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Colorado State University, Ph.D., 1971
Zoology

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DISSERTATION

LIFE HISTORY OF PHYSALOPTERA RARA HALL AND WIGDOR, 1918, IN DEFINITIVE, INTERMEDIATE, AND PARATENIC HOSTS

Submitted by

John L. Olsen

In partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Colorado State University

Fort Collins, Colorado

August, 1971

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COLORADO STATE UNIVERSITY

August, 1971

WE HEREBY RECOMMEND THAT THE DISSERTATION PREPARED UNDER OUR SUPERVISION BY JOHN L. OLSEN ENTITLED LIFE HISTORY OF PHYSALOPTERA RARA HALL AND WIGDOR, 1918, IN DEFINITIVE, INTERMEDIATE, AND PARATENIC HOSTS BE ACCEPTED AS FULFILLING IN PART REQUIREMENTS FOR THE DEGREE OF DOCTOR OF PHILOSOPHY.

Committee on Graduate Work

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ABSTRACT OF DISSERTATION

LIFE HISTORY OF PHYSALOPTERA RARA HALL AND WIGDOR, 1918, IN DEFINITIVE, INTERMEDIATE, AND PARATENIC HOSTS

Adults of Physaloptera rara inhabit the stomach and anterior third of the small intestine of canids and felids. Embryonated eggs are voided with the feces of the host. Upon ingestion of the eggs by a suitable orthopteran intermediate host, the larvae hatch and migrate into the wall of the midgut and rectum where they develop to the infective third stage.

The common field cricket, (Gryllus assimilis) was used as experimental intermediate hosts, and domestic cats (Felis domestica) served as the experimental definitive host.

The first stage of larval development was completed between the 11th and 12th days postinfection. The body of the larva was uniform in width, with a tapered, pointed tail flexed dorsally. The tooth-like spine at the anterior extremity of the body was lost between the 5th and 11th days of development. The esophagus was straight and well developed. Strands of nerve fibers made up the nerve ring. The excretory pore was visible, and slightly posterior to the nerve ring. The intestine was continuous to the anus. An anal plug was present.

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Development of the second stage occurred during the 13th and 14th days postinfection. The nerve ring, excretory pore, esophagus, and intestine were well developed in second-stage larvae.

Third-stage larvae were first observed 15 days postinfection. The pseudolabia and teeth were developed more than in second-stage larvae. The tail was more pointed, and no longer flexed dorsally, and the anal plug was absent. Other than an overall increase in body size, no further development was observed in third-stage larvae.

Prairie rattlesnakes (Crotalus viridis) in Colorado are naturally infected paratenic hosts of P. rara.

Frogs and mice were successfully infected as paratenic hosts of P. rara. Third-stage larvae recovered from feces of an experimentally infected frog 21 days postinfection were fed to a cat. Mature worms were recovered from it 156 days later. Larvae of P. rara did not establish themselves in experimentally infected chickens.

A survey of wild carnivores to determine the incidence of physalopteran infections revealed: 40 (38.8%) of 103 coyotes positive for P. rara, five red foxes negative for physalopterans, 9 (81.8%) of 11 bobcats positive for P. praeputialis, and one skunk with P. maxillaris. A badger and three raccoons were negative for physalopteran infections.

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CHAPTER I

INTRODUCTION

More than 150 species of the genus Physaloptera have been described throughout the world. They have been reported from amphibians, reptiles, birds, and mammals. Although the majority of species of Physaloptera utilize wild animals as definitive hosts, there are numerous reports of them from domestic carnivores. Only one species has been reported from man.

All Physaloptera are thought to have an indirect life cycle, using arthropods as an intermediate host. Reports of larval Physaloptera encysted in various organs and locations in the musculature of different vertebrates suggests that they have evolved a high degree of efficiency in making use of paratenic hosts to facilitate reaching the definitive host.

Life history studies have been conducted on a limited number of species (Alicata, 1937; Hobmaier, 1941b; Petri, 1950; Schell, 1952a; Zago, 1958). For this reason, there is need for greater understanding of the biology of these parasites, especially with respect to their ability to use paratenic hosts.

In examining over 300 dogs from the Detroit area, Hall and Wigdor (1918) recovered a single female specimen which they

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described and named Physaloptera rara. Since publication of the original description of P. rara, it has been reported from numerous wild and domestic carnivores.

The purposes of this study are to add to the present knowledge of the life cycle and the biology of P. rara. The investigations include: (1) description of the adult of P. rara; (2) description of the larval stages; (3) role of paratenic hosts in the life cycle; (4) investigation of natural incidence of infection in wild canids and felids; (5) ecology of P. rara.

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CHAPTER II

REVIEW OF LITERATURE

The family Physalopteridae was erected by Leiper in 1908. Yamaguti (1961) uses the classification of Stossich (1898), in which all known genera of the family are included in the subfamily Physalopterinae. Of the nine genera of physalopterids recognized by Yamaguti, only the genera Physaloptera and Pseudophysaloptera occur in mammals.

Morgan (1941) allocated the species of physalopterids from North America to the following four genera: Abbreviata (Travassos, 1920) Schulz, 1927; Skrjabinoptera Schulz, 1927; Physaloptera Rudolphi, 1819; and Pseudophysaloptera Baylis, 1934. Abbreviata, Physaloptera, and Pseudophysaloptera occur in mammals.

Since Rudolphi described the type genus Physaloptera in 1819, Yamaguti (1961) has listed over 90 species described from mammals, of which 15 species are found in mammals of the United States.

P. praeputialis Linstow, 1899 and P. rara Hall and Wigdor, 1918, are the two members of the genus most commonly found in domestic cats and dogs. In addition, they have been reported from numerous wild carnivores. P. praeputialis is of world wide distribution, while P. rara is known only from North America.

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Some surveys of domestic and wild carnivores in the United States for helminths often failed to turn up Physaloptera. They included 50 dogs from the vicinity of Washington, D.C. (Sommer, 1896), 74 dogs from Michigan (Hall, 1917), 50 dogs and 30 cats from Oklahoma (Guberlet, 1923), 150 dogs from the Washington, D.C. area (Wright, 1930), 42 dogs from Kansas (Gaafar and Ameel, 1950), 75 coyotes from Utah (Butler and Grundman, 1954). Baker (1941) suggested that due to a superfical resemblance, many Physaloptera were probably misidentified as immature ascarids.

In other surveys of carnivores from the United States, Physaloptera sp. were commonly found. Ackert (1941) found 88 of 193 cats from the Manhattan, Kansas, area infected with Physaloptera. Later, Ackert and Furumoto (1949), in a second survey from Kansas, found 51% of 131 cats with Physaloptera. Of 186 dogs from the north central United States, Ehrenford (1953) found 30.1% infected with Physaloptera. Over a 11 year period Gier and Ameel (1959) reported Physaloptera in 51% of 1,850 Kansas coyotes. Stanley (1963) recovered Physaloptera from 3 of 18 red fox from Kansas. Of the 320 raccoons from South Carolina, Georgia, Florida, and Virginia, Harkema and Miller (1964) reported that 25% harbored Physaloptera.

In surveys of cats from areas other than the continental United States, Physaloptera sp. were commonly found. Chen (1934) found 36.8% of 89 cats from China infected with Physaloptera. Pinto (1936)

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stated that 35% of the cats examined from Rio de Janeiro were infected with Physaloptera. Calero, Ortiz, and Souza (1951) examined 93 cats from Panama, of which 29.7% were positive for Physaloptera. Yutuc and Cosio (1953) reported 53% of 51 cats in the Philippines harbored Physaloptera. Ash (1962b) recovered Physaloptera from 23% of 107 cats examined in Hawaii. Costa, Costa, and Freitas (1966) examined 63 cats from Belo Horizonte, Brazil and found 77.8% infected with Physaloptera.

Most cases of Physaloptera infection are relatively light, but extremely heavy infections have been reported. Harrison and Hall (1909) diagnosed the death of a captive tiger as resulting from a heavy infection of Physaloptera, but did not give the number of worms found. Pinto (1936) stated that infections of Physaloptera were often fatal to domestic cats in Brazil. Ehlers (1931) found large numbers of Physaloptera in captive badgers which had died. He recovered over 200 from the stomach of one animal. Baker (1941) recovered 140 immature Physaloptera from the stomach of a dog. Schiller and Morgan (1949) found 105 Physaloptera in the stomach and small intestine of a raccoon. Ameel (1955) recovered 73 Physaloptera from a coyote.

Two instances of Physaloptera occurring in unusually abnormal locations have been reported. Ingram (1941) found 16 Physaloptera in the postcaval vessel of a raccoon. Shumard and Bolin (1958),

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while collecting tissue for a rabies test, found two adult female physalopterids in the brain of a skunk.

Alicata (1937) was the first to have any success in elucidating the life cycle of Physaloptera. He recovered third-stage larvae of Physaloptera turgida Rudolphi, 1819 from the German cockroach (Blatella germanica) 27 days after they were fed embryonated eggs. The larvae were found loosely coiled in cysts in the tissue surrounding the body cavity. He was unable to obtain adult P. turgida after feeding third-stage larvae to a dog, cat, rabbit, guinea pig, rat, and chick. However, third-stage larvae were recovered encysted in the stomach wall of the rat.

Hobmaier (1941b) found that eggs of Physaloptera maxillaris Molin, 1860 did not hatch outside the body of the intermediate host. He also used the German cockroach as the intermediate host, and recovered encysted larvae in 4-6 weeks. They were fed to cats, dogs, and guinea pigs. Upon examination of these animals six weeks later, they were negative for Physaloptera species.

Petri (1950) was the first to successfully complete the life cycle of a species of Physaloptera. He experimentally infected German cockroaches and confused flour beetles (Tribolium confusum), from which larvae of P. rara were recovered. He stated that "The eggs were hatched easily at room temperature in 0.8 per cent saline." He fed third-stage larvae from the roaches to cats and dogs, from which immature P. rara were recovered one month later.

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Petri and Ameel (1950) experimented with various intermediate hosts. They were able to infect ground beetles (Harpalus sp.), and field crickets (Gryllus assimilis) with P. rara. Using embryonated eggs of P. praeputialis, they infected German cockroaches, camel crickets (Ceutophilus sp.), and field crickets.

The life cycle of Physaloptera hispida Schell, 1950 from the cotton rat, Sigmodon hispidus, was worked on by Schell (1952a). He fed embryonated eggs to a variety of arthropods in an attempt to determine those most suitable for intermediate hosts. German cockroaches, ground beetles (Harpalus sp.), and European earwigs (Forficula auricularia) were the only arthropods in which larvae were able to develop to the infective stage.

In contrast to Petri's findings, Schell was unable to hatch embryonated eggs in either saline solution or tap water. They hatched only in the digestive tract of the intermediate host. The first-stage larvae penetrated into the wall of the digestive tract posterior to the mesenteron. No evidence was found to indicate that larvae passed through the wall into the body cavity, as reported by Alicata (1937). Soon after penetration, the larva was enclosed by a membrane of host origin (Schell, 1952b). Within the cyst, the larva or larvae, as the case may be, developed to third-stage in 30-35 days. He was able to infect cotton rats by feeding second-stage larvae, but in these cases development of the parasite was retarded. When cotton

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rats were fed third-stage larvae, sexually mature adults appeared in 73-90 days.

Deer mice (Peromyscus sp.), Norway rats (Rattus norvegicus), rice rats (Oryzomys palustris), and albino rats (Rattus norvegicus), were fed encysted larvae of P. hispida. P. hispida were recovered only from the Norway and albino rats (Schell, 1952a).

Zago (1956) collected and examined 86 field crickets, and found 7 of them naturally infected with third-stage larvae of P. praeputialis. The larvae were identified by comparing them with third-stage larvae obtained from experimental infections.

In order to determine the natural intermediate host, Zago (1957) used cat feces as bait to attract arthropods to traps. Following capture, they were fed eggs of P. praeputialis, and later examined for larvae. The field cricket and gray cricket (Miogryllus verticalis) proved to be the most successful intermediate hosts.

While working on the life cycle of P. praeputialis, Zago (1958) found that female worms placed in physiological saline solution would release an average of 4,500 eggs in a 12-hour period. He estimated that an average of an additional 154,000 eggs both, embryonated and unembryonated, were retained within the female. Embryonated eggs kept in physiological saline solution at 4° C remained viable for periods up to 60 days. Hatching did not occur when the eggs were exposed to room temperature or refrigeration.

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Twenty-three days were required, from the time of ingestion of P. praeputialis eggs by crickets until third-stage larvae had developed. It was noted that time required for development of the larvae increased when the intermediate hosts were kept at lower than room temperature (Zago, 1958).

Third-stage larvae began to show sexual differentiation within a few days following ingestion by the definitive host. Sexually mature worms were recovered from cats 131 to 156 days after having been fed third-stage larvae (Zago, 1958).

The literature contains numerous reports of larval helminths encysted in the musculature and organs of animals which are not considered as either intermediate or final hosts. Joyeux and Baer (1934) were the first to realize the potential of these hosts, and referred to them as "hôtes d'attente". In using this term, they implied that the host was not essential, and neither aided nor hindered in the completion of the life cycle.

Subsequent investigators added such terms as "accumulator", "collector", "reservoir", "storage", and "transport hosts" in designating this type of host.

In Russia, Rizhikov (1954) defined a reservoir host as an animal which took up infective helminth larvae and assisted in their transfer to the final host, without being an obligatory part of the life cycle. This host classification was further elaborated by

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Shumakovich and Rizhikov (1954), who divided reservoir hosts into two groups: one in which the reservoir is always a vertebrate, and the second in which it is either an invertebrate or a vertebrate.

Baer (1951), in order to reduce synonomy and confusion, proposed the term "paratenic host" to replace "hôtes d'attente", which lost its true meaning when translated into other languages.

Czaplinski (1963) defined paratenic parasitism "as the inclusion in the parasite life cycle of a host which only collects and as if (sic) stores infective stages for long periods of time." He suggested that the suspended development of larvae allowed survival over long periods with reduced metabolic rate. This is advantageous to the parasite by increasing its chance of reaching a final host, and by increasing the area of distribution.

In discussing host-relationships, Sprent (1963) stated that paratenic hosts are often merely resting places for the parasite, and not essential for development to infectivity. He cited the case of spiruroid nematodes utilizing beetles as a true intermediate host. If an infected beetle is eaten by an animal other than the final host, the larvae freed in the intestine migrate from it and reencyst in the tissue. In the paratenic host, they are provided a second chance to reach the true final host. The definitive host thus readily acquires infection by ingesting paratenic hosts which constitute a part of its natural food. In this respect, the paratenic host is essential as an

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ecological source. Baer (1951) uses the life cycle of Diphyllobothrium latum as an example of an essential paratenic host. The first intermediate host, a copepod, is ingested by plankton-feeding fish which constitute the second intermediate host. When these are ingested by piscivorous fish the plerocercoids migrate from the gut and reencyst in various tissues. These fish now function as true paratenic hosts. This second fish, while not necessary in the life cycle, is more apt to be eaten by man or bear.

In discussing the trematodes, Baer (1951) stated that there is a complete lack of paratenic hosts in digenetic trematodes. In the same year, Potekhina (1951) fed metacercariae of Alaria alata to white mice, and recovered them encysted in the intercostal muscles. These larvae developed to adults when fed to foxes and dogs. Pearson (1954) fed mesocercariae of Alaria arisaemoides to mice, ferrets, ducklings, and chicks. He recovered living mesocercariae from the birds and ferrets one month later, and in mice one year after feeding. When he (Pearson, 1956) fed mesocercariae of A. arisaemoides to frogs, garter snakes, domestic chickens and ducks, house mice, deer mice, a ferret, and a raccoon, encysted mesocercariae were recovered from the frogs, snakes, chickens, and mice. He also fed mesocercariae of A. canis to goldfish, frogs, garter snakes, ducks, chickens, deer mice, a chipmunk, a ferret, and a raccoon. Encysted mesocercariae were recovered from all

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except the chipmunk, raccoon, and goldfish. He also found large numbers of mesocercariae encysted in the tissue of a captured otter. Some of these were fed to a dog and mature A. canis were recovered later. Johnson (1968) found that white mice, white rats, and chicks served as paratenic hosts, when experimentally fed mesocercariae of A. marcianae.

A number of investigators have reported animals harboring immature larvae of nematodes, which normally use arthropods as their intermediate hosts. Chandler (1932) found gnathostome larvae, a spirurid using an arthropod as the normal intermediate host, in the liver of an opossum. Arita (1953) reported encysted larvae of Gnathostoma spinigerum in the tissue of weasels. Salamanders in Japan are naturally infected with gnathostome larvae (Miyazaki, 1954). Babero and Shepperson (1959) found gnathostome larvae encysted in the abdominal wall of a water moccasin (Agkistrodon piscivorous). Ash (1960) experimentally infected rats and mice with larvae of G. procyonis. Later, he (1962a) fed third-stage larvae of G. procyonis to guppies and recovered larvae which were subsequently fed to a snake. Six weeks post infection encysted larvae were found in the intercostal muscles. In addition, he was able to pass third-stage larvae serially from one snake to another. This suggested to him that gnathostome larvae are able to infect a series of cold blooded animals, thus demonstrating a well developed paratenic

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host-relationship. Daengsvang (1968) found third-stage larvae of G. spinigerum to be more versatile than those of G. procyonis in their ability to use a large variety of paratenic hosts. He experimentally infected such paratenic hosts as fish, amphibians, reptiles, birds, and mammals. When the encysted larvae from these hosts were fed to various other animals of the same class, they readily reencysted.

Alicata (1962) found land planaria (Geoplana septemlineata) serving as a natural paratenic host for Angiostrongylus cantonensis. In Tahiti, Alicata and Brown (1962) found fresh water prawns acting as paratenic hosts for A. cantonensis, and later Alicata (1964a) recovered encysted third-stage larvae from the stomach wall of burrowing land crabs. He (1964b) force fed third-stage larvae of A. cantonensis to young pigs and a calf. Upon examination, two weeks post-infection, encysted larvae were recovered from these animals. Natural infections of encysted A. cantonensis larvae were observed in frogs by Ash (1968). He postulated that cold-blooded animals are the most efficient paratenic hosts for this parasite.

Hobmaier (1937) reported that frogs, toads, lizards, snakes, sparrows, chickens, ducks, and rodents served as paratenic hosts for Aelurostrongylus abstrusus. He further determined experimentally that encysted larvae of A. abstrusus remained viable longer in cold-blooded animals, and suggested that they represented the most important paratenic host for this species.

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Hobmaier (1941a) reported that the Pacific garter snake (Thamnophis sp.) served as a natural paratenic host for Crenosoma mephitidis. Encysted larvae were visible in the wall of the stomach and intestine.

The livers of 573 small wild mammals from Canada were examined for encysted lungworm larvae. Of the four species of mammals found harboring larvae, the short-tailed shrew (Blarina brevicauda) was most frequently infected (Lankester and Anderson, 1966).

Bailey, Morgan, and Cabrera (1963) found Spirocerca sp. larvae encysted in the wall of the crop of several chickens in regions where the incidence of Spirocerca lupi was high. They postulated that chickens, which feed on dung beetles, fulfill the role of paratenic hosts in making the infective larvae of S. lupi readily available to dogs.

Huizinga (1967) reported larvae of Contracaecum sp. encysted in the mesenteries of piscivorous fish in Florida. Guppies experimentally infected with third-stage larvae were fed to largemouth bass and larvae were recovered from the mesenteries of the bass 7 days later.

Rizhikov (1952), using infective larvae of Physocephalus sexalatus obtained from beetles, infected fish (Carassius carassius, and Misgurnus fossilis), amphibians (Rana temporaria and Bombina bombina), and a turtle (Testudo horsfieldii).

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Shmitova (1963) succeeded in infecting mice, rats, lizards, frogs, and fish with third-stage larvae of Ascarops strongylina, but birds of various species were refractive. He found variation in rate of capsule formation in the different paratenic hosts. In rodents, the capsules were formed in 18-36 hours, while in lizards and frogs 3-5 days were required.

The following encysted spirurid nematode larvae were reported from reptiles in the U.S.S.R.: Eustrongylides sp., Contracaecum sp., Spirocerca lupi, Physocephalus sexalatus, Ascarops strongylina, and Agamospirura sp. (Sharpilo, 1964).

Basir (1948) was the first to report a natural infection of Physaloptera larvae in an intermediate host. He recovered larvae free in the body cavity of an earwig from India.

Ehlers (1931), while working with captive badgers, suggested that the high incidence of Physaloptera in his captive animals might have resulted from feeding them wild rodents. Both Erickson (1944) and Kilgore (1969) implied that small animals and birds, which make up the bulk of the diet of wild canids act as important paratenic hosts for Physaloptera. This assumption is supported by the analysis of stomach contents of deer mice by Johnson (1961), who found that arthropods, especially insects when available, were consumed in large numbers.

In early reports of Physaloptera larvae encysted in abnormal hosts, Walton (1931) described a physalopterid larvae from the

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stomach and intestinal wall of a bull frog (Rana catesbiana) from Indiana. Cram (1932) found Physaloptera larvae encysted in the breast and leg muscles of bob-white quail (Colinus v. virginiania) and ruffed grouse (Bonasa umbellus) from Minnesota and Wisconsin. In a later survey conducted on ruffed grouse and sharp-tailed grouse from Minnesota and neighboring states, Boughton (1937) reported Physaloptera larvae encysted in the breast and leg muscles. He suggested that this location was unusual, since most encysted spirurid larvae are found either near the wall of the digestive tract or in it. Campbell and Lee (1953) examined 16 scaled quail (Callipepla squamata pallida) from New Mexico, and found Physaloptera sp. larvae encysted in the breast muscles. Hibler (1970) found 10 of 14 scaled quail from New Mexico infected with larvae of Physaloptera. They were encysted in the breast muscles, and the number of larvae per bird ranged from one to many. These reports were followed by that of Dixon and Roberson (1967), who found larvae of Physaloptera encysted in a bob-white quail from Louisiana. They were concentrated in the breast muscles in the area of the shoulder girdle. Mirza (1934) found physalopterid larvae encysted in the stomach wall and body cavity of the Indian squirrel (Sciuris palmarum).

A species of Physaloptera from domestic pigs was reported by Reid (1943). He recovered 20 immature worms deeply embedded in the stomach mucosa with only their posterior ends visible.

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Widmer (1967) reported Physaloptera sp. larvae encysted in the wall of the digestive tract of prairie rattle snakes (Crotalus viridis) from Colorado. Later he (1970) fed these larvae to cats, and recovered adult Physaloptera rara.

An interesting phase of physalopteran biology was encountered during an examination of wild mammals in Israel for leptospires. Numerous Physaloptera clausa Rudolphi, 1819 were recovered from a hedgehog, and the celomic cavity of the worms contained large numbers of leptospires. Due to the numbers of leptospires present, Torten, Beemer, and van der Hoeden (1966) postulated that the bacteria were multiplying in the worms. They were unable to determine if P. clausa served as a reservoir or definite host for the leptospires.

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CHAPTER III

METHODS AND MATERIALS

Snakes

Prairie rattlesnakes, Crotalus viridis Rafinesque, 1818, are known to act as a paratenic host for Physaloptera rara Hall and Wigdor, 1918. They provided one source of the larval P. rara used in this study.

Collection of prairie rattlesnakes.--The snakes were collected from five different prairie dog towns in Weld County, Colorado. Snakes were taken alive, using a snake stick for restraint. They were placed in burlap feed sacks, the sacks tied securely around the middle with a short rope, and transported to the laboratory. Collections were made in the fall (October-November) and spring (April-May) of the years 1968-1969. Fall collections consisted of snakes returning to prairie dog towns for hibernation and spring collections were snakes leaving the denning sites.

Maintenance of snakes in laboratory.--Burlap sacks containing ten snakes each were kept in a large wire cage 21 x 38 x 96 inches in size, constructed of angle iron, and covered on all sides with quarter inch wire mesh. It was located in a cool basement room. The sacks were frequently moistened to reduce dehydration of the snakes.

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Examination of snakes for larvae.--Snakes were examined for larvae as soon after collection as possible. They were removed from the sacks with a noose-stick and decapitated. After skinning each snake, the esophagus, stomach, small intestine, and mesenteries surrounding these organs were removed and examined for larvae visually and by means of a tissue press.

The tissue press consisted of two $\frac{1}{4}$ inch thick glass plates, measuring $3\frac{1}{2}$ inches wide by $4\frac{1}{4}$ inches long. Small pieces of tissue placed between the plates were pressed together and examined with a dissecting microscope. Following microscopic examination, all of the viscera and flesh of each snake were fed to domestic cats.

Cats

Members of the families Felidae and Canidae normally serve as the final host for P. rara. Due to their small size and ready availability, domestic cats, Felis domestica, were chosen as the final host to be used in this study.

Source and care of experimental cats.--Cats obtained from the Veterinary Clinic at Colorado State University were vaccinated for distemper and maintained in individual cages meeting government requirements. The cats were fed dry commercial dog food. To determine if natural infections of Physaloptera were present, feces from each cat were examined by a fecal flotation procedure.

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Examination of feces for eggs.--In preparation for examination, cat feces were collected and soaked in water for 24 hours. Coarse material was removed from the sample by straining the feces through a series of three graded screens with mesh sizes of 1.68 mm, 0.595 mm, and 0.074 mm. After final screening, the fecal suspension was placed in bottles for 10 hours to allow the eggs to settle. The colored water and fine suspended matter were decanted.

Various flotation solutions of different specific gravities were tried in an effort to determine the most suitable for use in the recovery of Physaloptera eggs. Magnesium sulfate with a specific gravity of 1.3, was the solution of choice. For examination, a one ml sample of the fecal sediment was pipetted from the bottle into a 12-ml centrifuge tube. The tube was then filled with magnesium sulfate solution. A coverslip was placed on top of the tube, and the tube was centrifuged for 10 minutes at 900 rpm to float the eggs. Eggs will float to the top, but all other debris will be pulled down. After centrifugation, the coverslip was transferred to a glass slide and examined with a compound microscope for eggs.

Purpose for infecting cats.--Cats were fed infected snake tissue to obtain adult worms for identification, and as a source of infective eggs. Experimental paratenic hosts, which had previously been fed third-stage larvae were examined both visually and microscopically to determine if they were infected. These animals were then fed to cats in order to recover any worms which may have been overlooked.

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Infection of cats.--Cats were infected by feeding them third-stage larvae from naturally infected rattlesnakes, and from experimentally infected crickets. Infective larvae collected from crickets were embedded in balls of meat and fed to cats in order to maintain a supply of worms.

Recovering adult worms.--Cats were euthanized by injecting 3 cc of lethol solution (see Appendix) into the heart. Immediately after death, a midventral incision was made from the pubic region to the base of the lower jaw. The esophagus, stomach, and first three feet of the small intestine were removed. Each of these three parts was opened with scissors and examined visually. All worms were removed with forceps, placed in clean tap water, washed and transferred to physiological saline solution.

Male worms were preserved and stored in Ward's fixative (see Appendix). Female worms were left in saline solution at room temperature for periods up to a week. During this time, they released many larvated eggs. These were used to infect the intermediate hosts employed in this study. Following egg collection, the females were preserved and stored in Ward's fixative.

Insects

Insects in the order Orthoptera serve as the intermediate host for many species of Physaloptera. Five different species of insects were tried as intermediate hosts. They included the American

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cockroach, Periplaneta americana, Madeira cockroach, Leucaphaea maderae, tenebrionid beetle, Eleodes tuberculata, common field cricket, Gryllus assimilis, and the red-legged grasshopper, Melanoplus femur-rubrum. Only the last two were found to be successful intermediate hosts.

Intermediate host.--Grasshoppers are difficult to maintain in the laboratory, therefore, the common field cricket was chosen as the intermediate host. Crickets were collected in areas remote from human dwellings, to reduce the possibility of natural infections of Physaloptera from feral cats and dogs.

Collections were made early in the morning or on cold days so the crickets would be torpid and easily captured.

Maintenance of crickets.--When crickets were brought into the laboratory, they were promptly separated into small groups to reduce or prevent cannibalism, which invariably occurs when large numbers are confined in a small container. Groups of five crickets each were placed in one-gallon jars, the bottoms of which were covered with $1\frac{1}{2}$-2 inches of sand. Water for drinking was supplied in a small dish filled with moist cotton, the cotton prevented drowning. A piece of crumpled paper towel was provided for the crickets to perch on and hide under. The sand was kept moist to maintain a relative humidity of 38-42%. The room temperature ranged between 73-80° F, over a two month period.

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Crickets were fed a mixture of rolled oats, dried milk, and sugar. These ingredients were dried, and ground into a fine powder with a mortar and pestle. The powder was kept in a dry form. When needed to feed the crickets, sufficient water was added to form a soft paste-like material which was placed on a piece of waxed wrapping paper. In addition, they were fed lettuce leaves. The lettuce was washed thoroughly to remove any insecticides which may have been present.

Adult crickets mated in the gallon jars, and the females laid eggs. When the eggs hatched, adults were removed to prevent the young from being eaten. These young crickets served as a source of parasite-free intermediate hosts.

A stock of crickets was kept in a clean five-gallon lard drum, with 2-3 inches of sand covering the bottom. Water and moisture were supplied as described above for the small containers.

Method of infecting intermediate host.--Preparatory to infection, each cricket was isolated in a pint jar, supplied with a small dish of drinking water but deprived of food for 6-10 days. The mouth of the jar was covered with a piece of gauze to keep the crickets from escaping.

Eggs of P. rara are normally covered with a sticky substance that causes them to adhere to objects with which they come into contact. When left in saline solution at room temperature, a light

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growth of bacteria and fungal contaminents soon surround the eggs. The contaminant "containing" the eggs facilitated separation of the egg masses with teasing needles and also permitted easy positioning of the egg masses on the lettuce, using teasing needles and a dissecting microscope. To insure that the eggs adhere to the lettuce, and that the crickets would eat them, excessive water was removed from the surface of the lettuce with small strips of absorbent paper.

Lettuce bearing Physaloptera eggs was placed in a jar containing a starved cricket. After the crickets ingested the lettuce, they were maintained in gallon jars with five individuals per jar. Crickets that did not eat the infested lettuce were discarded.

Collection of third-stage larvae.--Infected crickets were decapitated with scissors, the legs removed, and a lateral incision made from the thorax to the posterior part of the abdomen. The entire gastro-intestinal tract was teased free and transferred to a clean petri dish.

Third-stage larvae were collected by allowing the digestive tract to soak in physiological saline solution over night. The larvae excysted and migrated into the saline. They were transferred to clean saline solution the following day.

Experimental paratenic hosts

Snakes, representing the class Reptilia in this study, were proven paratenic hosts for P. rara. To determine if other terrestrial

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vertebrates would serve as paratenic hosts, a representative of the classes Amphibia, Aves, and Mammalia, was subjected to infection with third-stage larvae of P. rara.

Amphibia.--The leopard frog, Rana pipiens, was selected as a representative of the Amphibia. A total of 20 frogs was collected from abandoned gravel pits near Fort Collins.

Ten of the frogs were examined for natural infections. The viscera, muscle tissue, skin, and body cavity were examined visually and with a dissecting microscope. The different organs were placed in individual jars containing a pepsin-hydrochloric acid digestive solution, as used by Morgan and Hawkins (1949), kept at 38° C, for 12 hours. Digestion was halted by removing the excess solution and replacing it with 10% formalin. The material was treated with the iodine-thiosulfate method described by Whitlock (1948), and examined for larvae with a dissecting microscope.

The remaining ten frogs were divided into two groups of five each. One group served as a control group, and the other five were force-fed third-stage larvae.

Each frog was kept separately in a disposible plastic mouse cage. Immature cockroaches were supplied as food, and the feces of each infected frog were collected every other day and examined for larvae.

Experimental frogs were killed and examined on the 76th and 78th days postinfection. The viscera, muscle tissue, and body

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cavity were examined both grossly and with a dissecting microscope. A tissue press was used to make a more detailed study of the digestive tract.

Following microscopic examination, one frog was digested with pepsin-hydrochloric acid solution, and examined by the iodine-thiosulfate technique. After gross and microscopic examination of the viscera and muscle tissue, the remaining four frogs of the infected group were fed to a cat.

All frogs in the control group were examined grossly and microscopically, and then fed to a cat.

Aves.--Chickens were selected as representatives of the class Aves. Nine 10-day old cornish rock chicks were obtained from a local hatchery. Four chicks were used as the control group, and five were force-fed third-stage Physaloptera larvae. The larvae were drawn into a rigid plastic tube by means of a rubber squeeze bulb, the tube inserted down the esophagus of the chick, and the larvae flushed out.

Beginning on the 42nd day and terminating on the 57th day post-infection, the chickens were killed and the viscera, muscle tissue, and body cavity were examined grossly and with a dissecting microscope. A more complete examination of the digestive tract was made with the aid of the tissue press.

One chicken was digested with pepsin-hydrochloric acid solution, and the residue was examined, using the iodine-thiosulfate

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technique. The remaining four chickens in the infected group were fed to two cats after gross and microscopic examination of viscera and muscle tissue.

The control group of chickens, with the exception of pepsin digestion, was examined in the same manner as the infected group. Following examination, they were fed to two cats.

Mammalia.--CFW strain White Swiss-Webster mice, purchased from Carwood Farms, New City, New York, were the mammals used in this experiment. A total of eleven 10-week old mice was used. The control group consisted of five mice and the experimental group of six mice all of which were fed Physaloptera larvae.

Two of the mice were force-fed larvae using a syringe fitted with a stomach tube. The other four mice were starved for two days, and then fed the digestive tract of crickets containing encysted Physaloptera larvae.

One of the mice that was force-fed larvae, and one mouse that ingested larvae were examined and digested in pepsin-hydrochloric acid solution. The remaining four mice were killed and examined grossly and microscopically before being fed to a cat. The entire control group was necropsied, examined, and then fed to a cat.

Examination of wild hosts for natural infections

The carcasses of 1 badger, Taxidea taxus; 11 bobcats, Lynx rufus; 103 coyotes, Canis latrans; 3 raccoons, Procyon lotor; 5 red

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foxes, Vulpes fulva; and 1 striped skunk, Mephitis mephitis, obtained from trappers, fur buyers, and hunters were examined for adult Physaloptera. The esophagus, stomach, and first three feet of the small intestine were examined.

Fixation and clearing of Physaloptera

Adult and larval Physaloptera were killed in hot Ward's fixative, in which they were stored at room temperature. Worms were cleared in glycerine-alcohol solution, to which a drop of dilute fast green stain was added to aid in microscopic observation of external morphological characteristics.

The Physaloptera were prepared as temporary mounts in glycerine jelly for microscopic examination and study. Following examination, they were removed from the slides, and returned to the fixative.

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CHAPTER IV

PRESENTATION OF DATA

The results of this study are presented as 1) brief history of taxonomy, 2) basic life cycle of Physaloptera rara, 3) description of the adult P. rara, 4) description of the larval stages, 5) role of paratenic hosts in the life cycle, 6) investigation of natural incidence of infection in wild canids and felids.

Brief history of taxonomy

Physaloptera rara was described by Hall and Wigdor (1918) from a single female specimen collected from a dog raised in Detroit. The first report of a physalopteran nematode from a domestic cat in North America was that of Ackert (1936) who described it as a new species Physaloptera felidis. Following the examination of specimens of P. rara and P. felidis, Morgan (1944) considered them to be the same and placed P. felidis in synonomy with P. rara.

Basic life cycle of P. rara

Sexually mature worms inhabit the stomach and the first part of the small intestine of canids and felids (Plate IV, Fig. 1). Embryonated eggs released by the female are voided with the feces of the host, and the larva is infective at the time of passage. Upon ingestion

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of the eggs by a suitable orthopteran intermediate host, the larvae hatch and migrate into the wall of the midgut and rectum. Following penetration, cysts are formed around the larvae, and in these cysts development to the infective third-stage occurs (Plate IV, Fig. 2).

In certain cases, when infected intermediate hosts are eaten by animals unsuitable as final hosts, the larvae freed in the digestive tract apparently migrate through the gut and reencyst in various tissues of the new host. Thus, these animals become and serve as paratenic hosts, which may or may not be essential in the life history of the nematode, but are an ecological expediency for it. When either an infected intermediate or paratenic host is ingested by a canid or felid definitive host, the larvae excyst in the stomach, attach, undergo two molts, and develop to sexual maturity, mate, and begin reproducing, thus completing the life cycle.

Morphology of the adult nematode

Physaloptera rara Hall and Wigdor, 1918

(Plate 1, Figs. 1-3; Plate II, Figs. 1-2)

The cuticle has fine transverse striations the entire length of the body. A pair of lateral cephalic papillae is located just anterior to the nerve ring. Two large lateral lips, or pseudolabia, surround the mouth. Each lip bears two teeth, the inner one has three points of approximately equal height, whose overall length is slightly greater

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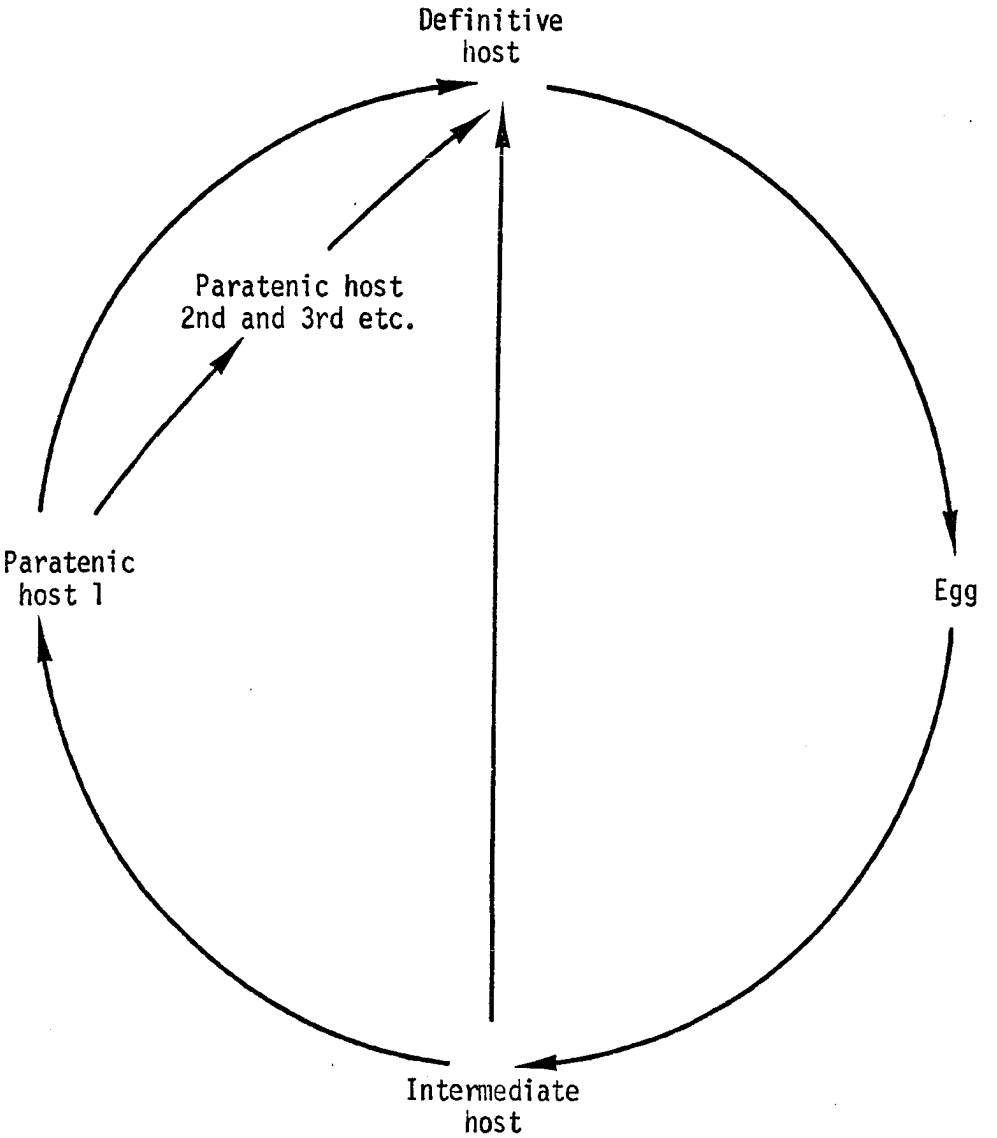

Basic life cycle of Physaloptera rara including postulated 2nd and 3rd, etc., paratenic hosts.

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than that of the single outer tooth. Each lip bears one median amphid and two papillae, one subdorsal and the other subventral.

Male (10 randomly selected specimens).--Length* 31.0 (28.0-36.0) mm. Maximum width of body, just posterior of esophageo-intestinal junction 911.0 (343.0-1067.0) μ. Length of esophagus 5.9 (5.0-6.9) mm. Width of collarette 257.0 (218.0-295.0) μ. Nerve ring to anterior extremity of body 498.0 (429.0-569.0) μ. Excretory pore to anterior extremity of body 666.0 (600.0-709.0) μ. Length of tail 1.21 (0.999-1.544) mm. The spicules are unequal in length with the left 686.0 (608.0-725.0) μ, and the right 585.0 (515.0-647.0) μ (Table 1).

Bursa moderate in size, with the tail flexed ventrally. There are 21 posterior papillae consisting of 1) four pairs of long peduncled lateral papillae supporting the bursa, of which 2 pairs are anterior to the anus and 2 pairs posterior to the anus; 2) three sessile preanal papillae forming a triangle, with the middle papilla closest to the anus, 3) four sessile postanal papillae aligned on the border of the anus, and 4) three pairs of sessile caudal papillae on the anterior three-fourths of the tail (Plate I, Figs. 4-6).

Female (10 randomly selected specimens).--Length of body 40.3 (30.0-46.0) mm. Maximum width of body just posterior to

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*Measurements include average with range inclosed in parentheses.

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esophageo-intestinal junction 1.262 (0.931-1.521) mm. Length of esophagus 6.8 (5.0-7.7) mm. Width of collarette 295.0 (257.0-359.0) μ. Nerve ring to anterior extremity of body 465.0 (343.0-507.0) μ. Excretory pore to anterior extremity 793.0 (601.0-1030) μ. Vulval opening to anterior extremity 5.1 (3.9-6.3) mm. Length of tail 641.0 (507.0-811.0) μ (Table 2). Uteri didelphic, each branch attached to lateral side of posterior end of egg chamber. A long muscular duct extends from the anterior end of the egg chamber to the vulva (Plate II, Figs. 3, 5-6).

General morphology of the egg

Egg.--The oval, thick, smooth shelled eggs contain a first stage larva when released by the female. Egg 46.1 (43.8-48.8) μ long by 31.9 (30.1-33.8) μ wide (Plate II, Figs. 4, 7).

Morphology of larval stages from experimentally infected crickets

First-stage larvae.--Attempts to free larvae from eggs by mechanical pressure resulted in ruptured specimens unsuitable for study. The first intact larvae were recovered from crickets 36 hours after the eggs had been ingested. Length of body 252.0 (223.0-270.0) μ. Width of body at esophageo-intestinal junction 15.0 (14.0-17.0) μ. Length of esophagus 79.0 (71.0-86.0) μ. Nerve ring to anterior extremity of body 31.0 (27.0-33.0) μ. Length of tail 13.0 (11.0-14.0) μ. Width of body at anus 5.0 (4.0-5.0) μ. Average measurements of first stage and subsequent stages appear in Table 3.

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Table 1. Measurements of mature males of *Physaloptera rara*

Worm No.	Length of Body	Length of Esoph.	Width of Body	Width of Collarette	Distance, anterior extremity to Nerve ring	Distance, anterior extremity to Excretory pore	Length of spicule L	Length of spicule R	Length of tail
1	29.0	5.9	0.976	0.265	0.507	0.663	0.710	0.647	1.23
2	29.0	6.4	0.976	0.226	0.429	0.640	0.702	0.515	1.15
3	32.0	6.1	1.022	0.242	0.538	0.694	0.608	0.530	1.11
4	30.0	6.0	0.976	0.257	0.491	0.663	0.663	0.569	1.01
5	33.0	5.9	0.953	0.295	0.507	0.710	0.663	0.593	1.14
6	36.0	6.9	0.999	0.273	0.468	0.702	0.702	0.640	1.54
7	28.0	5.4	0.343	0.218	0.507	0.600	0.694	0.624	1.17
8	29.0	5.4	0.885	0.250	0.484	0.608	0.671	0.585	1.25
9	34.0	6.2	1.067	0.289	0.569	0.710	0.725	0.593	1.50
10	30.0	5.0	0.908	0.250	0.476	0.0	0.725	0.554	1.00

* Measurements in millimeters

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Table 2. Measurements of gravid females of Physalopera rara.

Worm No.	Length of Body	Length of Esoph.	Width of Body	Width of Collarette	Distance, anterior extremity to Nerve ring	Distance, anterior extremity to Excretory pore	Distance, anterior extremity to Vulva	Length of tail
1	46.0	7.5	1.45	0.359	0.484	0.889	6.2	0.613
2	39.0	6.7	1.09	0.273	0.460	0.702	3.9	0.585
3	44.0	7.1	1.34	0.289	0.484	0.788	6.0	0.671
4	45.0	6.7	1.36	0.281	0.484	0.819	5.4	0.585
5	40.0	6.8	1.02	0.312	0.491	0.718	4.0	0.671
6	30.0	5.0	0.93	0.257	0.343	0.601	3.9	0.507
7	46.0	7.4	1.52	0.359	0.507	1.030	6.3	0.811
8	38.0	7.7	1.36	0.289	0.0	0.936	5.4	0.647
9	39.6	6.7	1.36	0.273	0.0	0.889	5.4	0.725
10	35.5	6.4	1.18	0.257	0.0	0.554	4.5	0.591

* Measurements in millimeters

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Table 3. Average measurements of larvae of Physaloptera rara at various intervals from 36 hours to 159 days old, and encysted larvae from rattlesnakes.

Larvae age in days	Length of		Width of body at Esoph. intest. junction and Anus		Distance, anterior extremity to		Length of tail
	Body	Esoph.			Nerve ring	Excret. pore	
1½	0.252	0.079	0.015	0.005	0.031	0.0	0.013
5	0.273	0.095	0.030	0.018	0.0	0.018	0.046
11	0.714	0.207	0.054	0.034	0.066	0.108	0.057
13	1.585	0.384	0.071	0.042	0.092	0.163	0.087
14	2.363	0.515	0.113	0.062	0.094	0.0	0.122
15	2.079	0.603	0.103	0.049	0.093	0.157	0.115
16	2.630	0.857	0.122	0.060	0.133	0.231	0.127
21	4.412	1.334	0.164	0.063	0.135	0.256	0.119
24	4.547	1.379	0.174	0.066	0.143	0.275	0.133
33	4.313	1.240	0.162	0.071	0.142	0.258	0.133
129	4.801	1.623	0.184	0.072	0.153	0.260	0.154
152	6.095	1.873	0.217	0.075	0.167	0.353	0.163
from rattlesnake	6.901	1.818	0.219	0.087	0.187	0.331	0.152

*Measurements in millimeters

The body of a larva 36 hours old is filiform, tapering to a pointed tail which is flexed dorsally. The cuticle is finely striated transversely. A prominent tooth-like structure occurs at the anterior extremity. The esophagus is recognizable, but undeveloped. The nerve ring appears as two large cells, one on each side of the esophagus. The excretory pore was not seen. The intestine consists of refractile granules, extending from the posterior end of the esophagus to the anus (Plate II, Fig. 8).

The following measurements are of larvae five days old. Length of body 273.0 (249.0-307.0) μ. Width of body at esophageo-intestinal junction 30.0 (26.0-34.0) μ. Length of esophagus 95.0 (81.0-103.0) μ. Length of tail 46.0 (44.0-47.0) μ. Width of body at anus 18.0 (17.0-19.0) μ.

Five-day old larvae were quite well developed: the esophagus was more definite, and compressed into an S-shaped structure. A large excretory cell was located in the region of the distal third of the esophagus. The intestine extended to the border of the large well-defined rectum, but at this time the two structures had not united. The intestinal wall consisted of large cells with prominent centrally located nuclei. Numerous refractive granules were dispersed throughout the cytoplasm. An anal plug protruded from the anus (Plate II, Fig. 9).

The first stage terminated and was ready to molt to the second stage between the 11th and 12th days postinfection in experimental

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crickets kept at room temperature. The following measurements are an average of larvae collected at this time. Length of body 670.0 (554.0-803.0) μ. Width of body at esophageo-intestinal junction 53.0 (49.0-56.0) μ. Length of esophagus 205.0 (188.0-234.0) μ. Excretory pore to anterior extremity of body 106.0 (98.0-117.0) μ. Nerve ring to anterior extremity 64.0 (55.0-68.0) μ. Length of tail 57.0 (51.0-64.0) μ. Width of body at anus 35.0 (30.0-38.0) μ.

The bodies of these larvae were uniform in width, with a tapered, pointed tail flexed dorsally. The tooth-like spine at the anterior extremity was lacking. The esophagus was straight and well developed, with the muscular portion a little more narrow than the glandular portion. Strands of nerve fibers making up the nerve ring encircled the esophagus near the junction between the muscular and glandular portions. The excretory pore was readily visible and slightly posterior to the nerve ring. The intestine was continuous to the anus, and the intestinal cells were more numerous and smaller in size. An anal plug was still present (Plate II, Fig. 10).

Second-stage larvae.--Development of the second-stage occurs during the 13th and 14th days postinfection; by the end of the 14th day they are mature and ready to molt to the third stage. The following measurements were made on larvae collected on the 13th and 14th days. Length of body 1.662 (1.009-2.363) mm. Width of body at esophageo-intestinal junction 75.0 (45.0-113.0) μ. Length of

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esophagus 397.0 (252.0-515.0) μ. Excretory pore to anterior extremity of body 163.0 (139.0-184.0) μ. Nerve ring to anterior extremity 92.0 (80.0-102.0) μ. Length of tail 91.0 (81.0-122.0) μ. Width of body at anus 44.0 (38.0-62.0) μ.

In the second-stage larvae, a pair of cephalic glands develop at the anterior extremity of the esophagus. The pseudolabia begin to acquire the shape characteristic of those of the adult. The nerve ring increased in size. The excretory pore and duct were well developed and clearly visible. The esophagus, intestine, rectum, and anal plug were similar to those structures described for the terminal phase of the first-stage larvae (Plate III, Fig. 1).

Third-stage larvae.--Measurements were made on a series of third-stage larvae taken at various intervals between the 15th and 152nd days postinfection, and from encysted third-stage larvae collected from rattlesnakes. Averages for each age group were made from 4 to 10 larvae, depending on number available.

15 days postinfection.--Length of body 2.079 mm. Width of body at esophageo-intestinal junction 103.0 μ. Length of esophagus 603.0 μ. Excretory pore to anterior extremity of body 157.0 μ. Nerve ring to anterior extremity 93.0 μ. Length of tail 115.0 μ. Width of body at anus 49.0 μ.

Other than increase in size, very few morphological changes occurred during the third stage. The few changes noted were in

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15- and 21-day-old larvae. Genital primordia could not be seen in any of the larvae.

In 15-day old third-stage larvae, the teeth on the pseudolabia were starting to develop. The rectum was the same width as the intestine. The tail was both more tapered and pointed, and no longer flexed (Plate III, Fig. 2).

16 days postinfection.--Length of body 2.630 mm. Width of body at esophageo-intestinal junction 122.0 μ. Length of esophagus 857.0 μ. Excretory pore to anterior extremity of body 231.0 μ. Nerve ring to anterior extremity 133.0 μ. Length of tail 127.0 μ. Width of body at anus 60.0 μ.

21 days postinfection.--Length of body 4.412 mm. Width of body at esophageo-intestinal junction 164.0 μ. Length of esophagus 1.334 mm. Excretory pore to anterior extremity of body 256.0 μ. Nerve ring to anterior extremity 135.0 μ. Length of tail 119.0 μ. Width of body at anus 63.0 μ.

In larvae of this age, the pseudolablia, teeth, and collarette were well developed, and the anal plug no longer present (Plate III, Fig. 3).

24 days postinfection.--Length of body 4.547 mm. Width of body at esophageo-intestinal junction 174.0 μ. Length of esophagus 1.379 mm. Excretory pore to anterior extremity of body 275.0 μ. Nerve ring to anterior extremity 143.0 μ. Length of tail 133.0 μ. Width of body at anus 66.0 μ.

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33 days postinfection.--Length of body 4.313 mm. Width of body at esophageo-intestinal junction 162.0 μ. Length of esophagus 1.240 mm. Excretory pore to anterior extremity of body 258.0 μ. Nerve ring to anterior extremity 142.0 μ. Length of tail 133.0 μ. Width of body at anus 71.0 μ.

129 days postinfection.--Length of body 4.801 mm. Width of body at esophageo-intestinal junction 184.0 μ. Length of esophagus 1.623 mm. Excretory pore to anterior extremity of body 260.0 μ. Nerve ring to anterior extremity 153.0 μ. Length of tail 154.0 μ. Width of body at anus 72.0 μ.

152 days postinfection.--Length of body 6.095 mm. Width of body at esophageo-intestinal junction 217.0 μ. Length of esophagus 1.873 mm. Excretory pore to anterior extremity of body 353.0 μ. Nerve ring to anterior extremity 167.0 μ. Length of tail 163.0 μ. Width of body at anus 75.0 μ.

Encysted third-stage larvae from rattlesnakes.--Length of body 6.90 mm. Width of body at esophageo-intestinal junction 219.0 μ. Length of esophagus 1.82 mm. Excretory pore to anterior extremity of body 332.0 μ. Nerve ring to anterior extremity 187.0 μ. Length of tail 152.0 μ. Width of body at anus 87.0 μ (Plate III, Fig. 4).

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Naturally infected paratenic host of Physaloptera rara

Prairie rattlesnakes from Colorado are known paratenic hosts for P. rara and the only animals considered as such in this study. Third-stage larvae are encysted in the wall of the esophagus, stomach, first part of the intestine, and in the mesenteries surrounding these organs.

A total of 160 rattlesnakes was fed to experimental cats. Of this number, 88 snakes were dissected and examined both grossly and microscopically for encysted third-stage larvae. Thirteen of the snakes dissected harbored physalopteran larvae. Only encysted larvae were encountered. They numbered from 2 to many per snake. Without examination, the remaining 72 snakes were killed, cut into small pieces, and fed to cats. The first recovery of eggs of P. rara occurred 75 days after the cat had been fed infected snake tissue.

Experimentally infected hosts of Physaloptera rara

Representatives of several groups of vertebrate animals were experimentally fed third-stage larvae of P. rara to determine which might serve as paratenic hosts.

Frogs.--From 20 leopard frogs collected locally, 10 were chosen randomly and examined for natural infections of physalopteran larvae. All were negative. Of the remaining 10 frogs, five receiving no larvae served as a control group, and five were force-fed third-stage larvae of P. rara and constituted the experimental group.

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The experimentally infected group received a total of 62 third-stage larvae, as shown in Table 4. Feces from the frogs of this group were examined daily for the first four days following infection. After this period, feces were examined at four-day intervals until the frogs were killed. A total of 25 larvae was recovered from the feces with the first appearing on the second day postinfection, and the last on the 21st day.

Nine larvae recovered from the feces on the 21st day post-infection were placed in a ball of meat and fed to a cat. When this cat was euthanized 156 days later, two P. rara were recovered from the first two inches of the small intestine.

Experimentally infected frogs were killed and examined for larvae of P. rara 42, 44, 45, 59, and 77 days postinfection. Upon examination, no encysted nor unencysted larvae were found. Following examination, all the frogs were fed to one cat. This cat was euthanized 119 days later and examined for worms. Three adult P. rara were recovered (Table 7).

The cat fed the group of control frogs was negative for P. rara when examined.

Chickens.--Nine chicks purchased from a hatchery were used in this part of the experiment. Four served as controls, and five were force-fed larvae. The experimentally infected group was given a total of 90 third-stage P. rara larvae (Table 5). From feces

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Table 4. Experimental infection of frogs with third-stage larvae of Physaloptera rara.

Frog No.	No. of third-stage larvae fed to animal	No. of larvae recovered from feces	Age of infection in days	No. of third-stage larvae recovered from animal at necropsy
Experimental				
1	15	6	77	0
2	10	2	59	0
3	12	1	42	0
4	15	12	44	0
5	8	7	45	0
Controls				
6	0	0	--	0
7	0	0	--	0
8	0	0	--	0
9	0	0	--	0
10	0	0	--	0

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Table 5. Experimental infection of chickens with third-stage larvae of *Physaloptera rara*.

Chicken No.	No. of third-stage larvae fed to animal	No. of larvae recovered from feces	Age of infection in days	No. of third-stage larvae recovered from animal at necropsy
Experimental				
1	20	5	26	0
2	20	0	42	0
3	20	4	47	0
4	18	2	53	0
5	12	0	44	0
Controls				
6	0	0	--	0
7	0	0	--	0
8	0	0	--	0
9	0	0	--	0

collected for 12, 24, and 36 hours after the chicks were infected, a total of 11 larvae was recovered from feces passed during the first 12 hours postinfection. Feces collected during the 24 and 36 hour-periods postinfection and examined for Physaloptera larvae were all negative.

The chickens were killed 26, 42, 44, 47, and 53 days after infection. The chick killed on the 26th day postinfection was examined grossly and microscopically, then digested in pepsin-hydrochloric acid solution in an effort to recover larvae from the tissues. The residue from digestion was examined by the thiosulfate-iodine method. No Physaloptera larvae were found. The remaining four chickens were examined grossly and microscopically without finding larvae. Their carcasses were fed to two cats.

One hundred and twenty nine days after the last experimentally infected chicken had been fed to the cats, they were euthanized and the esophagus, stomach, and small intestine were examined for Physaloptera. Both cats were negative for Physaloptera (Table 7). From this experiment none of the chickens became infected.

The control group of chickens was examined in the same manner as the infected group except for the pepsin-hydrochloric acid digestion, which was omitted. No larvae were seen. Following examination, the chickens were fed to two control cats. Upon examination, after euthanasia the two control cats were negative for Physaloptera infection.

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Mice.--Four white laboratory mice served as a control group, and six other mice were fed a total of 45 third-stage larvae of P. rara. The results of this experiment are presented in Table 6.

Two of the mice were force-fed larvae. One of these mice received three larvae recovered from frog feces nine days after the frog was force-fed third-stage larvae, and the second mouse received 11 third-stage larvae collected from an experimentally infected cricket. The remaining four mice of the experimental group were starved two days and then fed digestive tracts of crickets containing encysted third-stage larvae.

Feces of the experimentally infected group were collected continuously during 24 and 48 hour periods postinfection, and examined for larvae. One 24-hour collection, from a mouse which had ingested an infected digestive tract of a cricket, contained nine pieces of larvae. These consisted of five tails, three heads, and one mid-section of body. All of the other fecal collections were negative for Physaloptera larvae.

The mouse that was force-fed 11 third-stage larvae was injured during the feeding process. It was killed and examined four days after being fed larvae. One larva was recovered from the lumen of the stomach. Following examination, the animal was dissected and digested with pepsin-hydrochloric acid solution. Nine larvae were recovered from the digest. Three larvae came from the back and ribs, three from the neck, and three from the right leg.

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Table 6. Experimental infection of mice with third-stage larvae of Physaloptera rara

Mouse No.	No. of third-stage larvae fed to animal	No. of larvae recovered from feces	Age of infection in days	No. of third-stage larvae recovered from animal at necropsy
Experimental				
1	3	0	89	0
2	11	0	4	10
3	5	0	77	0
4	10	0	63	0
5	10	9	57	0
6	8	0	68	0
Controls				
7	0	0	--	0
8	0	0	--	0
9	0	0	--	0
10	0	0	--	0

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Table 7. Summary of experimentally infected frog, chicken, and mouse paratenic hosts with third-stage larvae of Physaloptera rara from laboratory reared and infected field crickets (Gryllus assimilis).

Cat fed experimentally infected	No. of experimental infected paratenic hosts fed to cat	No. of days after cat fed experimental paratenic hosts until euthanized	No. of P. rara recovered from cat at necropsy
Frogs	5	119	3
Chickens	5	129	0
Mice	5	117	1

The other mouse, which was force-fed larvae, was killed 89 days postinfection. Both the gross and microscopic examination failed to reveal Physaloptera larvae. Following examination, the mouse was fed to a cat which was necropsied 126 days later. No Physaloptera were found on examination.

The remaining four mice of the experimentally infected group were killed and examined for encysted larvae 57, 63, 68, and 77 days after they had ingested the third-stage larvae. No larvae were found upon gross and microscopic examination. The mouse killed on the 57th day postinfection was digested in the usual manner followed previously, and examined by the iodine-thiosulfate method. The results of this examination were negative for physalopteran larvae. Following examination of the other three mice, they were fed to a cat. It was euthanized and necropsied 117 days later, and one female P. rara was recovered (Table 7).

Investigations on natural incidence of Physaloptera infection of wild canids and felids

A survey of wild carnivores, with emphasis on canids and felids, was made to determine the prevalence of physalopteran infections.

Canids.--Of 103 coyotes examined for Physaloptera, 40, or 38.8%, were positive for P. rara. The number of worms per animal ranged from 1 to 38.

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Of the five red foxes examined, all were negative for phyalopteran infection.

Felids.--Nine of the 11, or 81.8%, bobcats examined were positive for P. praeputialis. The number of worms per animal ranged from 2 to 73.

Other carnivores.--One badger, one skunk, and three raccoons were examined for Physaloptera. Of these animals, only the skunk was infected with Physaloptera. A large number of P. maxillaris was recovered from the stomach.

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CHAPTER V

DISCUSSION

Morgan (1944) presented a table comparing the measurements of adult P. rara collected from dogs with the measurements Ackert (1936) had made on adult P. felidis from cats. From the similarities of the measurements and morphological features, Morgan concluded that P. felidis was synonymous with P. rara. Measurements of adult P. rara from cats experimentally infected during this study are listed with those made by Ackert and Morgan in Table 8. From these measurements, it is evident that P. rara can utilize both canids and felids as the definitive host with equal success. This lack of host specificity greatly enhances the probability of completion of the life cycle.

Petri (1950) used the german cockroach as an experimental intermediate host for P. rara, and Zago (1958) found crickets naturally infected with third-stage larvae of P. praeputialis. Since german cockroaches are uncommon in the northern part of Colorado, the cricket or possibly grasshoppers were considered to be a more normal intermediate host.

While no naturally infected crickets were found, this can be partly explained by the area from which they were collected.

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Table 8. Comparative measurements of adult Physaloptera rara.

Author and Host	Sex	Length of Body	Length of Esoph.	Width of Body	Distance, Head to Vulva	Eggs	Spicule Length Left	Spicule Length Right
Ackert	M	25.0-29.0	4.7-5.3	0.710-0.803	-------	----------	0.671-0.830	0.513-0.603
(cats)	F	27.0-44.0	6.6-7.8	0.958-1.100	3.2-5.8	0.045 x 0.032	----------	----------
Morgan	M	26.0-28.0	4.8-6.0	0.800	-------	----------	0.796	0.482
(dogs)	F	30.1-41.0	7.0	1.000	4.8	0.043 x 0.032	----------	----------
This	M	28.0-36.0	5.0-6.9	0.343-1.067	-------	----------	0.608-0.725	0.514-0.647
study	F	30.0-46.0	5.0-7.7	0.931-1.521	3.9-6.3	0.046 x 0.032	----------	----------
(cats)								

* Measurements in millimeters

Collections were made in areas unlikely to be frequented by either domestic or wild canids or felids.

In describing the larvae of P. praeputialis, Zago (1958) found that very little development occurred during the first two days after hatching in the intermediate host. He noted the following structures in two-day-old larvae: 1) three cephalic spines at the anterior extremity, 2) esophagus visible, but poorly developed, 3) intestine consisting of a delicate membrane and not yet having attained full length, 4) anus not visible, and 5) excretory cell in the region of the posterior third of the esophagus. Measurements of two-day-old larvae were given as total length 273 μ, width of body at esophageo-intestinal junction 16 μ, and length of esophagus 117 μ.

Petri (1950) stated that differentiation of the esophagus and intestine began in larvae of P. rara at two days of age. The two larvae of this age which he measured were 256 μ and 290 μ in length. By comparison, the average length of nine 36-hour old larvae from the present study was 252 μ, width of body at esophageo-intestinal junction 15 μ, and length of esophagus 79 μ.

Petri (1950) described the cuticle as smooth and noted a single spine-like structure at the anterior extremity of the body in larvae five to seven days old. The esophagus, which had started to differentiate into the muscular and glandular portions, and the intestine were well developed. Total lengths of his larvae ranged from 260 to

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455 μ, with lengths of the esophagi ranging from 79 to 142 μ. The excretory pore was prominent and located 63 to 112 μ from the anterior extremity of the body. The length of the tail was 50 to 76 μ.

These measurements are in the range of those made of five-day-old larvae in this study. The average length of four five-day-old larvae was 273 μ, esophageal length 95 μ, and length of tail 46 μ. In these larvae, the excretory pore and gland had not yet developed. It was represented by a large triangular shaped cell in the region of the distal third of the esophagus. This suggests that Petri was referring to larvae older than five days when he reported a prominent excretory pore.

The fifth to twelfth days were considered by Zago (1958) as a period of rapid growth and development for P. praeputialis. By the twelfth day, which terminated the first stage, the larvae had reached a length of 524 μ. The esophagus was 172 μ long and well developed. The excretory pore was 128 μ from the anterior extremity of the body and clearly visible. The nerve ring was 98 μ from the anterior extremity, and the intestine had extended to the anus. The tail was 76 μ in length.

Petri (1950) found the first stage terminating between the 11th and 16th days postinfection. Sixteen-day-old larvae ranged in total length from 350 to 625 μ with an esophageal length of 168 to 191 μ. He also stated that the esophagus had grown to the extent that it had doubled up on itself.

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In this study the first stage was found to end between the eleventh and twelfth days. The average measurements of the body were 700 μ, length of esophagus 205 μ. The nerve ring and excretory pore were 64 and 106 μ, respectively, from the anterior extremity of the body. The length of the tail was 57 μ. Rather than the cuticle being smooth as reported by Petri (1950), it showed transverse striations in all larval stages. A single spine-like process was present at the anterior extremity of larvae in eggs and remained up to the fifth day in the cricket. Zago (1958) in describing early first-stage larvae of P. praeputialis, reported three anterior spine-like structures. This difference suggests a morphological characteristic that could be used to distinguish first-stage larvae of P. rara from those of P. praeputialis.

While Petri (1950) reported that the esophagus doubled upon itself in 12-day-old larvae, this characteristic was seen only in larvae five days old or less in the present study.

Zago (1958) found that the second stage of larval development of P. praeputialis took place from the 13th to 23rd days postinfection. He considered this period as a stage of rapid growth. The excretory gland continued development and became distinct in shape. The glandular and muscular portion of the esophagus had began to differentiate. A bud-like growth, lacking cellular structure, in the region of the esophageo-intestinal junction was identified as the genital

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primordium. The intestine was a straight tube. Neither lips nor cephalic papillae were seen in second-stage larvae. He also noted that second-stage larvae lacked movement when dissected from the cyst, and were not infective when fed to cats. Measurements of second-stage larvae 15 days old were body length 649 μ, and esophagus 207 μ. Distance from anterior extremity of body to nerve ring and excretory pore were 99 and 138 μ, respectively; width of body at esophageo-intestinal junction 52 μ, and length of tail 71 μ.

Petri (1950) reported that the second stage of P. rara lasted from the 17th to 21st days postinfection. He described them as more slender than first-stage larvae and with the esophagus more uniform (sic) throughout its length. Two lateral lips were evident. The prominent tooth-like spine at the anterior extremity of the first-stage larvae was lacking in second-stage larvae. The nerve ring had continued to develop and marked the line of demarcation between the muscular and glandular portions of the esophagus. He noted inconspicous cuticular striations in late second-stage larvae, and observed the germinal primordium some distance below the equator of the intestine. His measurements of second-stage larvae were as follows: total length of body 920 to 1700 μ, length of esophagus 248 to 497 μ, distance from anterior extremity of body to excretory pore 149 to 175 μ.

In this study, the second stage of larval development took place during the 13th and 14th days postinfection. A pair of large cephalic

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glands was located at the anterior extremity of the esophagus. The two lateral pseudolabia had began to develop their characteristic shape, and the nerve ring had increased in size. Both the excretory pore and duct were well developed and clearly visible. The esophagus was well developed, and the esophageo-intestinal junction was clearly evident. Both the dorsal flexing of the tail and the anal plug were retained. The rectum was greater in width than the intestine, and its boundaries were well defined. The following measurements are combined averages of 13- and 14-day-old larvae: length of body 1662 μ and of esophagus 397 μ, distance from anterior extremity of body to excretory pore and nerve ring 163 μ and 92 μ, respectively, width of body at esophageo-intestinal junction 75 μ, and length of tail 91 μ.

While the measurements of second-stage larvae from this study, and those from Petri's (1950) study fall into a comparable range, his required an additional seven days to reach third stage. This implies that the common field cricket is a more efficient intermediate host than the german cockroach, assuming that room temperature (which was not given by Petri) was the same in both cases.

Zago (1958) observed third-stage larvae of P. praeputialis at 23 days postinfection. The head and pseudolabia were well developed, and the cephalic glands had disappeared. The esophagus showed a marked size differentiation, with the anterior muscular portion being

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smaller in diameter than the posterior glandular portion. The excretory pore and duct were well developed, and the intestine continued as a straight tube. The genital primordium remained the same as described for second-stage larvae. He noted that third-stage larvae were able to remain alive long after the intermediate host had died. When the digestive tract of crickets containing encysted third-stage larvae were placed in physiological saline, the larvae actively left the cyst. He (1958) found larvae of P. praeputialis to be infective as soon as they reached the third-stage. The following measurements are of larvae 23 days postinfection: length of body 2.094 mm, of esophagus 776 μ, distance from anterior extremity of body to nerve ring and excretory pore 161 μ and 253 μ, respectively, width of body at esophageo-intestinal junction 137 μ, and length of tail 109 μ.

Petri (1950) found third-stage larvae at 21 days postinfection. In this stage, he noted definite cuticular striations, and well developed pseudolabia and collar. The nerve ring marked the junction of the muscular and glandular portions of the esophagus, and the two oral glands were still visible. He stated that sexual differentiation of third-stage larvae was possible by the location of the genital primordium. In males, the genital primordium was located in the posterior half of the body, and in females in the esophageal region. His measurements of third-stage larvae were: length of body 1.6 to 4.0 mm, width of body 112 to 199 μ, length of esophagus 539 to 1550 μ,

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distance from anterior extremity of body to excretory pore 224 to 298 μ.

Third-stage larvae in this study were observed 15 days post-infection. The pseudolabia and teeth were more prominent and further developed than in the second-stage. The tail appeared to be more pointed, and was no longer flexed dorsally. The rectum had decreased in width, being the same as the distal portion of the intestine. The anal plug was lacking. Other than an overall increase in body size, and the morphological changes mentioned above, third-stage larvae examined at various times up to 152 days postinfection retained the same morphological characteristics as those described for the second-stage larvae.

The following average measurements of third-stage larvae were taken from four individuals 15 days old: length of body 2.079 mm, of esophagus 603 μ, distance from anterior extremity of body to nerve ring and excretory pore 93 μ and 157 μ, respectively, width of body at esophageo-intestinal junction 103 μ, and length of tail 115 μ.

While Petri (1950) first recovered third-stage larvae 21 days postinfection, and Zago (1958) at 23 days postinfection, their measurements fall into the range of the third-stage larvae recovered at 15 days postinfection in this study.

Petri (1950) stated that sexual differentiation occurs as early as that of the third-stage larva of P. rara. Zago (1958), on the other

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hand, examined over 100 third-stage larvae of P. praeputialis and was unable to distinguish sexual differences, which is in agreement with the observations made in the present study.

Zago (1958) found the prepatent period of P. praeputialis in experimentally infected cats to range from 132 to 154 days. Petri (1950) experimentally infected a cat and dog with third-stage larvae of P. rara. He necropsied them 29 and 30 days postinfection, respectively, and recovered only immature worms. Widmer (1970) fed rattlesnake tissue containing encysted larvae of P. rara to a cat, and reported recovery of eggs from the feces 35 days later. In this study, P. rara eggs were first recovered from the feces of experimentally infected cats 75 days after being fed infected rattlesnake tissue, and 79 days after being fed third-stage larvae collected from crickets. The prepatent period reported by Zago for P. praeputialis, and the recovery of immature P. rara by Petri fall within the time limitations of the prepatent period observed in this study. It is not clear how Widmer (1970) was able to recover eggs of P. rara in the feces of experimentally infected cats in such a short time.

The role of the intermediate host in the life cycle of a helminth parasite consists of two major physiological categories (Croll, 1966). In P. rara it provides, first, the stimulus for the egg to hatch and, second, nutrition and protection for the larva during the period of growth to, and including, the infective stage. Since P. rara is a

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parasite of large carnivores, the insect intermediate is not an important item in their food, and constitutes an ecological deterrant to infection. The presence of a suitable paratenic host in the food chain is of great importance.

In considering paratenic hosts, Croll (1966) lists three major functions which they perform. They are 1) providing the larvae a second chance to reach a suitable definitive host; 2) serving as a collector of larvae; and 3) serving as a normal constituent of the food chain when the intermediate host is not an important food item for the definitive host.

A required arthropod intermediate host may be regarded as an ecological barrier in the life cycle of P. rara, whose definitive host is a carnivore. Physaloptera rara has overcome this obstacle by developing the ability to utilize paratenic hosts.

In this study, frogs and mice were successfully infected as paratenic hosts. This is of ecological significance, since these animals are more important in the food chain of canids and felids than the arthropod intermediate host. Being insectivorous, frogs may accumulate large numbers of larvae in their intestine. It was shown that larvae freed in the gut are infective to cats. Thus the frogs might serve as efficient paratenic hosts without the necessity of the larvae encysting.

Although reports of encysted physalopteran larvae using birds as paratenic hosts appear in the literature, P. rara larvae did not

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occur in experimentally infected chickens in this study. Whether they are able to establish themselves in chickens is uncertain due to the limited material available. On the basis of natural infections with third-stage larvae in wild birds, it is suspected that P. rara might likewise infect chickens.

Rattlesnakes were naturally infected paratenic hosts as observed in this study, and also reported by Widmer (1970). Since insects constitute a large part of the diet of small birds, reptiles and rodents, these animals are the first line of paratenic hosts in the life cycle of P. rara. Being the major part of the food chain of wild canids and felids, these paratenic hosts bridge the ecological barrier between the insect intermediate and the definitive hosts. Rattlesnakes feeding on this first line of paratenic hosts, become a second line of paratenic hosts, acquiring large numbers of infective third-stage larvae. Thus, as the various paratenic hosts ascend in the food chain they become more important in that role.

While canids and felids may not actively hunt rattlesnakes, coyotes readily ingest dead snakes when available. Observations supporting this concept were made upon visiting an area two days after more than 100 rattlesnakes had been killed by hunters. Only a few remnants of snake carcasses remained. All the others had been eaten by coyotes, as indicated by the signs of their activities.

In the survey of wild canids and felids, for infections of Physaloptera there were 40 (38.8%), of 103 coyotes were positive for

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P. rara, five red foxes were negative for Physaloptera, and nine (81.8%), of 11 bobcats were positive for P. praeputialis. The coyotes were collected from the plains and foothills, and the bobcats from mountain areas. Since there was little if any overlapping in the areas from which the coyotes and bobcats were collected, this may explain in part why P. rara was recovered only from coyotes, and P. praeputialis only from bobcats. This suggests that P. rara is more ecologically adapted to utilize plains animals as paratenic hosts, than P. praeputialis. While both bobcats and coyotes are carnivorous, coyotes consume large amounts of carrion, which in turn would subject them to a greater range of potential paratenic hosts other than their normal prey.

The basic life cycle of P. rara is similar to the life cycles known for other species of Physaloptera, such as P. turgida according to Alicata, 1937, P. maxillaris by Hobmaier, 1941b, P. rara by Petri, 1950, P. hispida studied by Schell, 1952a, and P. praeputialis investigated by Zago, 1958. While the occurrence of paratenic hosts in the life cycles of species of Physaloptera has been suggested by other investigators, this study is the first experimental demonstration of various animals serving in this role.

Inasmuch as this study is by no means definitive, additional investigations should be directed toward the demonstration of serial transfer from one paratenic host to the next. Additional experiments

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using various birds should be done to determine if they can serve as paratenic hosts.

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CHAPTER VI

SUMMARY

1. Investigations were conducted on the life cycle and role of paratenic hosts, and the incidence of natural infections in wild carnivores of Physaloptera rara Hall and Wigdor, 1918, which occurs in the stomach and the anterior third of the small intestine of canids and felids.

2. Domestic cats (Felis domestica) served as the experimental definitive host.

3. Common field crickets (Gryllus assimilis) were used as the experimental intermediate host.

4. Development of the first-stage larvae was completed between the 11th and 12th days postinfection. Their body was uniform in width, with a tapered, pointed tail flexed dorsally. The tooth-like spine at the anterior extremity of the body was lost between the 5th and 11th days of development. The esophagus was straight and well developed. Strands of nerve fibers made up the nerve ring. The excretory pore was visible and slightly posterior to the nerve ring. The intestine was continuous to the anus. An anal plug was present.

5. Development of the second-stage larvae was completed between the 13th and 14th days postinfection. In these larvae, a pair

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of cephalic glands developed at the anterior extremity of the esophagus. The pseudolabia were acquiring the shape characteristic of the adult. The nerve ring had increased in size. The esophagus, excretory pore, intestine, anal plug, and tail were similar to those described for the first-stage larvae.

6. Third-stage larvae were first observed 15 days postinfection. A number of larvae of this stage collected at various intervals between the 15th and 152nd days postinfection, and encysted third-stage larvae from rattlesnakes were measured and compared in size. Other than an overall increase in body size, very few morphological changes occurred in the third stage. The few changes noted were in 15- and 21-day-old larvae. In 15-day-old larvae, the teeth and pseudolabia were starting to develop. The tail was more tapered, and no longer flexed. In larvae 21-days old, the pseudolabia, teeth, and collarette were well developed, and the anal plug was no longer present.

7. Thirteen of 88 rattlesnakes examined were naturally infected with encysted third-stage larvae of P. rara. Thus, they were naturally infected paratenic hosts of P. rara.

8. In an effort to determine which animals could serve as paratenic hosts, representatives of several groups of vertebrates were experimentally fed third-stage larvae of P. rara. Frogs and mice were successfully infected with third-stage larvae, and served

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as paratenic hosts for P. rara. Chickens were refractory to experimental infection.

9. Eggs of P. rara were first recovered from cat feces 75 days after the cat was fed infected snake tissue, and 79 days after a cat was fed third-stage larvae collected from an experimental cricket.

10. A survey of natural incidence of physalopteran infections in wild carnivores showed 40 (38.8%) of 103 coyotes positive for P. rara, and nine (81.8%) of 11 bobcats for P. praeputialis. Of five red foxes, one badger, one skunk, and three raccoons examined for physalopterans, all were negative except the skunk which harbored P. maxillaris.

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*Abbreviations used here are in accordance with those in the Index Catalogue of Medical and Veterinary Zoology. U.S. Govt. Printing Office, Washington.

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APPENDIX

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WARD'S FIXATIVE

Alcohol, 95%	24 ml
Formalin, 100%	15 ml
Acetic acid, glacial	5 ml
Glycerine	10 ml
Water, distilled	46 ml

LETHOL

Sodium pentabarbital	130 gm
Sodium chloride	15 gm
Alcohol, 95%	200 ml
Sodium hydroxide, 10%	15 ml
Water, distilled, bring total volume to	1,000 ml

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ABBREVIATIONS USED IN ILLUSTRATIONS

A - amphid

AP - anal plug

B - bursa

C - collarette

CA - caudal papilla

CG - cephalic gland

CP - cephalic papilla

CU - free cuticule

E - esophagus

EC - egg chamber

EP - excretory pore

EX - excretory cell

GE - glandular portion of esophagus

I - intestine

IT - inner teeth

LP - lateral papilla

M - mouth

ME - muscular portion of esophagus

N - nerve ring

NC - cells of beginning nerve ring

OT - outer tooth

P - labial papilla

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ABBREVIATIONS USED IN ILLUSTRATIONS (Cont.)

PA - preanal papilla

PO - postanal papilla

PS - pseudolabium

R - rectum

S - anterior tooth-like spine

SP - spicule

U - uterus

V - vulva

VA - vagina

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DESCRIPTION OF PLATE I

Fig. 1 -- Ventral view of head of adult P. rara.

Fig. 2 -- Lateral view of head of adult.

Fig. 3 -- En face view of adult.

Fig. 4 -- Ventral view of posterior end of male.

Fig. 5 -- Lateral view of posterior end of male, showing parts labelled in Fig. 4.

Fig. 6 -- Anus with three pre- and four postanal papillae.

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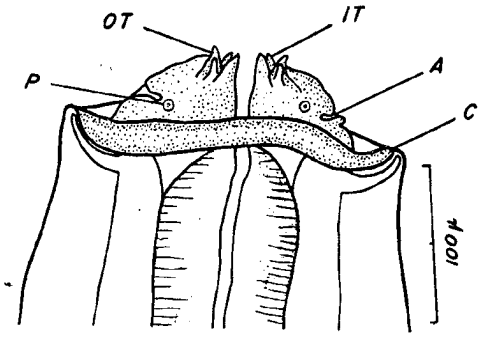
PLATE I



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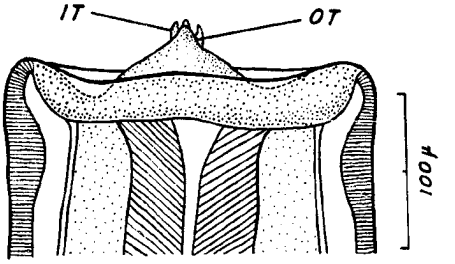

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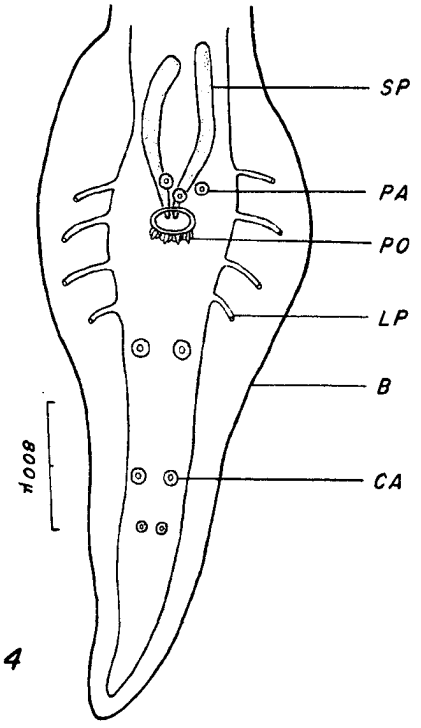

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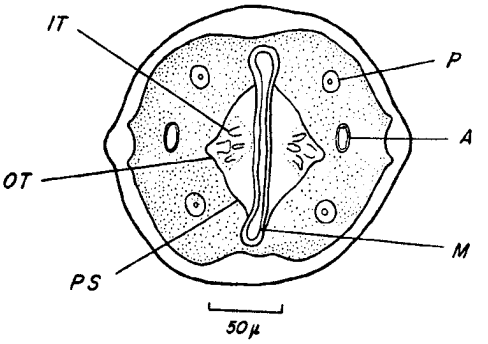

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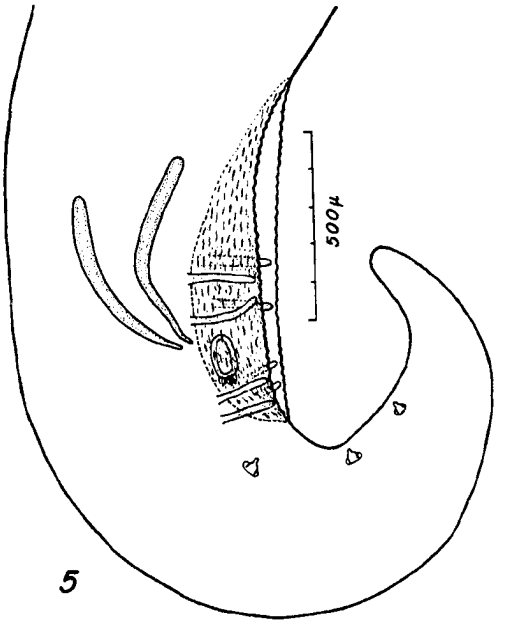

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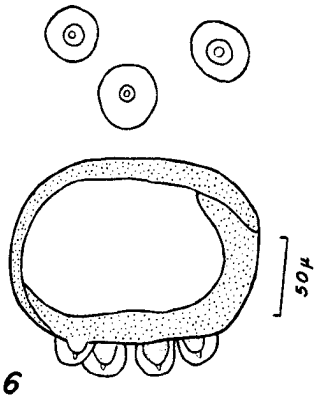

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DESCRIPTION OF PLATE II

Fig. 1 -- Dorsal view of anterior end of adult P. rara.

Fig. 2 -- Lateral (right) view of anterior end of adult.

Fig. 3 -- Lateral view of posterior end of adult female, showing rectum, anus, and anal muscles.

Fig. 4 -- Egg containing first-stage larva.

Fig. 5 -- Reproductive system of adult female, showing uterus (didelphic), egg chamber, vagina, and vulva.

Fig. 6 -- Dorsal view of posterior end of egg chamber with didelphic uterus.

Fig. 7 -- Ruptured egg shell with freed first-stage larva.

Fig. 8 -- Lateral view of first-stage larva 36 hours old from cricket.

Fig. 9 -- Lateral view of first-stage larva 5 days old from cricket.

Fig. 10 -- Lateral view of first-stage larva 11 days old from cricket.

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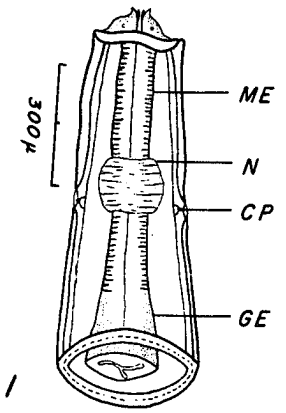
PLATE II



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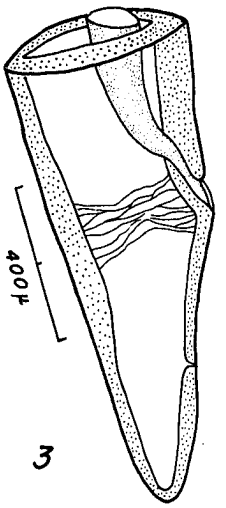

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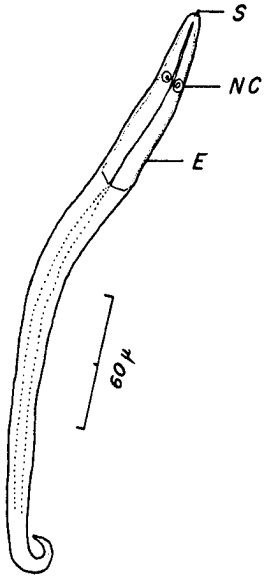

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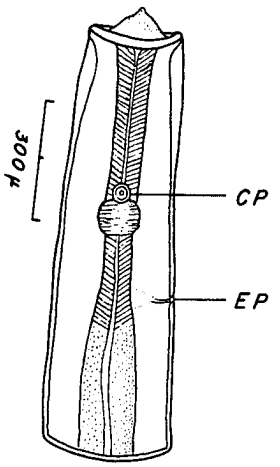

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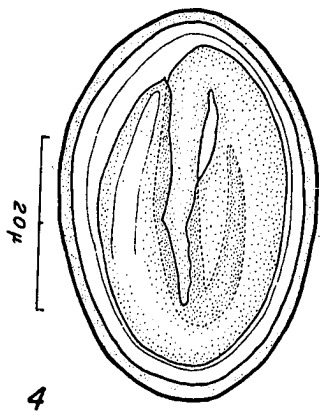

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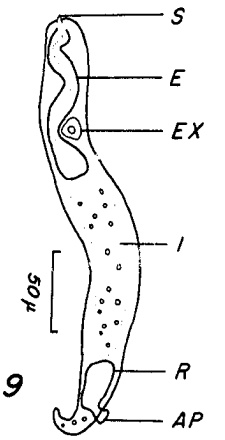

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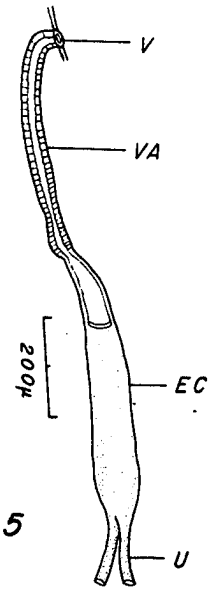

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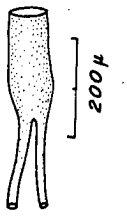

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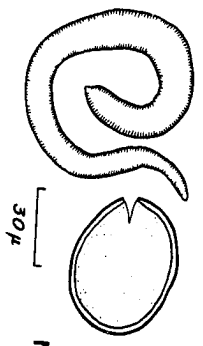

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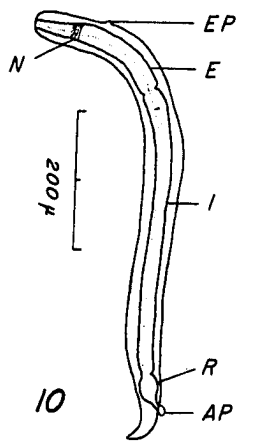

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DESCRIPTION OF PLATE III

Fig. 1 -- Lateral views of anterior and posterior ends of second-stage larva 13 days old from cricket.

Fig. 2 -- Lateral views of anterior and posterior ends of third-stage larva 15 days old from cricket.

Fig. 3 -- Lateral views of anterior and posterior ends of third-stage larva 21 days old from cricket.

Fig. 4 -- Lateral views of anterior and posterior ends of third-stage larva encysted in rattlesnake.

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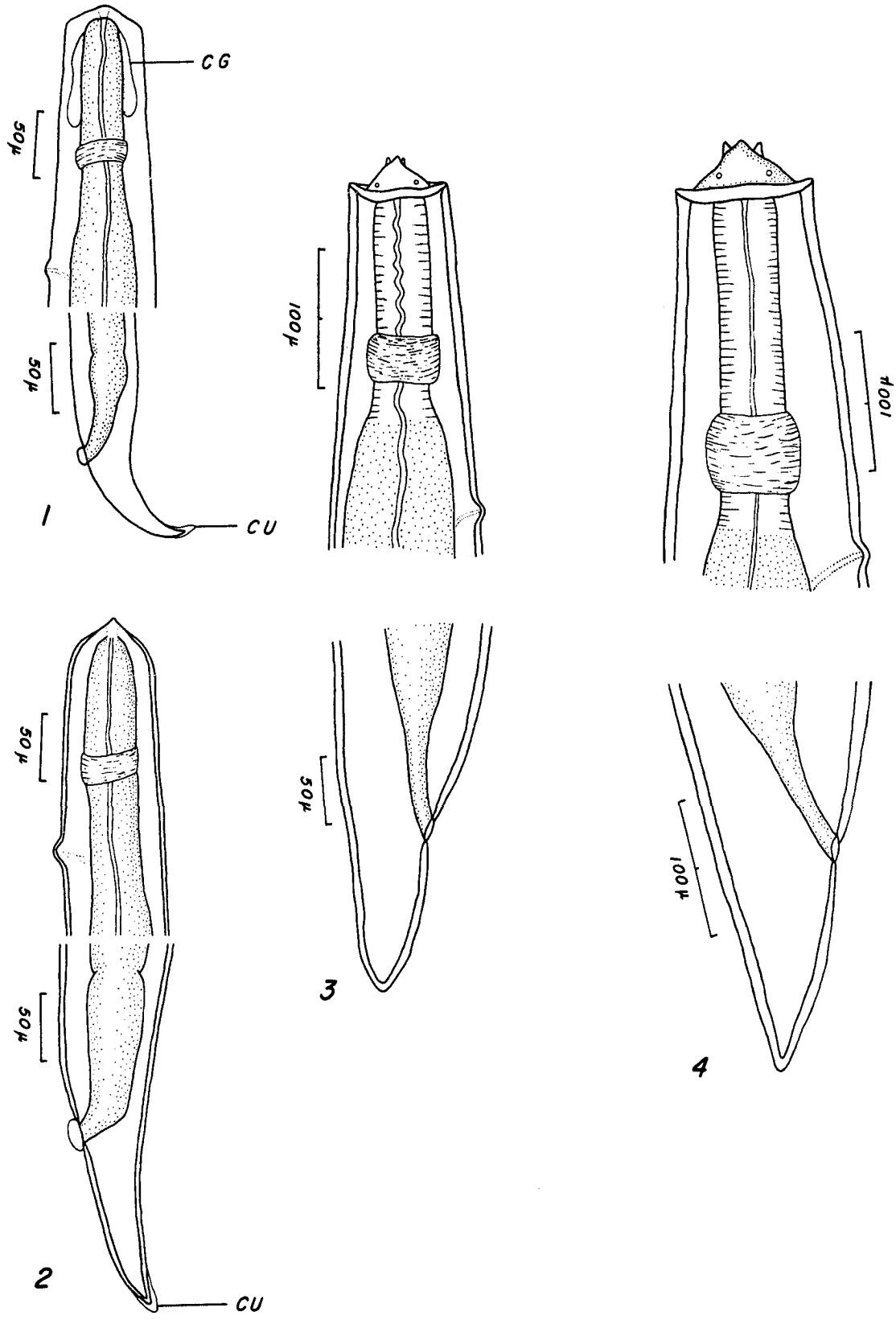
PLATE III



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DESCRIPTION OF PLATE IV

Fig. 1 -- Posterior part of stomach and anterior part of duodenum of experimentally infected cat showing attached adults of *P. rara* (X 1.5).

Fig. 2 -- Lateral view of midgut, hindgut, and rectum of experimentally infected cricket showing cysts containing third-stage larvae of *P. rara* (X 14).

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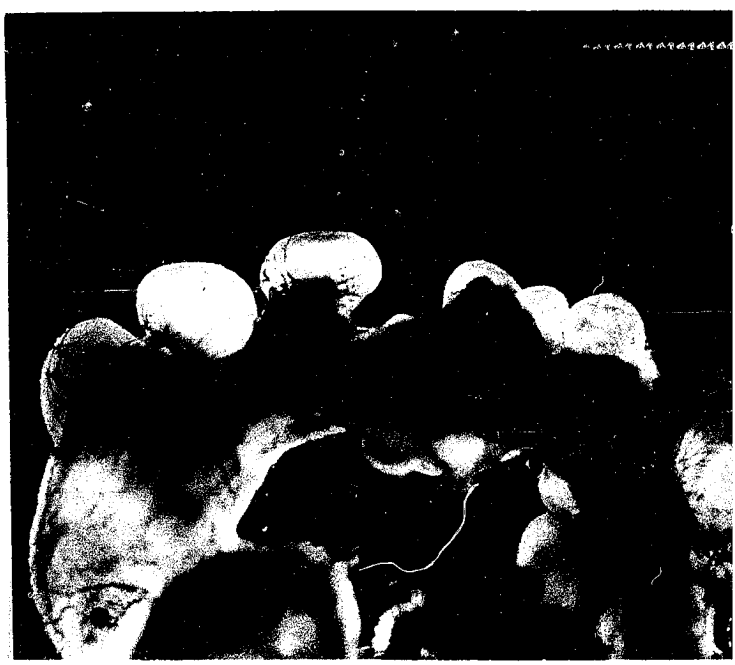
PLATE IV



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