
5
10
15
20
25
30
35
40
45
50
55
60
65
70
75
80
85
90
24·00
24·00
24·00
TIME OF DAY

Table 12. Re-establishment of normal periodicity[a] of Microfilaria quiscali after reversal and 5 days of continuous exposure to light

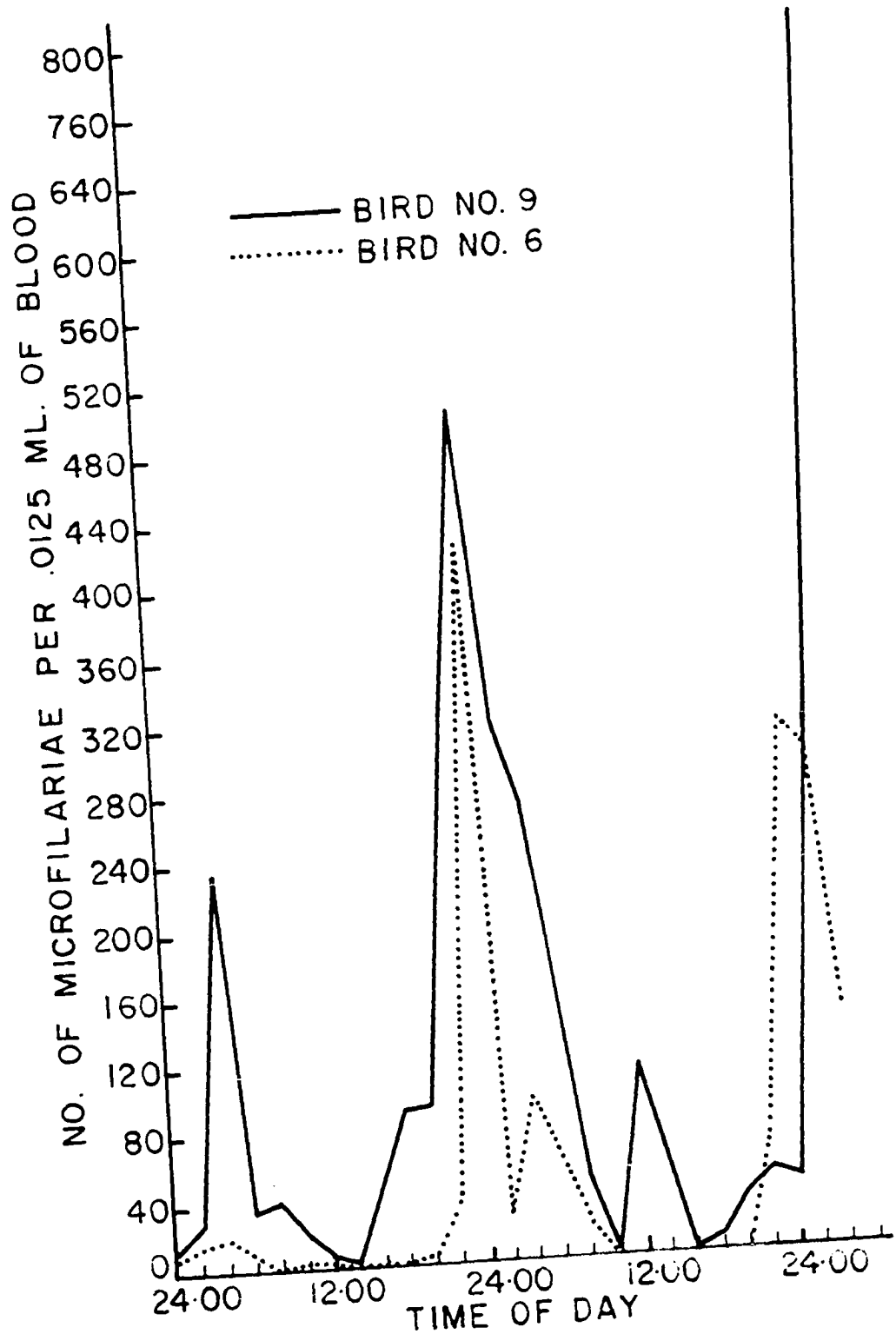
Date	Time	Room condition	Grackle number 6	8	9
March 7, 1960	1400	Artificial light	0	0	18
"	1600	"	1	0	9
"	1800	Darkness	6	1	19
"	2000	"	4	1	48
"	2200	"	6	3	*[b]
"	2400	"	4	2	28
March 8, 1960	200	"	14	4	18
"	400	"	10	16	9
"	600	"	3	6	1
"	800	Artificial light	1	0	0
"	1000	"	0	0	2
"	1200	"	0	0	2
"	1400	"	2	0	3
"	1600	"	3	0	2
"	1800	"	2	0	1
"	2000	Darkness	10	29	8
"	2200	"	19	17	6
"	2400	"	11	20	9
March 9, 1960	200	"	11	9	6
"	400	"	10	20	3
"	600	"	2	1	2
"	800	Artificial light	Preparation not made		
"	1000	"	0	0	4
"	1200	"	0	0	2
"	1400	"	3	0	4
"	1600	"	1	0	2
"	1800	"	4	0	2
"	2000	Darkness	21	2	10
"	2200	"	35	10	7
"	2400	"	*	17	8
March 10, 1960	200	"	38	46	36
"	400	"	8	17	96
"	600	"	18	6	10
"	800	Artificial light	1	0	4
"	1000	"	0	0	1

[a]Approximately 0.0125 ml. blood was withdrawn from metatarsal vein of each bird every two hours.

[b]Slide of prepared smear was broken.

Graph 11. Effect of continuous darkness on periodicity of *Microfilaria quiscali*

(All birds remained in darkness from 6 P.M. on March 30, 1960 to 4 A.M. on April 4, 1960.)


BIRD NO. 9
BIRD NO. 6
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
800
760
640
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
24·00
12·00
24·00
12·00
24·00
TIME OF DAY

Graph 12. Effect of continuous darkness on periodicity of *Microfilaria quiscali*

(The bird remained in darkness from 6 P.M. March 30, 1960 to 4 A.M. on April 4, 1960.)

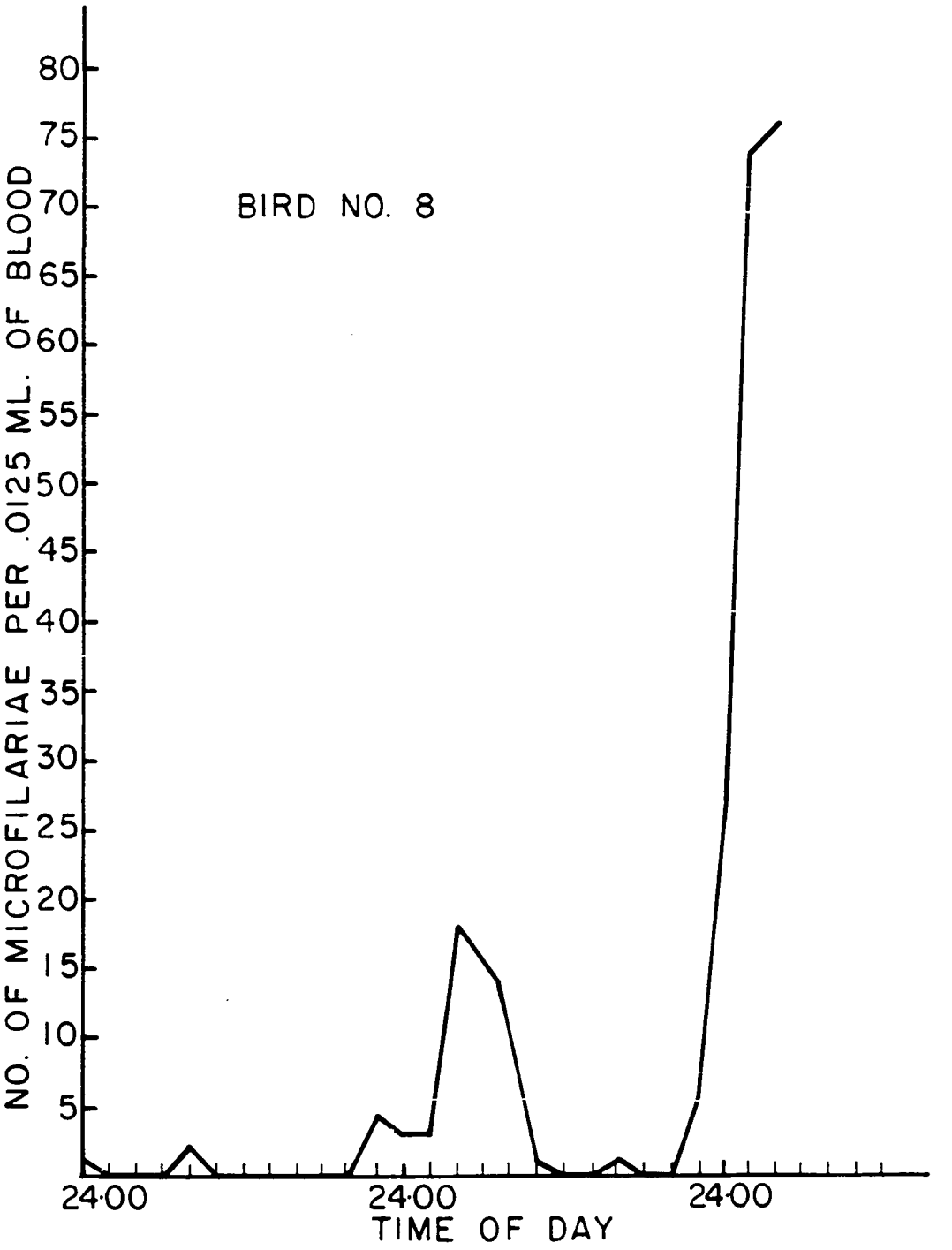

BIRD NO. 8
NO. OF MICROFILARIAE PER .0125 ML. OF BLOOD
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
24·00
24·00
24·00
TIME OF DAY

Table 13. Effect of continuous darkness[a] on periodicity[b] of Microfilaria quiscali

Date	Time	Grackle number 6	8	9
April 2, 1960	0005	4	1	12
"	200	15	0	28
"	400	19	0	235
"	600	10	0	34
"	800	1	2	41
"	1000	3	0	20
"	1200	3	0	7
"	1400	0	0	3
"	1600	0	0	19
"	1800	2	0	90
"	2000	6	0	93
"	2230	36	4	313
"	2400	419	3	499
April 3, 1960	200	29	3	317
"	400	97	18	267
"	600	Preparation not made		
"	830	16	14	45
"	1000	0	1	7
"	1200	0	0	114
"	1400	1	0	57
"	1600	0	1	2
"	1800	0	0	8
"	2000	2	0	29
"	2200	60	6	45
"	2400	310	27	41
April 4, 1960	200	293	74	311
"	400	141	76	805

[a]All birds remained in darkness from 1800 (6 P.M.) on March 30, 1960 to 400 (4 A.M.) on April 4, 1960.

[b]Approximately 0.0125 ml. blood was withdrawn from metatarsal vein of each bird every two hours.

Transfusion Experiments

Transfusion into natural hosts

Lane's theory of simultaneously-timed daily production of microfilariae as a possible cause of periodicity has been challenged by many parasitologists despite (O'Connor's claims (1931, 1932) of histological evidence in support of the theory. Low and Manson-Bahr (1933) and many others criticised the theory because of the effects of daily absorption of millions of dead microfilariae, and because microfilariae can be kept alive outside the host for many days. However, convincing proofs of Lane's theory were sought from results of experiments involving the injection of microfilariae into filaria-free animals. A brief review of such transfusion experiments follows.

Fülleborn (1908) injected 6.5 cc of blood containing approximately 468,000 microfilariae of Dirofilaria immitis into a young puppy. The microfilariae persisted for nine months. Fülleborn, however, did not take into account the possibilities of natural infection by mosquitoes or by prenatal infection via the placenta, as Hinman, Faust and DeBakey (1934) later pointed out.

Fülleborn (1929) further recorded that microfilariae of Dirofilaria repens persisted in the blood of a recipient dog for two years and in the lungs for 2-3/4 years.

Murgatroyd (1933) injected a human volunteer intravenously with blood containing about 720,000 microfilariae of Wuchereria bancrofti but failed to recover any from ear-blood of the recipient.

Hinman, Faust, and DeBakey (1934) made a mutual exchange of 140 cc

of blood of two dogs (A and B) weighing 12 and 10 pounds respectively. At the time of the exchange, Dog A's blood contained 27,475 microfilariae of Dirofilaria immitis per cc whereas Dog B's had none. Hinman and his colleagues discovered that 92% of the injected microfilariae never showed up in the peripheral blood of Dog B, but a few active microfilariae persisted for ten weeks. The level of microfilarial content was lowered for six succeeding days in Fog A but returned to the original level in two weeks.

Low and Manson-Bahr (1933) injected into the heart of each of two guinea pigs 1 cc of human blood containing approximately 139,000 microfilariae of Wuchereria bancrofti. No microfilaria was ever recovered from blood obtained from pricking the ears of these animals.

Augustine and Drinker (1935), working with Dirofilaria immitis, injected 1,000 cc of heparinized blood containing approximately 310,000 microfilariae per cc (on the morning of transfusion) into a "clean" dog after withdrawing 450 cc of its blood. They established the fact that within the hour, microfilariae migrated into the lymph, where 400 per cc were found. However, no study was made of the longevity of the transfused microfilariae.

Knott (1935) while working in the Virgin Islands on Wuchereria bancrofti, performed eight transfusion experiments with humans, the blood of six of whom revealed no evidence of microfilariae before transfusion, while the other two had known numbers of microfilariae per cc of blood. Knott set out to discover how long microfilariae would live in a "clean" host, and whether the injection of large numbers of microfilariae (2,000,000 and 900,000 in this instance) into a patient could cause a

corresponding rise of microfilariae in the circulating blood. His experiments led him to conclude (1) that microfilariae injected into uninfected hosts are "filtered out" completely from blood circulation from within a few minutes to 14 days after injection, and (2) that injected microfilariae could cause a temporary rise of them in the blood. Such an increase, however, lasted for only two and one-half hours in a patient. Knott reported that he observed an apparent periodicity in one of his experiments, but considered his results as inconclusive.

Rao (1936) injected citrated blood containing 120,000 microfilariae of Wuchereria bancrofti into a human volunteer, but failed to recover any despite many examinations.

Hawking (1940) performed transfusion experiments on four humans, some mice, rats, rabbits, a guinea pig and a monkey. Microfilaria bancrofti was used in all these experiments. Humans were injected intravenously, but some of the experimental animals were injected intraperitoneally or subcutaneously. Hawking discovered that two of the humans never exhibited any microfilariae in their blood while the blood of the other two retained the parasites for nine and "more than 8" days, respectively. No characteristic periodicity was observed. Microfilariae could not be demonstrated in the blood of animals for more than 30 hours after intravenous injection. Animals injected intraperitoneally and subcutaneously never exhibited microfilariae in their blood but retained them alive for about seven days and 40 hours, respectively.

Gönnert (1942) reported that the microfilariae of Loa loa persisted in recipient human volunteers for only three days, while those of Acanthocheilonema perstans persisted for three years.

Hawking (1953) proved through a transfusion experiment (already noted in a previous section) that microfilariae of Dirofilaria repens can be successfully transfused from one dog to another. He was able to transfer large numbers from an infected dog into an uninfected one where they remained 68 days. Moreover, he showed graphically the very close similarity between the periodicity exhibited by the donor and that of the recipient after transfusion. Unfortunately, Hawking was not certain whether or not the recipient dog was free from infection, neither was he quite sure of its clinical history at the onset of his experiment. However, he stated: "This dog was bought in England (where Dirofilaria repens does not occur) and owing to quarantine regulations, it is extremely improbable that it had ever been out of the country."

It is apparent from the foregoing that results of transfusion experiments vary greatly, not only among different species of microfilariae, but even when microfilariae of the same species are employed.

In these studies, transfusion experiments were carried on by obtaining blood from recently-killed, naturally-infected grackles. Blood was taken from the heart, lungs, and adjoining large arteries and veins and was diluted either with sodium citrate solution, or with the blood diluting fluid already described in a previous section. This citrated blood was then injected into a recipient grackle by the use of a 27-gauge hypodermic needle. All recipient birds were carefully checked to make certain they were free from microfilarial infection. Donor birds were never killed with anaesthetics for fear of their possible deleterious effects on microfilariae. Injected grackles received only local anaesthetics for fear of their possible deleterious effects on microfilariae.

Injected grackles received only local anaethetics at the site of inoculation. Since the size of the veins of grackles made intravenous injections very difficult, many injections were made intraperitoneally and occasionally intramuscularly.

Four transfusion experiments were performed between May and November, 1959. The birds used in these experiments, although not laboratory-reared, had been checked repeatedly for presence of microfilariae, but in all cases the peripheral blood was found to be free from them.

Grackle No. 20 was injected intraperitoneally on May 5, 1959, with 0.4 ml. of citrated microfilaremic blood containing an undetermined number of microfilariae while No. 21 received 0.8 ml. partly intravenously, but mainly intraperitoneally. When both were sacrificed on May 22, neither showed any microfilariae in the peripheral circulation. Grackle No. 22 was transfused intraperitoneally on July 1, 1959, with 2 ml. of citrated blood containing approximately 20,000 microfilariae. Daily examination of the peripheral blood of this bird never revealed the presence of any microfilariae even after 31 days. It was sacrificed in May, 1960, and necropsied. On November 10, 1959, the fourth bird, No. 23, was inoculated with approximately 100,000 microfilariae, partly intravenously and partly intramuscularly. No microfilariae were ever recovered from the peripheral blood circulation between November 12, 1959, and March 15, 1960.

Despite the apparent lack of microfilariae in the peripheral blood in all these experimental birds, some microfilariae were found in two of them after the animals had been sacrificed. In birds Nos. 20 and 22, for example, both of which were transfused entirely intraperitoneally, neither

microfilariae nor adults were found anywhere in their bodies, and no unusual pathological effects attributable to filarial infection were observed. However, in the other two birds (Nos. 21 and 23) which had been injected in part intravenously, some living microfilariae were observed in blood taken from their hearts and lungs. In each case, one adult female worm was recovered from the brain. The female worms recovered from the brains of these transfused birds (at least in the case of No. 23, which was very carefully studied) could not have produced the microfilariae found in these birds at necropsy, for these females, although full of eggs, contained no recognizable microfilariae.

The occurrence of only female worms had been noticed in three other birds during this investigation, and in all these, no microfilariae were present anywhere in the blood or tissues of the birds. It is therefore unlikely that the adult worms recovered from the cranial region of the transfused birds developed from microfilariae experimentally injected. Three possible explanations for the presence of microfilariae in these birds (Nos. 21 and 23) are (1) that adult Splendidofilaria quiscali inhabit some parts of the host's body other than the brain, (2) that copulation takes place before adults reach the brain, and (3) that some adults (including all the males) died and were completely absorbed after reaching the brain, and that females recovered from the injected birds were not inseminated before these deaths occurred.

Until the above can be proven experimentally, it appears likely that microfilariae injected into a natural host may live for at least four months.

Transfusion into unnatural hosts

Transfusion of filaremic blood into natural hosts has been performed by many investigators with varying results, but very few successful experiments of transfusion into unnatural hosts have been reported. Hawking (1940), working with Wuchereria bancrofti, performed many experiments on transfusion into mice, guinea pigs, monkeys, rabbits, and rats. He, like his predecessors, concluded that microfilariae lived in the recipients for a short period of time. From his experiments, he concluded that microfilariae lived for a maximum of seven days in animals injected intraperitoneally, but they "rapidly disappeared from the circulating blood within 20-30 hours" in animals injected intravenously. However, in animals injected intramuscularly, microfilariae were never observed in the blood, and of the 7,000 microfilariae he injected intramuscularly only one was found in the scrapings from under the surface of the skin at the site of inoculation.

In this investigation, five experiments were performed to determine (1) how long microfilariae of S. quiscali could be observed in the peripheral blood of inoculated chickens and (2) whether any periodicity could be observed in the recipient birds.

Experiment 1 A chicken, 15 days old, was transfused on June 5, 1959, with approximately 10,000 microfilariae of S. quiscali. Injections were in part intravenous and in part intraperitoneal. The original intention was to inject 2 ml. of citrated filaremic blood into the brachial vein, but because the unanesthetized bird moved during the operation, only about 0.75 ml. was injected intravenously. The remaining 1.25 ml. was injected intraperitoneally.

Stained preparations of drops of blood withdrawn from the metatarsal vein were made daily until July 3, 1959. All smears contained between one to seven microfilariae per slide.

On July 4, 1959, it was decided to check whether microfilariae were still alive at the time the smears were made. Hence, fresh preparations were observed daily until July 20, 1959. The results of this experiment are presented in Table 14.

Table 14. Results of blood examination of chicken No. 1, injected in part intravenously and in part intraperitoneally with approximately 10,000 Mf. on June 3, 1959

Date	No. of smears	Time of day	Type of smear	No. of Mf. present
June 10, 1959	1	12:05 A.M.	Stained	2
" 15 "	1	"	"	3
" 18 "	1	"	"	3
" 19 "	4	"	"	3,2,2,1
" 25 "	1	"	"	1
July 1, 1959	1	"	"	2
" 3 "	1	1:30 A.M.	"	7
" 4 "	1	"	Fresh	0
" 4 "	2	"	"	0.1 (living)
" 6 "	1	"	"	1 (living)
" 9 "	2	"	"	0
" 12 "	2	"	"	0
" 17 "	1	"	"	0
" 20 "	2	"	"	0,0

From this experiment it is evident that microfilariae injected intravenously into a 15-day-old chicken lived in the unnatural host for at least 31 days.

Experiment 2 Two chickens (Nos. 2 and 3) aged five and six weeks, respectively, were each transfused on July 1, 1959, with 2 ml. of citrated filaremic blood from grackles. This amount of blood contained approximately 20,000 microfilariae. A 27-gauge hypodermic needle was used. Bird No. 2 was injected intraperitoneally, while No. 3 was inoculated intravenously.

Blood smears of these birds were made at irregular intervals between July 1 and July 21, 1959. Both birds were sacrificed on August 6, 1959, and necropsied. The hearts, lungs, and spleens of both birds were fixed in AFA, embedded in paraffin, sectioned, stained with Delafield's haematoxylin and counterstained with fast green, eosin or erythrosin.

Chicken No. 2 injected intraperitoneally never showed any microfilariae in its blood throughout the duration of the experiment, neither were any found in the heart or lungs at necropsy. No blood clot was found in the peritoneal cavity. Sections of the various internal organs revealed no microfilariae, and both macroscopically and microscopically the internal organs were apparently normal.

Chicken No. 3, injected intravenously, showed actively moving microfilariae between July 3 and July 17, 1959, in blood removed from its metatarsal veins. Data obtained from this investigation are presented on Table 15. Microfilariae lived for at least 16 days in the injected chicken. No pathological lesions were discovered in histological sections of internal organs.

In intravenously injected bird No. 3, the microfilariae were observed in the peripheral circulation for at least 16 days after injection whereas no microfilaria was ever observed in the peripheral blood circulation of

chicken No. 2 injected intraperitoneally.

Table 15. Results of blood examination of Chicken No. 3 injected intravenously with approximately 20,000 microfilariae on July 1, 1959

Date of examination	No. of smears made	Time of day	Type of blood preparations examined	No. of mf. observed
July 3, 1959	1	1:30 A.M.	Stained	34
" 4 "	1	1:00 A.M.	Fresh	10
" 5 "	1	"	"	2
" 6 "	1	9:00 A.M.	"	5
" 8 "	3	"	"	3, 1, 1
" 12 "	1	"	"	2
" 17 "	1	"	"	1
" 17 "	1	1:00 P.M.	"	0
" 19 "	2	9:00 A.M.	"	0,0
" 20 "	3	1:00 A.M.	"	0,0,0
" 21 "	4	1:00 A.M.	"	0,0,0,0

Experiment 3 Results of Experiments 1 and 2 indicate that microfilariae of S. quiscali may live in a chicken for as long as 31 days. Two more experiments (Nos. 3 and 4) were therefore performed in order to determine whether transfused microfilariae in chickens would show the same nocturnal periodicity characteristic of infections in grackles.

On October 14, 1959, chicken No. 4 aged 32 days, was intravenously transfused with two ml. of citrated blood from an infected grackle. Approximately 350,000 microfilariae were injected. The bird was maintained in an animal room possessing no windows, so that during the daytime artificial light was employed.

Blood was withdrawn from the metatarsal veins for a period of three

days (between October 15 and 18) at 9 A.M. Examination of stained blood films revealed no microfilariae. However, fresh preparations of blood from the same chicken examined on subsequent days (October 19, 20, and 21) revealed 1, 3, and 3 actively moving microfilariae, respectively.

On October 22, 1959, the chicken was removed to a room with large glass windows and left undisturbed for four days to allow it to become accustomed to conditions ensuring normal daylight and darkness. At 12:05 A.M. on October 26 and on every succeeding two hours for 26 consecutive hours stained, (and sometimes fresh) preparations of approximately 0.01 ml. of blood withdrawn from the metatarsal veins were examined. All microfilariae on each slide were counted and recorded. Data obtained from this investigation are presented on Table 16.

Examination of about 0.02 ml. of blood withdrawn from the matatarsal vein of the chicken on November 12, 1959 revealed one actively moving microfilaria. Examinations of the bird's blood on six subsequent days, however, revealed no microfilariae and hence no further examinations were undertaken.

The data in Table 16 indicate that microfilariae of S quiscali show their characteristic nocturnal periodicity even in an unnatural host. There were more in the peripheral blood circulation of the chicken between 8 P.M. and 6 A.M. and they almost disappeared from the peripheral circulation between 8 A.M. and 6 P.M. When the number of microfilariae are expressed per millimetre of blood (as is often done by many parasitologists), this periodicity becomes more apparent. Furthermore, the experiment showed that some of the injected microfilariae were alive in

the chicken 29 days after injection.

Table 16. Observations on the periodicity of Mf. quiscali in chicken No. 4[a]

Date	Time of day	No. of mf. in fresh prep.	No. of mf. in stained slide[b]
October 26, 1959	12:05 A.M.	1	1
"	2:05 A.M.	4	12
"	4:00 A.M.	1	3
"	6:00 A.M.	?	3
"	8:00 A.M.	1	1
"	10:00 A.M.	0	1
"	12:00 Noon	0	0
"	2:00 P.M.	0	0
"	4:00 P.M.	Prep. not made	0
"	6:00 P.M.	"	1
"	8:00 P.M.	"	4
"	10:00 P.M.	"	2
October 27, 1959	12:15 A.M.	"	2
"	2:30 A.M.	"	3
November 12, 1959	(Not recorded)	1	Prep. not made
November 13-18, 1959	"	0	0

[a]Chicken No. 4 had been injected intravenously on October 14 with filaremic blood containing about 350,000 microfilariae.

[b]Each slide contained .01 ml. blood removed from the metatarsal veins.

Experiment 4 Since the results of Experiment No. 3 indicated that microfilariae of S. quiscali show characteristic nocturnal periodicity, even when in an unnatural host, it was decided to confirm these findings by another experiment.

On November 10, 1959, chicken No. 5 (aged 19 days) was injected with approximately 2 ml. filaremic blood containing about 200,000 microfilariae of S. quiscali, and placed in the animal room that ensured conditions of natural daylight and darkness. At 2:00 A.M. on November 12, 1959, and on succeeding two-hour intervals for 72 consecutive hours, approximately 0.01 ml. of blood was withdrawn in the manner described under methods of blood examination. Each slide was air-dried, fixed in absolute methyl alcohol and stained with Giemsa's stain. All microfilariae on each slide were carefully counted and recorded. The data obtained from the investigation are presented on Table 17.

This experiment (like the preceding one) indicates that Microfilaria quiscali when injected in sufficiently large quantity into a chicken will exhibit the same nocturnal periodicity characteristic of infections in grackles. The injected chicken was approximately four times the size of a grackle by weight and 2 ml. of the citrated blood was used, hence the comparatively few microfilariae observed in each 0.01 ml. sample of blood removed at each examination. It is significant, however, that the microfilariae were almost completely absent in the peripheral blood circulation between 8 A.M. and 6 P.M. as is the case in infected grackles.

Table 17. Result of periodicity experiment on the approximately 200,000 Mf. quiscali injected intravenously into chicken No. 5 on November 10, 1959

Date of examination	Time of day	No. of mf. per 0.01 ml. of blood
November 12, 1959	2:00 P.M.	0
"	4:00 P.M.	1
"	6:00 P.M.	3
"	8:00 P.M.	1
"	10:00 P.M.	2
"	12:00 P.M.	1
November 13, 1959	2:00 A.M.	2
"	4:00 A.M.	2
"	6:00 A.M.	1
"	8:00 A.M.	0
"	10:00 A.M.	0
"	12:00 Noon	0
"	2:00 P.M.	1
"	4:00 P.M.	0
"	6:00 P.M.	1
"	8:00 P.M.	2
"	10:00 P.M.	2
November 14, 1959	12:05 A.M.	2
"	2:00 A.M.	2
"	4:00 A.M.	2
"	6:00 A.M.	1
"	8:00 A.M.	0
"	10:00 A.M.	1
"	12:00 Noon	0
"	2:00 P.M.	0
"	4:00 P.M.	0
"	6:00 P.M.	0
"	8:00 P.M.	1
"	10:00 P.M.	1
November 15, 1959	12:05 A.M.	2
"	2:00 A.M.	3
"	4:00 A.M.	1
"	6:00 A.M.	3
"	8:00 A.M.	0
"	10:00 A.M.	0
"	12:00 Noon	0

Experimental Attempts to Determine Intermediate Hosts

One of the most puzzling problems facing parasitologists is that of elucidating life cycles of avian filarial worms. Although as early as 1878 according to Manson-Bahr (1954), Patrick Manson studied the microfilariae of "Filaria picae mediae" of the Chinese crow, the life cycle of but one member of the family Onchocercidae (= Dipetalonematidae) has been published, namely Ornithofilaria fallisensis by Anderson (1956). Many investigators have approached the problem epizootiologically, and a great number have unsuccessfully attempted to incriminate nest-inhabiting arthropods as intermediate hosts.

Dutton (1905) discovered microfilariae in lice (subfamily Leiothinae) taken from nests of swifts (Cypselus affinis Gray) in Africa, but nothing is known regarding the suitability of lice as transmitting agents of avian filarial worms. Thomas (1931), while working on Leucocytozoon parasites of ducks in Michigan, observed a microfilaria in the stomach of a simuliid fly, but did not pursue the investigation. Jellison (1940), in his studies on the fauna of birds' nest in Minnesota, reported the occurrence of 21 parasitic arthropods in 64 nests examined, and suggested that Culicoides might serve as vectors for filarial worms of magpies and crows (family Corvidae). Chernin (1953) observed that laboratory-reared white Pekin ducks (Anas boschas) became naturally infected with an unknown filarioid and showed microfilariae in their blood when these birds were left in the open where they were subject to attack by numerous invertebrates. He could not, however, recover adult worms at necropsy. Robinson (1955 a & b) made very intensive studies on the

epizootiology of avian filarial worms, attempted experimental transmission of dipetalonematid worms, but failed to discover the natural vectors involved. Although he allowed more than a thousand mosquitoes of 13 species and strains to bite infected crows and blue jays, he observed little or no subsequent development. Robinson also examined various species of mites, lice, ticks, and hippoboscid flies recovered either from the nests or from the bodies of crows and jays but found them all negative for microfilariae. From his studies he concluded that it was doubtful whether mites, ticks, tabanid flies, and other ectoparasites were involved in the transmission of avian filarial worms.

Anderson (1956) worked out the life cycle of Ornithofilaria fallisensis, described and illustrated the various developmental stages in the intermediate hosts. Anderson discovered that microfilariae of O. fallisensis develop to the infective stage in many species of Simuliidae but observed that mosquitoes and Culicoides did not serve as vectors for this species.

In the present investigations, many experiments were conducted in attempts to elucidate the life cycle of Splendidofilaria quiscali. Four species of mosquitoes (Culex pipiens, Culex tarsalis, Aedes triseriatus, and Aedes aegypti) and two species of mites (Ornithonyssus sylviarum and Dermanyssus gallinae) were studied intensively. Laboratory-reared Simulium spp. and Stomoxys calcitrans were also investigated as possible vectors. The use of corn borer larvae (Pyrausta nubilalis Hübner) as possible experimental hosts was suggested by the account of Yoeli et al. (1958) who reported partial development of microfilariae of Dirofilaria immitis in wax moth larvae (Galleria mellonella Linn.). Both

of these species of moth belong to the same family (Pyralididae). The availability of large numbers of corn borer larvae made it possible for the writer to utilise them in many injection experiments dealing with microfilariae of Splendidofilaria quiscali.

Indirect transmission experiments involving these arthropods were not performed during the course of this investigation. No grackles were reared in the laboratory from eggs, and hence it was not possible to make absolutely certain that parasite-free birds were being used. Even though blood films were frequently negative for microfilariae, it was found that birds might be harbouring adult worms. For example, the necropsy of five apparently uninfected birds revealed the presence of 4, 3, 2, 1, and 1 adult female worms respectively, in the cranial region. Birds collected in nature did not reproduce in the laboratory and many unsuccessful attempts were made to rear nestlings. Since microfilariae of dipetalonematid worms pass through similar developmental stages in their intermediate hosts as indicated by Feng (1936) in the case of D. immitis, and Anderson (1956) in the case of Ornithofilaria fallisensis, it was decided to look for comparable stages in the various arthropods employed in these experiments even though indirect transmission experiments were not performed.

Culicidae

The possibility of culicid flies acting as vectors for Splendidofilaria quiscali was not overlooked, since many species of dipetalonematid worms have been successfully transmitted by dipterous insects belonging to the family Culicidae. Highby (1943), for example, reported mosquitoes as vectors for Dipetalonema arbuta and Dirofilaria scapiceps.

Faust (1949) listed 74 species of mosquitoes of the genera Culex, Aedes, Mansonia, Anopheles, and Psorophora in which microfilariae of Wuchereria bancrofti develop, and Hawking and Thurston (1951) transmitted many species of Dirofilaria using culicid flies.

Various methods were employed to obtain mosquitoes for colonization. Some specimens of adult Culex tarsalis were collected from a drainage ditch near Gilbert, Iowa, but these proved difficult to rear in the laboratory since only a few of them fed on exposed birds. However, those reared from eggs in the laboratory did bite birds and transmission experiments were performed with them. Larvae of Aedes triseriatus were obtained from a water-filled tree-hole in the vicinity of Ames. The Aedes aegypti, Culex pipiens and Culex tarsalis used in these experiments were reared in the laboratory from eggs supplied by Dr. C. G. Huff.

The rearing of the various species of mosquitoes in the laboratory was done in the same manner. The eggs of each species were placed in separate white enamel pans of water and examined daily for developing larvae which were then placed in separate water-filled pans approximately 16 inches by 10 inches by 3½ inches in size. Powdered brewer's yeast and dried whole wheat bread crumbs sprinkled on the surface of the water served as adequate diet for the larvae. A minute portion of brain heart infusion (Difco) was added every other day to supplement this ration. These ingredients often formed a scum on the water surface, and hence a piece of paper towelling was drawn over the water surface whenever necessary, in order to prevent the high larval mortality resulting from suffocation by the scum. This procedure proved to be a satisfactory method for cleaning the larval rearing pans without undue disturbance of

the organisms.

For purposes of identification, fourth instar larvae inactivated by refrigeration were used. Species allocation was determined by using the publications of King et al. (1939), Ross (1947), Horsfall (1955) and Stone et al. (1959).

Pupae were removed with pipettes into small pans of water and placed in a wooden rearing cage measuring 12 inches by 12 inches and 15 inches high. This cage, which is without a bottom, has glass on two sides, wood on one side, while the fourth is covered with muslin cloth equipped with a sleeve. This latter opening permits easy access into the cage. This cage was placed, open end down, in a metal tank 16 inches square and five inches deep, and a layer of sand, three inches deep, was added into the cage. Paper towels were placed over the sand and the small pan containing the pupae introduced through the open sleeve. The open sleeve was closed by tying it. By adding water to the metal tank, moisture filtered under the bottom edges of the cage and eventually saturated the sandy floor. This technique provided relative humidity which could be easily maintained.

When adults began to emerge, nourishment was provided for them by suspending inside each rearing cage balls of cheese cloth soaked in saturated sugar solution or boiled raisins enclosed in cheese cloth. Adult females were periodically supplied with blood meals by suspending a grackle in the cage. Pans of fresh water were provided for oviposition, and eggs were transferred into larval rearing pans for continuation of the colony.

Techniques employed to determine whether Microfilaria quiscali

underwent developmental changes characteristic of other dipetalonematids were the same for the four species of mosquitoes used. An infected grackle was put into a tightly-fitting jacket made from cheese cloth. The legs and the partly deplumed head and chest were left uncovered. About midnight (when there were many microfilariae in the peripheral circulation), the incapacitated grackle was suspended in a cage containing mosquitoes that had been starved for 24 hours.

Engorged females were aspirated into a lantern chimney whose open ends were covered with cheese cloth. The chimney was then put in a petri dish containing pieces of cheese cloth soaked in unsaturated sugar solution. The apparatus was put in a cool place and the mosquitoes were examined periodically. Examination consisted of dissection of anaesthetized mosquitoes in a drop of saline put on a microslide. A dissecting microscope was employed during this process and a thorough search for microfilariae was made. The number of microfilariae found, their location in the vector's body and the stage of development were recorded. In most of these experiments, dissected mosquitoes were not stained, and by the use of a compound microscope the stage of development was determined and recorded.

Seven experiments were performed, involving the feeding of 239 female Aedes triseriatus on filaremic blood of a grackle. In four of these experiments, mosquitoes were fed after 9 P.M.; in two, they were fed around 2 P.M., and in one, they were fed at 10 A.M. For examination purposes, engorged females were anaesthetized with ether vapour and dissected in saline solution on micro slides. In many cases, careful search was made for microfilariae in the stomach, haemocoel, thoracic region,

head, salivary glands and Malpighian tubules; but often the insects were merely teased apart in a drop or two of saline and examined. Most of the mosquitoes were examined immediately after killing. A compound microscope was used for examination of stained and unstained preparations.

Those mosquitoes which fed after 9 P.M. ingested as many as 249 microfilariae each, those fed around 2 P.M. picked up as many as 207 each, but the maximum number of microfilariae observed in any of the mosquitoes fed at 10 A.M. was two. Most of the engorged mosquitoes died within the first two days after a blood meal (presumably of congestion due to the large number of microfilariae ingested). A few, however, lived for 14 days. Mosquitoes were not kept for more than 14 days after engorgement, since no living microfilariae was ever observed in A. triseriatus 48 hours after engorgement.

In all the mosquitoes dissected and examined for microfilariae, little or no development of microfilariae was ever noticed. Most of the microfilariae were found in the stomach, where they became partially encapsulated if not digested or voided with the blood within two days, but a few living ones were sometimes observed in the haemocoel. Although these living ones were usually unsheathed, and were apparently shorter and sluggish, no definite internal changes were observed in them. Moreover, because some of the microfilariae in the stomach were apparently also unsheathed, and since the length of microfilariae vary greatly even in blood from naturally infected grackles, these observations could not be regarded as significant.

What Robinson (1955a), referred to as "aimless and sporadic migrations to the thorax" was sometimes observed, but no microfilaria was ever

observed in the head. In a batch fed at 1:00 A.M., no microfilaria was found in any of the mosquitoes. This phenomenon might have been due to the fact that they either were voided with the blood or were "rapidly digested" as suggested by Robinson (1955a) who also observed that most of his culicine mosquitoes were negative for microfilariae despite the fact that they took blood meals.

In many of these experiments, specks of blood were observed on the sides of the lantern chimney and especially on the moist gauze in the petri dish supporting it. It is thus apparent that many of the insects eliminate the blood after engorgement.

Attempts to infect Culex pipiens, Culex tarsalis, and Aedes aegypti yielded results similar to those of Aedes triseriatus. Eight experiments involving the examination of 95 engorged Culex pipiens were performed, and 57 female Culex tarsalis fed on filaremic blood were examined for examined for developmental stages of Microfilaria quiscali. In the case of Aedes aegypti, over 90 engorged females were examined. In all these experiments, considerably fewer microfilariae were picked up with the blood meal. This was probably due in part to varying intensities of infection in the birds used, and in part to the sizes of the mosquitoes involved. In almost all these cases, mosquitoes were fed at night. Robinson (1955a) believed that examination of 15 individuals of a species should give sufficient indication of its suitability as a vector, and Anderson (1956) contended that an avian filarial worm would develop to the infective stage in insects belonging to the same genus even though only a few species may serve as the vectors in nature.

From the present investigations, it appears very unlikely that the

four species of culicid flies employed are vectors of Splendidofilaria quiscali unless certain environmental conditions necessary for their development were overlooked in the experiments.

Simuliidae

Many of the habits of grackles expose them to biting insects living near streams. Grackles, in general, prefer to roost near streams and lakes, and they frequent streams where considerable time is spent wading and bathing.

Anderson (1956) successfully transmitted Ornithofilaria fallisensis using many species of simuliid flies and also referred to unpublished manuscripts of Davies and Peterson who reported that they were able to collect swarms of Simulium venustum over nests containing unfeathered grackles. These authors also captured three females of the same species of flies that had fed on fledgling crows, and one engorged female from a great blue heron.

Since the species of mosquitoes employed in the preceding experiments did not encourage further experiments with culicids, the possibility of simuliids acting as vectors for Splendidofilaria quiscali was explored. Rocks with attached larvae and cocoons of simuliid flies were collected from a fast-flowing stream in the vicinity of Ames and were placed in a well-aerated aquarium covered by a screen cage. By the use of this apparatus, it was easy to obtain a regular supply of adults which could be used for feeding experiments. Six experiments involving the use of over 80 simuliids were performed. The feeding procedure was the same as with mosquitoes (described in a previous section). Infected

grackles were put in tightly-fitting jackets made from cheese cloth and suspended in cages containing the flies. Even though the legs and the partly-deplumed head and chest of infected birds were left uncovered, none of the simuliids took a blood meal in any of the six experiments.

The failure of laboratory-reared simuliids to bite is not unusual. Gibson (1952) in his studies on Onchocerca, reported that only three of the "several thousands" of black flies he reared in the laboratory took a blood meal. Thus, despite the unsuccessful results obtained from the writer's preliminary experiments, further investigations should be undertaken before any definite conclusions are drawn regarding the suitability of simuliids as intermediate hosts of Splendidofilaria quiscali. The use of wild flies should be investigated.

Acarina

A thorough search of the literature indicates that grackles very seldom harbour ectoparasites. Although Bequaert (1953) recovered some hipposcid flies from a grackle, none was found on any of the 112 grackles examined during this investigation. Hicks (1953) reported the recovery of Collembola, thrips and Corrodentia from nests of grackles.

The very high incidence of filarial infections of grackles suggests that the natural vector is probably quite abundant. Since mites are common parasites of birds' nests, the possibility that they might be the vectors for Splendidofilaria quiscali was investigated. Ornithonyssus sylviarum has been recovered from about 60 species of wild birds (Gordon Clark, personal communication), and it is also common in grackle nests.

Ornithonyssus bacoti was first incriminated by Williams and Brown

(1945) as a probable vector for Litomosoides carinii and this has been confirmed by various investigators (Bertram, Unsworth and Gordon, 1946; Scott, 1953; Scott and Macdonald 1953). In the present studies, two species of bird mites, Ornithonyssus sylviarum and Dermanyssus gallinae were investigated as possible vectors of Splendidofilaria quiscali.

Various methods have been suggested for the rearing of bird mites by many investigators such as Williams (1946), Scott, Stembridge and Sisley (1947), Wisseman and Sulkin (1947), Chamberlain and Sikes (1950) and Sikes and Chamberlain (1954). The method employed during this investigation was the simple one suggested by Dr. Gordon Clark in a personal communication. Into each of two cages 3 x 2 x 2 ft., liberally supplied with straw litter, were put three leghorn roosters. The roosters in one cage were infested with Dermanyssus gallinae and those in the other with Ornithonyssus sylviarum. The straw in the cages was kept fairly moist and the birds were supplied with an ample supply of food and water. Although only a few specimens of these mites were supplied by Dr. D. W. Strandtmann, they reproduced with great rapidity. Another source of a few specimens of O. sylviarum used during this investigation was a heavily infested dead young grackle. This colony was completely wiped out when some of the grackles were dusted with malathion in an attempt to control the infestation.

For feeding experiments, mites were recovered either from the roosters or from the straw litter. They were starved for about three days in a humidity chamber described by Scott (1952) and put on infected grackles for about 24 hours. Grackles were usually put into a cage lined with white paper and cheese cloth. Most of the engorged mites were

recovered from the cage, but a few were removed from birds either by merely brushing the feathers or allowing mites to crawl on the gloves while holding infested birds.

Engorged mites were transferred into the humidity chambers and maintained at a temperature of about 24°C. as suggested by Scott (1952). These engorged mites were examined periodically for the developmental stages of Microfilaria quiscali.

A series of 15 experiments were performed and many engorged mites were occasionally removed directly from grackles infested in the laboratory. Mites were dissected immediately. Of a total of over 250 examined, only 23 were discovered to have ingested one microfilaria each, and in none of these were any apparent developmental changes noted.

Stable flies

Only two experiments with stable flies (Stomoxys calcitrans) were undertaken because the flies did not bite infected grackles.

Corn borer larvae

The studies of Yoeli, Alger and Most (1958), dealing with the use of wax moth larvae (Galleria mellonella) as suitable experimental hosts for microfilariae of D. immitis, suggested the use of these larvae as possible hosts for microfilariae of Splendidofilaria quiscali and for investigations on developmental stages. Accordingly, over 200 corn borer larvae (Pyrausta nubilalis Hübner) were each injected with about 01. ml. of citrated blood from infected grackles in a series of experiments performed between April 29 and July 1, 1959. The aims of these experiments were (1) to discover whether microfilariae of S. quiscali when injected

into corn borer larvae, would undergo any developmental changes similar to those observed by Yoeli et al (1958) who injected moth larvae (Galleria mellonella) with Microfilaria immitis and (2) to observe how long the injected microfilariae could live in these larvae.

Larvae used in these investigations were supplied by Dr. Boyd George of Iowa State University, who raised them from eggs. These larvae were maintained in the laboratory in test tubes and were grown on an agar-base artificial medium used by Beck (1957). Not more than five larvae were put in each tube which was plugged with sterilized cotton. The larvae were stored in the refrigerator until required for experimental purposes, a procedure which retarded pupation considerably.

Filaremic blood used for injection was obtained and citrated in the manner already described in a preceding section, and the same size of hypodermic needle (27-gauge) was employed. Each larva was steadied between two fingers while blood was injected into its haemocoel.

Mortality of injected larvae varied greatly, from 5% to 90% during the 24 hours after transfusion. However, some larvae lived without pupating for at least 21 days after injection. A few were allowed to develop into adult moths.

Examination of injected larvae was done after dissecting them in a drop or two of saline solution and carefully examining them using a dissecting microscope. The thoroughly teased-out larvae were then covered with cover slips and examined under a compound microscope for living or dead microfilariae. Sometimes, stained smears of the haemolymph were made, and touch preparations were occasionally stained and examined.

A total of 123 injected larvae were examined between the second and fifteenth day after transfusion.

Results of these investigations shown in Table 18 indicate that microfilariae of Splendidofilaria quiscali can live in corn borer larvae for at least seven days. The maximum number of living microfilariae recovered from an injected corn borer was 12, after a period of six days. Apart from a slight decrease in length and shedding of the microfilarial sheath, no appreciable morphological changes were observed in any of the microfilariae observed alive in these larvae. Some larvae were permitted to pupate before being examined. None of these, however, harboured microfilariae. Most of those microfilariae observed within the first three days post-injection were dead. Such microfilariae were frequently coiled and apparently encapsulated.

Table 18. Summary and results of transfusions of Microfilaria quiscali into corn borer larvae

	Expt. 1	Expt. 2	Expt. 3	Expt. 4
Date of transfusion	Apr. 29, 1959	May 17, 1959	June 5, 1959	July 1, 1959
No. of larvae injected	25	50	40	100
Mortality of larvae within 24 hours of injection	20%	5%	87%	8%
Total larvae examined	20	30	10	63
Total living mf. recovered	15	12	2	88
Total dead mf. recovered	23	40	7	54
Observed longevity of mf. in corn borer larvae	3 days	5 days	4 days	7 days

SUMMARY AND CONCLUSIONS

1. Microfilariae are reported from the blood of the bronzed grackle (Quiscalus quiscula versicolor Vieillot, 1819) for the first time.
2. Examination of the blood of 112 bronzed grackles trapped in the vicinity of Ames, Iowa (1957-1959), revealed that 62% of these birds are hosts for microfilariae and adult onchocercid worms of at least two species. The first type, Splendidofilaria quiscali (von Linstow, 1904) n. comb., produces sheathed microfilariae that are blunt at both ends. The adults of the second type producing microfilariae that are pointed posteriorly, were not recovered during the present investigation, but their microfilariae were referred to in this manuscript as Microfilaria "X". Forty per cent were infected with only Splendidofilaria quiscali, 17% had both Microfilaria quiscali and Microfilaria "X", and 5% had only Microfilaria "X". Ten of the 42 uninfected birds were nestlings.
3. Adults and microfilariae of Splendidofilaria quiscali are fully described and illustrated for the first time.
4. Splendidofilaria quiscali is invariably localized in the cerebral hemispheres of the bronzed grackle. In birds with light infections adult worms are restricted to the lateral ventricles, but in heavily infected ones the presence of worms is easily discovered at the posterior border of the cerebrum where the parasites frequently lie immediately under the pia mater.
5. The microfilariae are released into the lateral ventricles from where they gain easy access into the lymphatic system and eventually into

the blood circulatory system of the host.

6. In general, female worms predominate, and it is not uncommon to discover infections involving only female worms. Thus, the absence of microfilariae from the blood is not always a sure indication that birds are uninfected with filarial worms.
7. Heavily infected grackles maintained in the laboratory for over two years showed no noticeable external effects attributable to the presence of worms in their cranial cavities. Macroscopically as well as microscopically, neither adult worms nor microfilariae produce any appreciable pathological lesions. Examination of cross, frontal, and sagittal serial sections of brains of infected birds showed no evidence of necrosis, hyperemia, external or internal hydrocephalus, haemorrhage, thrombosis, embolism, meningitis, encephalitis, myelitis, or encephalomyelitis, neither was there any signs of ependymitis despite occasional pressure exerted by worms on the ependyma. Apart from the usual eosinophilia, microfilariae produced no pathological lesions on cardiac or lung tissues although they occur in large quantities in these tissues.
8. Adult worms were kept alive <u>in vitro</u> for two and one-half days in saline solution containing some glucose. However, it was discovered that the worms could remain alive for as long as 16 days in the brain of sacrificed birds kept under refrigeration at approximately 5°C. (even when only the severed head is kept under refrigeration).
9. <u>Microfilaria quiscali</u> can live for at least 17 days <u>in vitro</u> (in citrated blood containing some glucose), when such microfilaremic blood is maintained at 3°C. under aseptic conditions. They can also

remain alive for as long as 18 days, in the heart of dead, infected birds maintained at a temperature of approximately 5°C.

10. Morphological differentiation of internal organs of adult Splendidofilaria quiscali is greatly facilitated by adding a drop of very dilute Giemsa stain to a preserved specimen on a slide. The reproductive system takes on the stain more rapidly than does the digestive tract, microfilariae and eggs become prominent, and the vulva and anus stand out clearly.

11. Detailed morphological study of microfilariae is best accomplished by examining either microfilariae in histological sections of cardiac muscles stained in Delafield's haematoxylin, or those in Giemsa-stained thin films of partially clotted blood from the heart of dead infected birds kept under refrigeration for a few days. The shape and location of individual nuclei in such preparations are distinct, and both the excretory cell and rectal cell 1 show clearly in most cases.

12. Microfilarial periodicity of Splendidofilaria quiscali was very intensively studied. Experiments were performed on normal periodicity, reversal of periodicity, effect of continuous light and continuous darkness on periodicity, and on the re-establishment of normal periodicity following reversal and exposure to continuous light. These experiments involved withdrawal of 0.0125 ml. blood from metatarsal veins of six infected birds every two hours for 48 to 72 consecutive hours. A thin film of each blood sample was made, air-dried, fixed in absolute methyl alcohol for at least three minutes and stained with Giemsa.

13. Microfilaria quiscali exhibits well-marked nocturnal periodicity with maximum numbers generally occurring around midnight.

14. When infected birds are supplied with artificial light between 8 P.M. and 8 A.M. of the following day, periodicity is reversed, there being more microfilariae in films made between 8 A.M. and 8 P.M., when birds were in darkness.

15. When infected birds were exposed to artificial light for five consecutive days and periodicity experiments conducted during the last two, normal periodicity was disrupted in all but one of six experimental birds. This grackle (Bird No. 10) demonstrated a regular maximum number of microfilariae at approximately 6 P.M.

16. Normal nocturnal periodicity was re-established when those birds which had been kept in continuous light were maintained for five days under normal conditions of light and darkness. However, maximum numbers of microfilariae in peripheral circulation were reduced considerably in comparison to the numbers present when birds were maintained under condition of continuous light.

17. When the same birds were later kept in the dark for five consecutive days, microfilariae maintained their normal nocturnal periodicity.

18. A method of blood examination for detecting microfilarial periodicity is described. This technique, involving the examination of blood taken from the metatarsal vein, is rapid, permits examinations at frequent intervals without undue discomfort to birds, and obviates the necessity of haemolysing or centrifuging the blood. Furthermore, it eliminates mathematical errors often associated with blood

dilution and concentration of microfilariae.

19. Four transfusions of microfilaremic blood of grackles into non-infected grackles were performed. Two grackles were transfused intraperitoneally, and two partly intramuscularly but mainly intravenously. None of the recipient grackles ever showed microfilariae in the peripheral circulation, but those injected partly intravenously exhibited living microfilariae in the heart at necropsy. Living microfilariae were recovered from one bird four months after being transfused.

20. Microfilaria quiscali transfused intravenously from grackles into chickens can be observed alive in the peripheral circulation of injected chickens for at least 31 days after injection. Those injected into chickens intraperitoneally never showed up in the blood circulation of the recipients.

21. Microfilaria quiscali, when injected in sufficiently large quantity into a chicken will exhibit the normal nocturnal periodicity characteristic of infection in grackles.

22. Experimental attempts to elucidate the life cycle of Splendidofilaria quiscali using four species of mosquitoes (Culex pipiens, Culex tarsalis, Aedes triseriatus and Aedes aegypti), two species of mites (Ornithonyssus sylviarum and Dermanyssus gallinae), laboratory-reared Simulium spp. and stable flies (Stomoxys calcitrans) were all unsuccessful. In none of the 481 engorged mosquitoes and 250 mites exposed to infected grackles were any apparent developmental changes noticed in ingested microfilariae. Some mosquitoes picked up as many as 249 microfilariae with the

blood, but in none of the 23 engorged mites was more than one microfilaria recovered. None of the laboratory-reared Simuliids nor the stable flies bit the grackles.

23. Injections of over 200 corn borer larvae with filaremic blood of grackles indicate that Microfilaria quiscali can remain alive for at least seven days in the larvae.

LITERATURE CITED

Ali, S. M. 1956. Studies on the nematode parasites of fishes and birds found in Hyderabad state. Indian J. Helminth. 8: 1-83.

Anderson, R. C. 1955. Ornithofilaria algonquinensis n. sp. from Hirundo erythrogaster with a revision of the genera Paramicipsella Chow, Gönnert, 1937. Can. J. Zool. 33: 107-112.

_____. 1956. The life cycle and seasonal transmission of Ornithofilaria fallisensis Anderson, a parasite of domestic and wild ducks. Can. J. Zool. 34: 485-525.

_____. 1958. On the classification of the Filarioidea with reference to the Filariidae and the Stephanofilariidae. Bull. Soc. Zool. France. 83: 144-159.

_____, and Chabaud, A. G. 1959. Remarques sur la classification des Splendidofilariinae I. Ann. Parasitol. 34: 53-63.

Augustine, D. L. 1937a. Description of a new filariid from ground doves of St. Croix, Virgin Islands. Trans. R. Soc. Trop. Med. (Hyg). 31: 47-54.

_____. 1937b. Observations on living "sheathed" microfilariae in the capillary circulation. Trans. R. Soc. Trop. Med. (Hyg). 31: 55-60.

_____, and Drinker, C. K. 1935. The migration of microfilariae (Dirofilaria immitis) from the blood vessels to the lymphatics. Trans. R. Soc. Trop. Med. (Hyg.). 29: 303-306.

_____, Field, M. E., and Drinker, C. K. 1936. Observations on living Microfilaria immitis in the capillary circulation of bats. Trans. R. Soc. Trop. Med. (Hyg.). 30: 231.

Beck, S. D. 1957. The European corn borer, Pyrausta nubilalis (Hübn.), and its principal host plant. IV. Larval saccharotrophism and host plant resistance. Ann. Ent. Soc. America 50: 247-250.

Bell, S. and Brown, H. 1945. Studies on the microfilarial periodicity of Litomosoides carinii, filariid parasite of the cotton rat. Amer. Jour. Trop. Med. 25: 67-71.

Bequaert, J. C. 1953. The Hippoboscidae or louse-flies (Diptera) of mammals and birds Part 1. Structure, physiology, and natural history. Ent. Amer. 33 (N.S.) : 211-442.

Bertram, D. S., Unsworth, K., and Gordon, R. M. 1946. The biology and maintenance of Liponyssus bacoti Hirst. 1913. and an investigation into its role as a vector of Litomosoides carinii to cotton rats and white rats, together with some observations on the infection in the white rats. Ann. Trop. Med. and Parasitol. 40: 228-254.

Boughton, D. C., Byrd, E. E., and Lund, H. O. 1938. Microfilarial periodicity in the crow. J. Parasitol. 24: 161-165.

Búsa, V. 1955. Prispervok K. dynamike výskytu mikrosetazil v krvi kani. Veterinarsky Casopis (in Russian) Bratislava. 4:99-109. (Original not available for examination; abstracted in Helminthological Abstracts. 24: 436. 1955.).

Caballero, E. y C. 1948. Nematodes de las aves de Mexico. Algunos nematodos de las aves del Estado de Nuevo León. Rev. Soc. Mexicana de Hist. Nat. 9: 264-268.

Chabaud, A. G. and Anderson, R. C. 1959. Nouvel essai de classification des filaires (Superfamille des Filarioidea) II. Ann. Parasitol. 34: 64-87.

_____, _____, and Brygoo, R. 1959. Sept filaires d'oiseaux malgaches. Ann. Parasitol. 34: 88-109.

_____ and Choquet, M. T. 1953. Nouvel essai de classification des filaires (superfamille des Filarioidea) Ann. Parasitol. 28: 172-192.

Chamberlain, R. W. and Sikes, R. K. 1950. Laboratory rearing methods for three common species of bird mites. J. Parasitol. 36: 461-465.

Chandler, A. C. 1924. New filariae from Indian birds. Parasitol. 16: 398-404.

Chatterjee, K. D. 1952. Human parasites and parasitic diseases. Calcutta, India, Sree Saraswaty Press, Ltd.

Chernin, E. 1953. The length of the prepatent period in a filarial infection of ducks. J. Parasitol. 39: 574-575.

Dutton, J. E. 1905. The intermediary host of Filaria cypseli (Annett, Dutton, Elliot); the filaria of the African swift Cypselus affinis. Thompson Yates (and Johnston) Lab. Rept. 6: 137-147.

Edeson, J. F. B., Hawking, F., and Symes, C. B. 1957. The periodicity of microfilariae. VI. The response of microfilariae of Wuchereria malayi and W. bancrofti, Pacific type, to various stimuli. Trans. Roy. Soc. Trop. Med. (Hyg.) 51: 359-365.

Faust, E. C. 1949. Human helminthology. A manual for physicians, sanitarians, and medical zoologists. London, Henry Kimpton.

Feng, L. C. 1936. The development of Microfilaria malayi in A. hycranus var. sinensis Wied. Chinese Med. J. Suppl. 1: 368-378.

Fülleborn, F. 1908. Über Versuche and Hundfilarien und deren Übertragung durch Mücken. Arch. Schiffs-u. Tropen-Hyg. 12: 313-351.

_____. 1912. Beiträge zur Biologie der Filarien. Centralbl. f. Bakter. 66: 255-267.

_____. 1913. Beiträge für Morphologie und Differentialdiagnose der Microfilarien. Arch Schiffs-u. Tropen-Hyg. 17: 7-72.

_____. 1929. Filariosen des Menschen. In Kolle and Wassermann ed., Handbuch der Pathogenen Mikroorganismen. 6: 1043-1224. Jena, Gustav Fischer.

Galliard, H. and Ngu, D. V. 1947. Recherches sur la filariose. Choix d'une technique de numeration des microfilaires du sang. Ann. Parasitol. 22: 158-163.

Gibson, C. L. 1952. Comparative morphology of the skin-inhabiting microfilariae of man, cattle, and equines in Guatemala. Am. J. Trop. Med. and Hyg. 1: 250-260.

Gönnert, R. 1942. Zur Lebensdaeur menschlicher Mikrofilarien. Zentralblatt für Bakteriologie. 149: 75-81.

Gordon, R. M., Kershaw, W. E., Crewe, W., and Oldroyd, H. 1950. The problem of loiasis in West Africa with special reference to recent investigations at Kumba in the British Cameroons and at Sapele in Southern Nigeria. Trans. Roy. Soc. Trop. Med. (Hyg). 44: 11-47.

_____ and Webber, W. A. F. 1955. A new technique for the concentration of microfilariae from the venous blood, and its application to their detection in persons harbouring them in low density; together with observations on the significance of such low densites. Ann. Trop. Med. and Parasitol. 49: 80-95.

Goubanov, N. M. 1954. Faune helminthologique d'animaux d'importance economique de la mer d'Okhotsk et de l'Ocean Pacifique. Trudi Gelminth. Labcrat. 7: 380-381.

Hanson, H. C., Levine, N. D. and Kantor, S. 1956. Filariae in a wintering flock of Canada geese. J. Wildl. Mgt. 20: 89-92.

Harley, G. W. 1932. A theory regarding the role of insect saliva in filarial periodicity. Trans. Roy. Soc. Trop. Med. (Hyg.) 25: 487-491.

Hawking, F. 1940. The transference of Microfilaria bancrofti into natural and unnatural hosts. Ann. Trop. Med. Parasitol. 34: 121-129.

_____. 1951. The periodicity of microfilariae. II. Explanation of the production. Trans. Roy. Soc. Trop. Med. (Hyg.). 45: 329-340.

_____. 1953. The periodicity of microfilariae. III. Transfusion of microfilariae into a clean host. Trans. Roy. Soc. Trop. Med. (Hyg.). 47: 82-83.

_____. 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. (Hyg.) 50: 397-417.

Hawking, F. and Thurston, J. P. 1951. The periodicity of microfilaria I. The distribution of microfilariae in the body. Trans. Roy. Soc. Trop. Med. (Hyg). 45: 307-328.

Hicks. E. A. 1953. Observations on the insect fauna of birds' nest. J. Kans. Ent. Soc. 26: 11-18.

Highby, P. R. 1943. Vectors, transmission, development, and incidence of Dirofilaria scapiceps (Leidy 1886) (Nematoda) from the snowshoe hare in Minnesota. J. Parasitol. 29: 253-259.

Hinman, E. H. 1936. Attempted reversal of filarial periodical in Dirofilaria immitis. Proc. Soc. Expt. Biol. Med. 33: 524-527.

_____, Faust, E. C., and DeBakey, M. E. 1934. Filarial periodicity in the dog heartworm, Dirofilaria immitis, after blood transfusion. Proc. Soc. Exptl. Biol. Med. 31: 1043-1046.

Horsfall, W. R. 1955. Mosquitoes--their bionomics and relation to disease. New York, N. Y., The Ronald Press Company.

Jellison, W. L. 1940. Biological studies on the fauna of nests of birds and rodents in relation to disease of animals and man. Summaries of Ph. D. thesis, 3: 53-56. University of Minnesota.

Joyeux, C. and Sautet, J. 1937. Contribution a l'étude de la culture des microfilaires. Compt. Rend. Sc. Soc. Biol. 126: 361-362.

Kazubski, S. L. 1958. Cerebrofilaria caprimulgi nov. gen., nov. spec., (Nematoda, Filarioridea), a parasite from the brain of the nightjar Caprimulgus europeaeus L. Bull. Acad. Polonaises des Sciences. 6: 73- 78.

Khalil, M. 1938a. The site of the adult filaria in the human body is the determining factor in the microfilaria being periodic or non-periodic even in the same species. J. Egyptian Med. Assoc. 21: 502-505.

_____. 1938b. Microfilariae disappear into the lymphatics when absent in the peripheral blood. J. Egyptian Med. Assoc. 21: 595-596.

_____. 1938c. Thermotropism in filariasis: the basis of the clinical and pathological manifestations and the rational methods of treatment. J. Egyptian Med. Assoc. 21: 597-602.

King, W. V., Bradley, G. H. and McNeel, T. E. 1939. The mosquitoes of the southeastern States. U. S. Dept. Agr. Misc. Pub. No. 336.

Knott, J. 1935. The periodicity of microfilaria of Wuchereria bancrofti. Preliminary report of some injection experiments. Trans. Roy. Soc. Trop. Med. (Hyg.) 29: 59-64.

Lane, C. 1929. The mechanism of filarial periodicity. Lancet. 216: 1291-1293.

_____. 1948. Bancroftian filariasis. Biological mechanisms that underlie its periodicity and other of its clinical manifestations. Trans. Roy. Soc. Trop. Med. (Hyg.) 41: 717-784.

Leidy, J. 1882. On some Entozoa of birds. (Abstract) Proc. Acad. Nat. Sci. Phila. 12: 109.

_____. 1886. Notices of nematoid worms. Proc. Acad. Nat. Sci. Phila. 16: 308-313.

Li, H. C. 1933. Report on a collection of parasitic nematodes, mainly from north China. Parasitol. 25: 192-210.

von Linstow, O. F. B. 1904. Beobachtungen an Nematoden und Cestoden. Arch. Naturg. Berlin 70: 297-309.

Lopez-Neyra, C. R. 1947. Parasitologia animal. Granada, Editorial Librerias Prieto.

_____. 1956. Revision de la superfamilia Filarioidea. Rev. Ib. Parasitol. 16: 3-225.

Low, G. C. and Manson-Bahr, P. H. 1933. Some recent observations on filarial periodicity. With a clinical and laboratory report by A. H. Walters. Lancet 224: 466-468.

_____ and _____. 1934. Further observations on filarial periodicity. Lancet 227: 531-535.

Mackenzie, S. 1882. A case of filarial haematochyluria. Trans. Path. Soc. London. 33: 394-410.

Manson, P. 1878a. The development of the Filaria sanguinis hominis. Med. Times and Gaz. 2: 731.

_____. 1878b. On the development of Filaria sanguinis hominis, and on the mosquito considered as a nurse. J. Linn. Soc. London. 14: 304-311.

_____. 1882. On the periodicity of filarial migrations to and from the circulation. China. Imp. Customs Med. Rep. (1881) 22: 63-68.

Manson-Bahr, P. H. 1912. Filariasis and elephantiasis in Fiji. J. Lond. Sch. Trop. Med. Suppl. 1: 1-192.

_____. 1954. The life history of avian filarial parasites. Bull. British Ornithologists' Club. 74: 75-77.

Mazzotti, L. 1953. Supervivencia de las microfilarias de Dirofilaria immitis y de Onchocerca verticulata a la temperatura de 25°C. bajo cero. Rev. Inst. Salub. y Enf. Trop. 13: 289-291.

McCarthy, D. 1956. Pseudo-periodicity in the Pacific variety of Wuchereria bancrofti: a study of fluctuations in the density of microfilariae in the peripheral blood of Samoans. Trans. Roy. Soc. Trop. Med. (Hyg.) 50: 66-71.

McFadzean, J. H. and Hawking, F. 1956. The periodicity of microfilariae. V. Stimuli affecting the periodic migration of the microfilariae of Wuchereria bancrofti and of Loa loa in man. Trans. Roy. Soc. Trop. Med. (Hyg.) 50: 543-562.

Molin, R. 1858. Prospectus helminthum, quae in prodromo faunae helminthologicae Venetaee continentur. Sitzungsb. K. Akad. Wissensch., Wien, Math.-Naturw. Cl. 30: 127-158.

Moorman, R. B. 1956. Nuisance sparrows and their control. Iowa State University Agri. Extension Service. Pamphlet 233.

Murgatroyd, F. 1933. Filarial periodicity. Lancet 224: 610.

Myers, W. W. 1881. Observations on Filaria sanguinis hominis in South Formosa. China. Imp. Customs. Med. Rep. 21: 1-25.

_____. 1886. Further observations on Filaria sanguinis hominis in South Formosa. China. Imp. Customs. Med. Rep. 32: 1-38.

Ochoterena, I. 1930. Contribuciones para el conocimiento de la oncocercosis en Mexico. Ann. Inst. Biol. Mexico 1: 309-311.

O'Connor, F. W. 1923. Researches in the western Pacific. Lond. Sch. Trop. Med. Res. Mem. Ser. 4: 1-57.

_____. 1931. Filarial periodicity with observations on the mechanism of the migration of the microfilariae from the parent worm to the blood stream. Porto Rico J. Pub. Health and Trop. Med. 6: 263-272.

_____. 1932. The aetiology of the disease syndrome in Wuchereria bancrofti infections. Trans. Roy. Soc. Trop. Med. (Hyg.) 26: 12-47.

_____, and Beatty, H. A. 1938. Filariasis of ground doves in St. Croix, Virgin Islands. Trans. Roy. Soc. Trop. Med. (Hyg.) 31: 407-412.

_____ and Hulse, C. R. 1932. Some pathological changes associated with Wuchereria (Filaria) bancrofti infection. Trans. Roy. Soc. Trop. Med. (Hyg.) 25: 445-454.

Odetoyinbo, J. A. and Ulmer, M. J. 1959. Studies on avian filarial worms of the subfamily Splendidofilariinae (Nematoda: Dipetalonematidae). J. Parasitol. 45, No. 4, Supp.: 58.

_____, and _____. 1960. Microfilarial periodicity of Splendidofilaria quiscali (von Linstow, 1904) n. comb. (Nematoda: Onchocercidae). J. Parasitol. 46, Supp. (in press).

Oschmarin, P. G. 1950. Connaissance de filaires animales de la zone meridionale de l'Extreme-Orient. Trudi Gelminth. Laborat. 7: 380-381.

Poynton, J. O. and Hodgkin, E. P. 1938. Endemic filariasis in the Federated Malay States. Bull. Institute for Medical Research, Federated Malay States. No. 1: 1-67.

Rao, S. S. 1936. Filariasis in Patnagarh (Orissa Fendatory State). Indian J. Med. Res. 23: 871-879.

Robinson, Jr., E. J. 1954a. Notes on the occurrence and biology of filarial nematodes in southwestern Georgia. J. Parasitol. 40: 138-147.

_____. 1954b. Additional data on filarial worm infections in vertebrates of southwestern Georgia. J. Parasitol. 40: 690-691.

_____. 1955a. A description of attempts to infect mosquitoes with avian filarial worms. J. Parasitol. 41: 176-178.

_____. 1955b. Observations on the epizootiology of filarial infections in two species of the avian family Corvidae. J. Parasitol. 41: 209-214.

Ross, H. H. 1947. The mosquitoes of Illinois (Diptera, Culicidae). Bull. Illinois Natural History Survey. 24: (Art. 1) 1-96.

Schnelle, G. B. and Young, R. M. 1944. Clinical studies on the microfilarial periodicity in war dogs. Bull. U.S. Army Med. Dept. 80: 52-59.

Scott, J. A. 1952. A humidity chamber for maintaining the tropical rat mite, Bdellonyssus bacoti. J. Parasitol. 38: 90-91.

_____. 1953. Recent developments in methods for maintaining and transmitting Litomosoides carinii in the laboratory. (Abstract) J. Parasitol. 39, No. 4, Supp.: 37.

_____ and Macdonald, E. M. 1953. Experimental filarial infections in cotton rats. Exptl. Parasitol. N. Y. 2: 129-140.

_____, Stembridge, V. A., and Sisley, N. M. 1947. A method for providing a constant supply of tropical rat mites, Liponyssus bacoti, infected with the cotton rat filaria, Litomosoides carinii. J. Parasitol. 33: 138-141.

Shipley (Sir), A. E. 1905. Notes on entoparasites from the Zoological Gardens, London and elsewhere. Proc. Zool. Soc. Lond. 1: 248-253.

Sikes, R. K. and Chamberlain, R. W. 1954. Laboratory observations on three species of bird mites. J. Parasitol. 40: 691-697.

Skrjabin, K. I. 1923. Beitrag zur Kenntnis der Vogelfilarien Russlands. Vestnik Mikrobiol. i Epidemiol. 2: 90-91.

_____ and Schikhobalova, N. P. 1936. Contribution au remaniement de la classification des nematodes de l'ordre des Filariata Shrjabin 1915. Ann. Parasitol. 14: 61-75.

_____ and _____. 1948. Filariae of animals and man, for veterinarians, medical men and biologists. (in Russian). Moscow, Ogiz-Selkhozgig. (Original not available for examination; cited in Ann. Parasitol. 28: 174.

Stone, A., Knight, K. L., and Starcke, H. 1959. A synoptic catalog of the mosquitoes of the world (Diptera, Culicidae). Baltimore, Md., The Horn-Shafer Company.

Taylor, A. E. R. 1960. Maintenance of filarial worms in vitro. Exptl. Parasitol. 9: 113-120.

Thomas, L. J. 1931. Note on filaria infecting ducks. Anat. Record. 51: 66.

Travassos, L. P. 1926. Filaires bresiliennes. Compte Rendu Soc. Biol. 94: 163-166.

Tschertkowa, A. N. 1946. A new filaria from the internal media of the eye in birds. C. R. (Doklady). Acad. Sc. U.R.S.S. 53: 863-865.

Vargas, L. 1952. Nota sobre la biologia de microfilaria de Onchocerca Volvulus. Medicina Rev. Mex. 32: 396-397.

Walton, A. 1927. A revision of the nematodes of the Leidy Collections. Proc. Acad. Nat. Sci. Phila. 79: 49-163.

Wehr, E. E. 1935. A revised classification of the nematode superfamily Filarioidea. Proc. Helminth. Soc. Wash. 2: 84-88.

_____ and Herman, C. M. 1956. Lophortofilaria californiensis n.g., n. sp. (Filarioidea, Dipetalonematidae) from California quail, Lophortyx Californicus, with notes on its microfilaria. J. Parasitol. 42: 42-44.

_____ and Hwang, J. C. 1957. Systemic position of Filaria helicina Molin, 1858, from the brain cavity of the snakebird, Anhinga anhinga. J. Parasitol. 43: 649-655.

Williams, R. W. 1946. The laboratory rearing of the tropical rat mite, Liponyssus bacoti (Hirst) J. Parasitol. 32: 252-256.

_____ and Brown, H. W. 1945. The development of Litomosoides carinii filariid parasite of the cotton rat in the tropical rat mite. Science 102: 482-483.

Wisseman, C. L., Jr., and Sulkin, S. E. 1947. Observations on the laboratory care, life cycle, and hosts of the chicken mite, Dermanyssus gallinae. Am. J. Trop. Med. 27: 463-469.

Wyman, J. 1868. On a thread worm (Filaria anhingae) infesting the brain of the snakebird, (Plotus anhinga Linn.). Proc. Boston Soc. Nat. Sci. 12: 100-104.

Yeh. L. S. 1957. On Chandlerella braziliensis n. sp. from a greenbilled toucan and a discussion on some related genera. J. Helminth. 31: 33-38.

Yoeli, M., Alger, N., and Most, H. 1958. Studies on filariasis. I. The behaviour of microfilariae of Dirofilaria immitis in the wax larva (Galleria mellonella). Am. J. Trop. Med. (Hyg.) 7: 249.

Yokogawa, S. 1939. Transmission of Wuchereria bancrofti. Trans. Roy. Soc. Trop. Med. (Hyg.) 33: 363-364.

Yorke, W., and Blacklock, B. 1917. Observations on the periodicity of Microfilaria nocturna. Ann. Trop. Med. and Parasitol. 11: 127-146.

_____ and Maplestone, P. A. 1926. The nematode parasites of vertebrates. London, J. and A. Churchill.

ACKNOWLEDGEMENTS

The author is much indebted to Dr. Martin J. Ulmer, under whose direction this work was conducted. His inspiration, interest, assistance and encouragement during this investigation, and during the preparation of this manuscript will always be gratefully remembered.

Special thanks are extended to Dr. Clay G. Huff for supplying mosquito eggs, to Dr. R. W. Strandtmann for the supply of mites, to Dr. Boyd George for the corn borer larvae, and to Dr. Allen McIntosh for bibliographic aid. Thanks are due also to Drs. Gordon Clark, Roy E. Anderson, William S. Monlux and Willard F. Hollander for their invaluable suggestions and advice during the course of this investigation.

The author wishes to express his sincere thanks to those members of the faculty and graduate students of the Department of Zoology and Entomology at Iowa State University for their help during various aspects of this investigation.

This study was supported in part by a National Science Foundation grant awarded to Dr. Martin J. Ulmer.

Plate I

Fig. 1. Photograph of the dorsal view of an infected grackle's brain

(The right lateral ventricle is exposed to show the adult filarial worms *in situ*.)

Fig. 2. Photograph of dorsal view of an infected grackle's brain with part of the pia mater removed to show adult filarial worms at the posterior part of the cerebrum

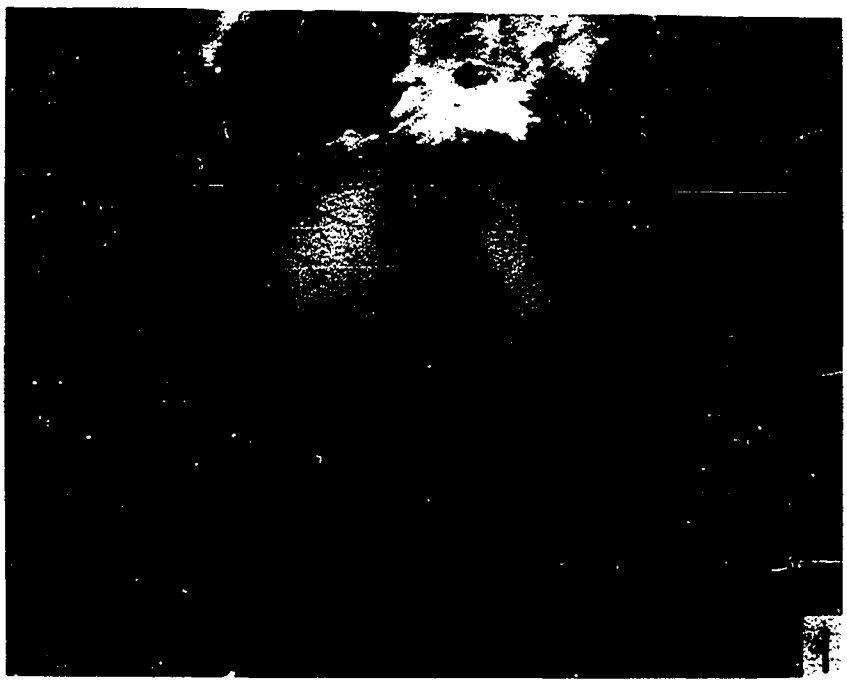

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Plate II

Figs. 3 and 4. Photomicrographs of cross sections of anterior portion of an infected grackle's brain showing sections of worms in the lateral ventricles

Figs. 5 and 6. Photomicrographs of cross sections of posterior portion of the cerebrum of an infected grackle

Abbreviations: W = Sections of filarial worms

PM = pia mater

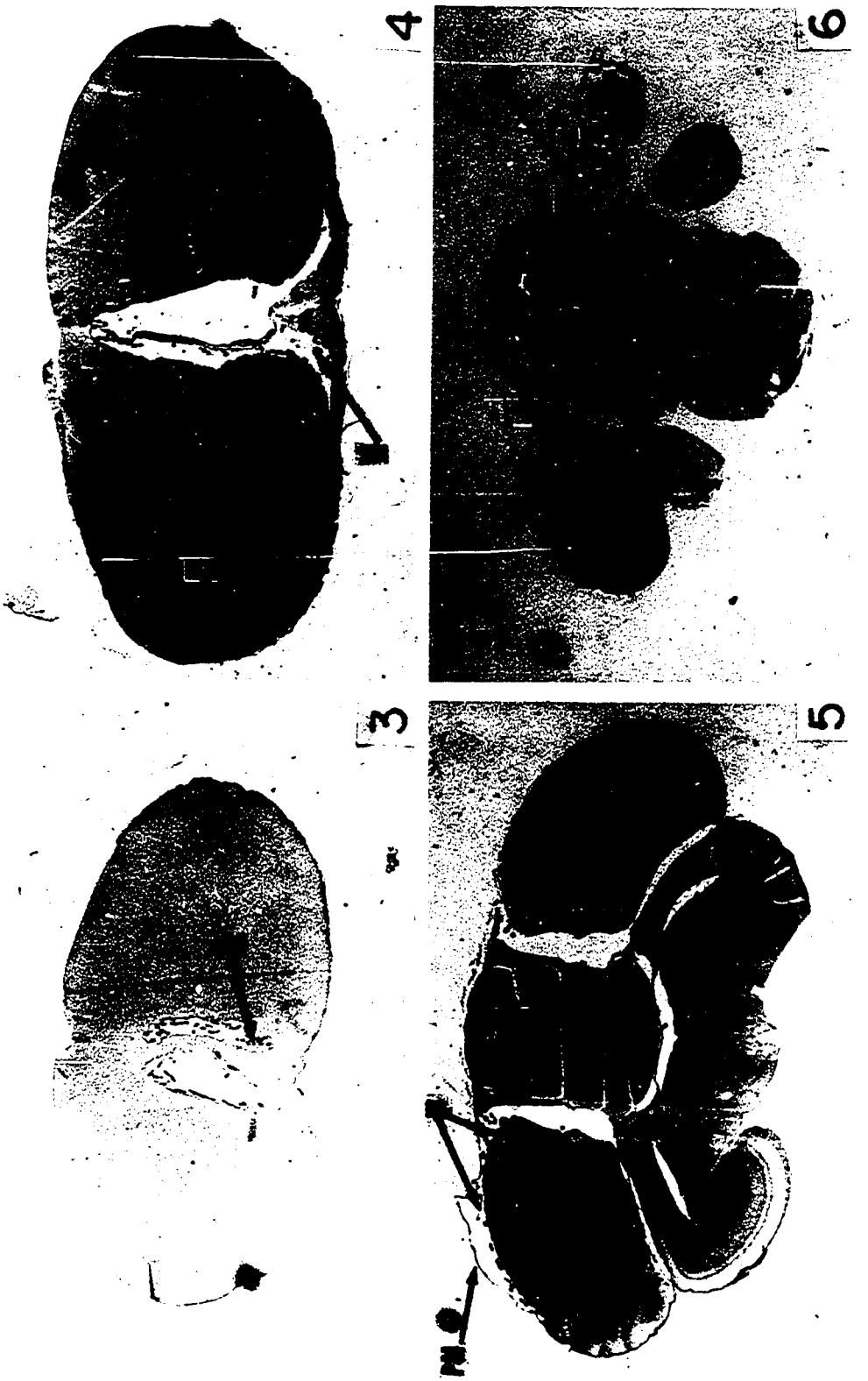
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Plate III

Fig. 7. Line drawing of anterior portion of male Splendidofilaria quiscali

Fig. 8. Line drawing of posterior portion of male Splendidofilaria quiscali

Abbreviations: A = Anus

C = Cloaca

CP = Cloacal papillae

E = Esophogus

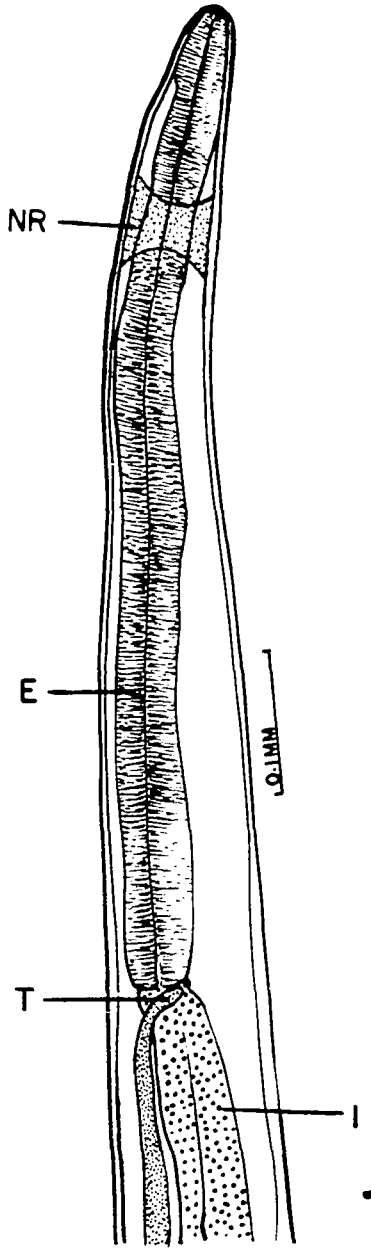
I = Intestine

NR = Nerve ring

S = Spicules

T = Testis

VD = Vas deferens



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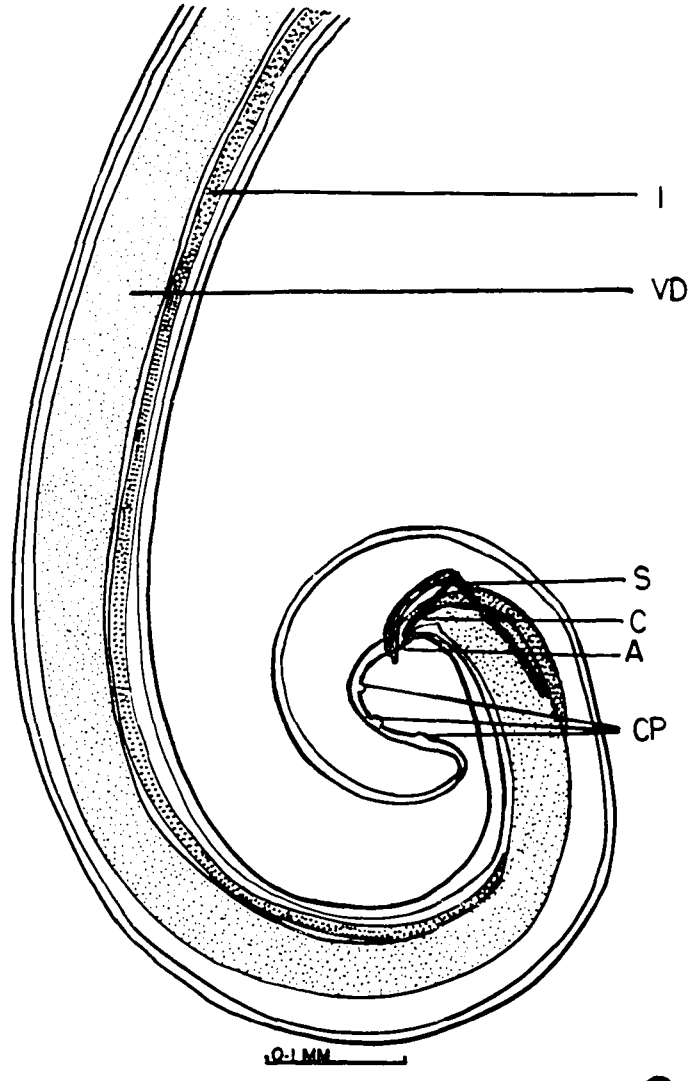

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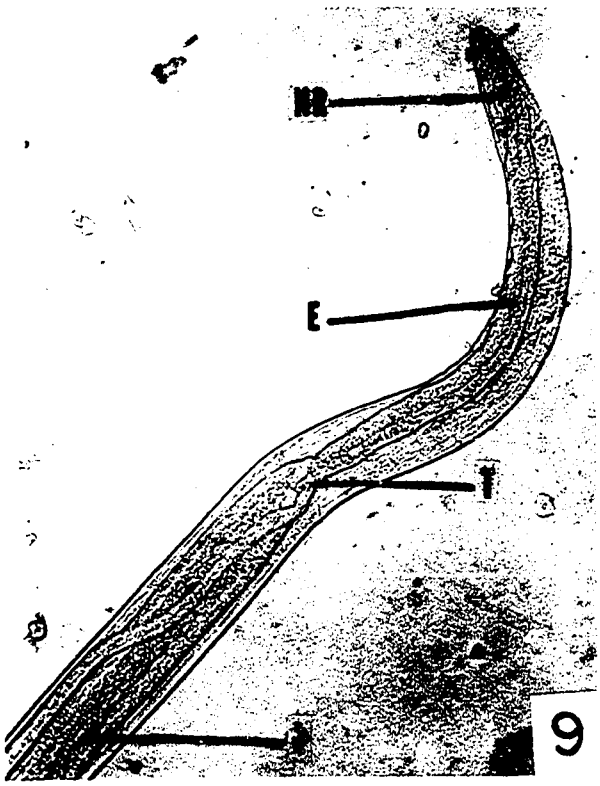
Plate IV

Fig. 9. Photomicrograph of anterior portion of male *Splendidofilaria quiscali*

Fig. 10. Photomicrograph of posterior portion of male *Splendidofilaria quiscali*

Abbreviations: CP = Cloacal papillae
E = Esophagus
NR = Nerve ring
I = Intestine
S = Spicules
T = Testis
VD = Vas deferens



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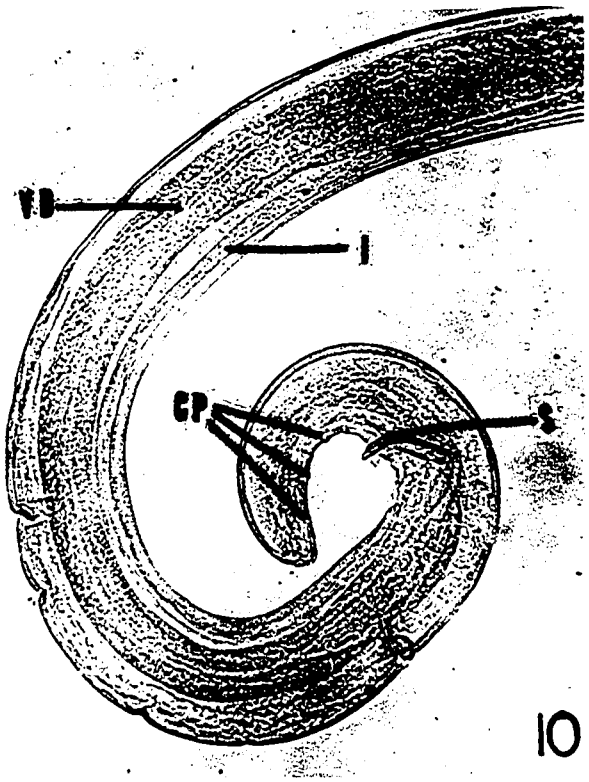

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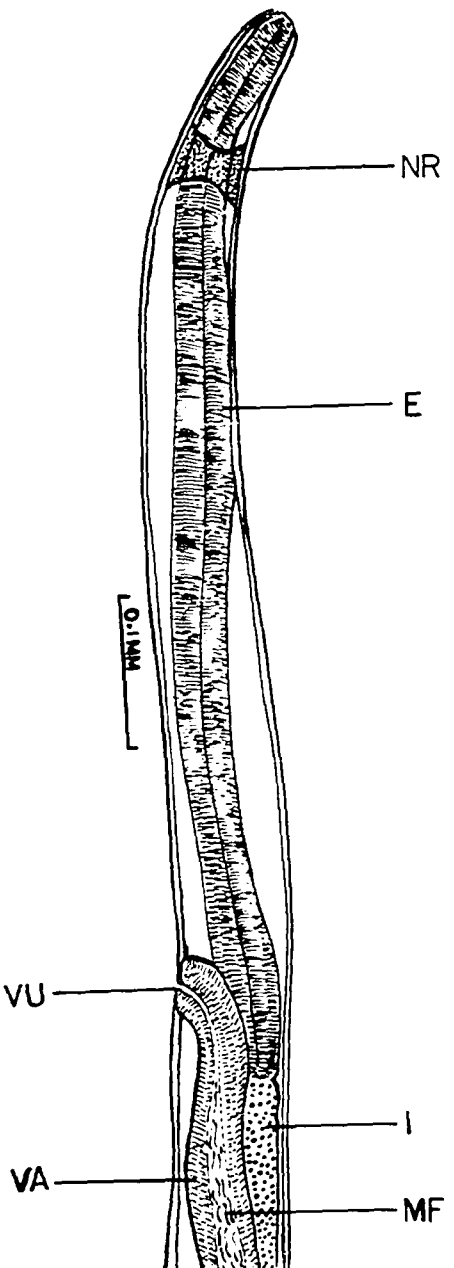
Plate V

Fig. 11. Line drawing of anterior portion of female Splendidofilaria quiscali

Fig. 12. Line drawing of posterior portion of female Splendidofilaria quiscali

Abbreviations: A = Anus
E = Esophagus
I = Intestine
MF = Microfilariae
NR = Nerve ring
O = Ovary
VA = Vagina
VU = Vulva



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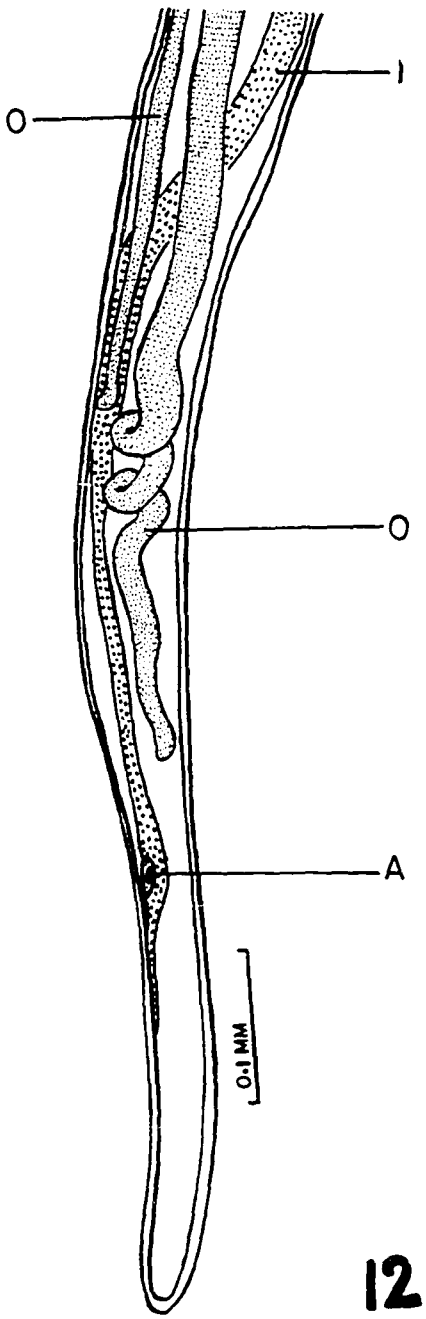

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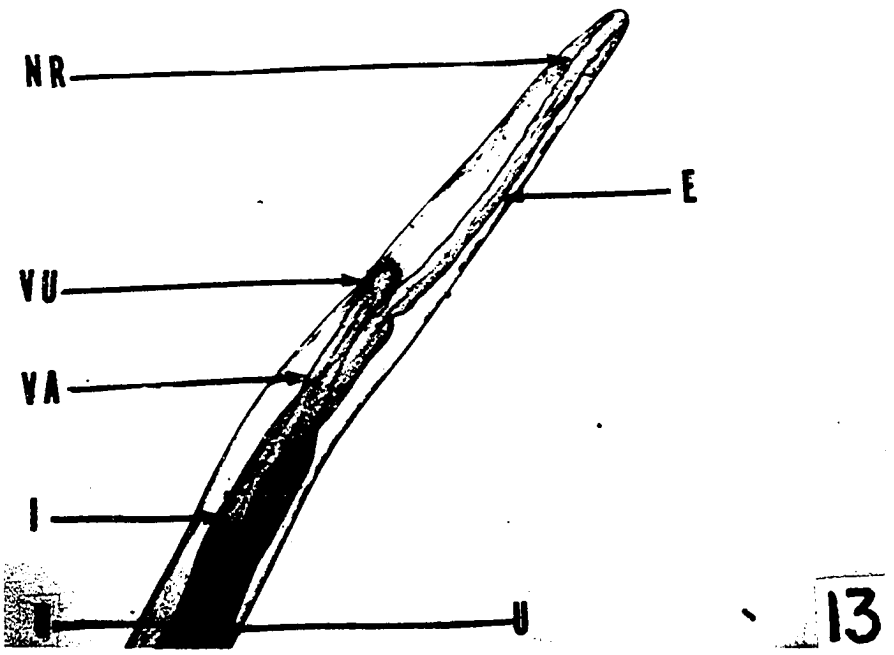
Plate VI

Fig. 13. Photomicrograph of anterior portion of female Splendidofilaria quiscali

Fig. 14. Photomicrograph of posterior portion of a gravid female Splendidofilaria quiscali

Abbreviations: A = Anus
E = Esophagus
I = Intestine
NR = Nerve ring
O = Ovaries
U = Uterus
VA = Vagina
VU = Vulva



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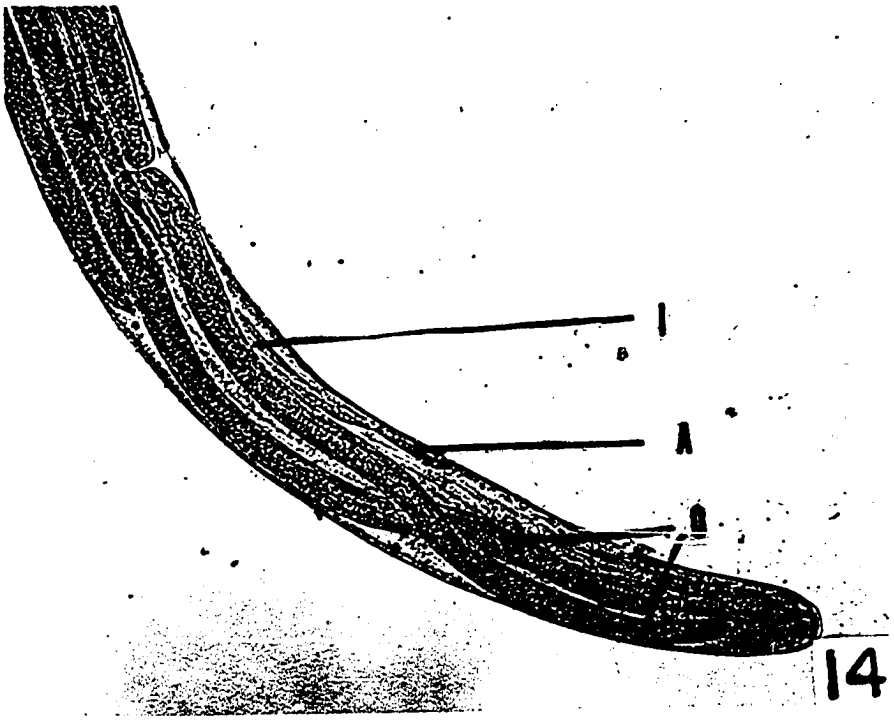

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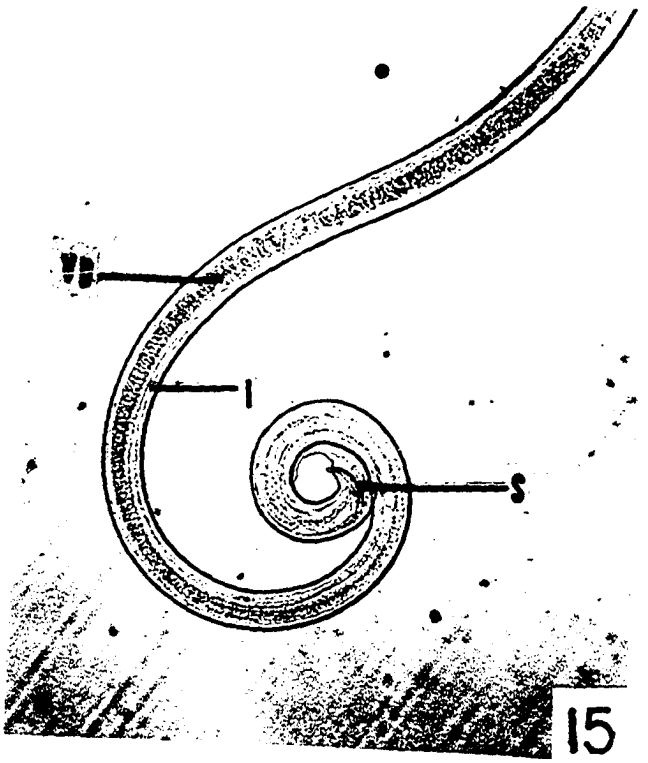
Plate VII

Fig. 15. Photomicrograph of posterior end of a male Splendidofilaria quiscali

Fig. 16. Photomicrograph of posterior end of a non-gravid Splendidofilaria quiscali

Abbreviations: A = Anus
I = Intestine
O = Ovary
S = Spicules
VD = Vas deferens



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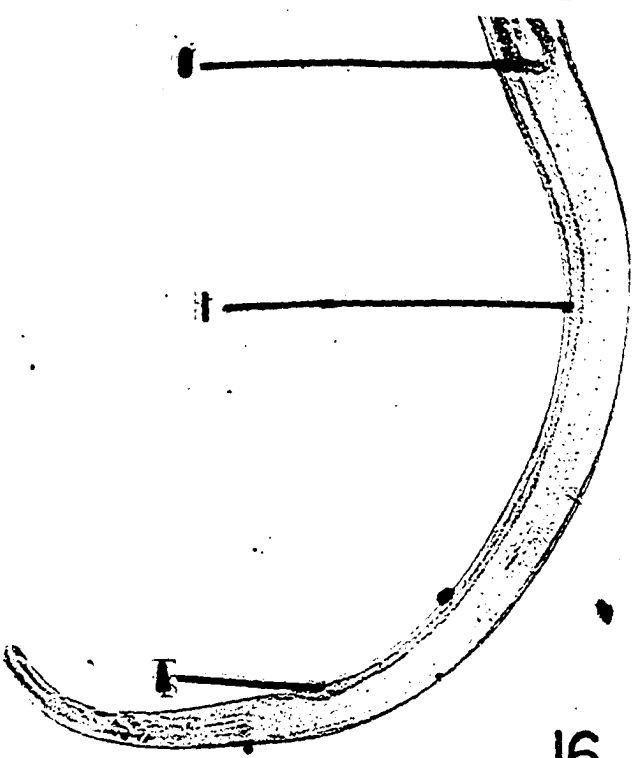

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Plate VIII

Fig. 17. Photomicrograph of histological section of cardiac muscle of an infected grackle showing microfilariae (MF) lying parallel to muscle fibres

Fig. 18. Photomicrograph of histological section of lung of an infected grackle showing microfilariae in the blood vessels

Abbreviation: MF = Microfilariae

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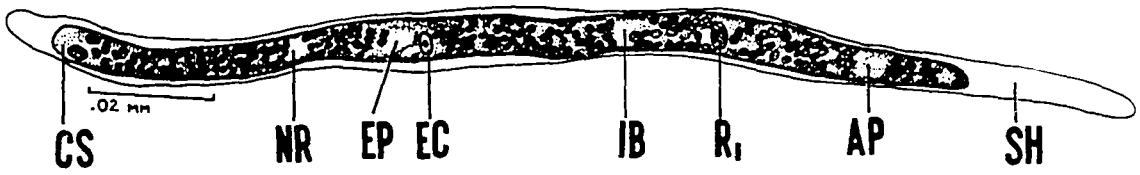
Plate IX

Fig. 19. Line drawing of *Microfilaria quiscali* showing the detailed morphology

Fig. 20. Photomicrograph of stained blood film of an infected grackle, showing microfilariae

Abbreviations:
AP = Anal pore
CS = Cephalic space
EC = Excretory cell
EP = Excretory pore
IB = Inner body
NR = Nerve ring
R1 = Rectal cell 1
SH = Sheath



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