1969-08-01

The migratory route of Toxocara canis in gerbils

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THE MIGRATORY ROUTE OF TOXOCARA CANIS IN GERBILS

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A Thesis

Presented to the

Department of Zoology and Entomology

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

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August 1969

This thesis by Thomas C. Baker is accepted in its present form by the Department of Zoology and Entomology of Brigham Young University as satisfying the thesis requirement for the degree of Master of Science.

Typed by Lucinda M. Nyberg

ACKNOWLEDGMENT

I am sincerely grateful to Dr. Ferron L. Andersen, my major professor who first interested me in parasitology, for his constant and careful guidance of this research project. He has provided helpful assistance and constructive criticism toward the preparation of this thesis. It is my pleasure to have been associated with him while a student these past years.

I am indebted to the members of my graduate committee, Dr. Vernon J. Tipton and Dr. Keith H. Hoopes, for their patience and encouragement during the preparation of this thesis. Also, I gratefully acknowledge the generous help and suggestions given me by Dr. Peter A. Nyberg whose assistance has made part of this project possible. I extend my thanks to Joseph Carl Fox and Thomas D. Bunch for caring for my animals in my absence.

I wish to express my heartfelt gratitude to my wife, Glenice, for her encouragement during the pursuit of this project and for her voluntary help in supporting our family while I have been in school. To our daughter, Cheryl, I give special thanks for her valuable assistance in the laboratory. Also, I express appreciation to our boys Ben and Dan for their help in caring for the animals.

The Toxocara canis eggs for this project were supplied by Dr.

Paul R. Fitzgerald, College of Veterinary Medicine, University of Illinois, Urbana, Illinois. Equipment and facilities were supplied by the Department of Zoology and Entomology, Brigham Young University. This project was supported in part by William Cooper and Nephews, Inc., Chicago, Illinois.

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INTRODUCTION

Toxocara canis (Werner, 1782) is the round worm most frequently found in dogs. From surveys in the United States, approximately 40-100% of young dogs and 5-70% of older dogs are infected with this parasite. Of 97 adult dogs surveyed in Provo, Utah, in 1968, 17.5% were infected with T. canis (Fox, personal communication). Adult dogs become infected by ingesting food contaminated with the infective eggs from this parasite. Puppies are most frequently infected prenatally from their mother (Nifontov, 1949; Yutuc, 1949; Sprent, 1958; Webster, 1958; Douglas and Baker, 1959; Scothorn, Koutz and Groves, 1965; Wiseman, 1969).

When T. canis accidentally parasitizes humans, the infection is known as "visceral larva migrans" (Beaver, et al., 1952), and is recognized as a frequent and serious disease. Cases have been reported in most parts of the world. The condition occurs primarily in children 15-36 months of age, who become infected apparently by ingesting soil containing eggs passed from infected dogs. After ingestion, the larvae hatch from the infective embryonated eggs and then subsequently invade such organs as the brain, spinal cord, eyes, liver, lungs, and kidneys (Beaver, et al., 1952; Beaver, 1958, 1962, 1969). Also, the invasion of these larvae may be responsible for the introduction or aggravation

of microbial agents (Wiseman, 1969).

In the eye, the larvae frequently cause symptoms similar to those observed in cases of retinoblastoma (Wilder, 1950; Beaver, 1958, 1962, 1969; Irvine and Irvine, 1959; Hogen, Kimura, and Spencer, 1965), which may result in excision of the eye because of incorrect diagnoses (Beaver, 1958). Other symptoms of the disease which may occur are hypereosinophilia, hyperglobulinemia, hepatomegaly, pneumonitis, fever, cough, loss of appetite, irritability and neurologic disturbances (Beaver, et al., 1952; Beaver, 1958, 1962, 1969).

Diagnosis of the disease in humans is difficult, since the parasite cannot be detected in the blood, body fluids or stool. Therefore, biopsy has been the accepted method of examination. Recently, Wiseman and Woodruff (1967) have developed an intradermal test using powdered desiccated adult worms for antigen in detecting T. canis infections. Also, an indirect fluorescent antibody test using second-stage T. canis larvae as antigen has been developed at the London School of Hygiene and Tropical Medicine. The results of these two different tests indicate that they are sensitive indicators of active infections and specific for this parasite (Wiseman, 1969).

In surveys in which the intradermal antibody test was used, the results showed that toxocariasis is a greater problem than previously suspected. Out of 156 apparently healthy persons tested in London, two persons were positive (Wiseman, 1969). At present there is no

known treatment for this disease and chronic symptoms may persist from 6 to 18 months (Chandler and Read, 1961). Also, the infective larvae are known to live up to ten years in experimental animals (Beaver, 1969).

Investigations have shown that the migratory pattern of T. canis larvae is similar in man and white mice (Beaver, et al., 1952). Additional information on its migratory route and location in the tissues of other experimental animals may be of value in an understanding of its control in human infections. Boisvenue (1965) found that the Mongolian gerbil, Meriones unguiculatus, was more susceptible to infection with the common round worm, Ascaris suum, than were white mice. Since a colony of gerbils was available at Brigham Young University for experimental research, the project herein reported was designed to study the migratory route of T. canis in these animals.

REVIEW OF LITERATURE

Life Cycle in Natural Host

Unembryonated eggs in the one-cell stage are passed in the feces of dogs and other carnivores which serve as natural hosts for T. canis. Under ideal conditions, the eggs embryonate in about five days to first-stage vermiform larvae. The larvae molt inside the eggs in approximately 9-15 days (Alicata, 1934) producing the infective second-stage (Nichols, 1956; Schacher, 1957; Sprent, 1958). Dogs usually become infected by ingesting the embryonated eggs containing the infective second-stage larvae in food contaminated by feces. The eggs hatch in the intestine, and the liberated larvae penetrate the mucosa; further migration and development depend upon the age, sex and immunity of the host (Levine, 1968). There are at least two different migratory routes that the infective second-stage larvae may follow: (1) migration to the liver and lungs, up the trachea and back to the intestine (tracheal migration); and (2) migration to the liver and lungs, to the somatic tissues (somatic migration) and possibly to the fetuses in utero (prenatal infection) (Beaver, 1969).

There is another type of transmission of T. canis which usually occurs in parturient female dogs. Augustine (1927), Webster (1958) and Douglas and Baker (1959) noted that pregnant bitches whose feces

were negative for T. canis infections before parturition, showed patent infections 25-46 days after whelping. Evidently, heavily infected pups pass third-stage larvae in their feces which develop beyond the stage requiring tracheal or somatic migration. The females swallow these third-stage larvae through licking the feces of their pups while caring for them. The larvae pass into the intestine and subsequently develop into adults with no further migration (Sprent, 1961). Infections are usually aborted spontaneously from 9-108 days (Douglas and Baker, 1959).

Tracheal Migration

Sprent (1958) and Webster (1958) found that tracheal migration predominates in dogs three months of age and younger. Basically, the ingested eggs hatch in the small intestine within 2-4 hours, after which the larvae penetrate the intestinal wall, enter the lymphatic system, migrate to the hepatic portal system and thence into the liver. The majority of larvae are found in the liver within two days. From the liver, they pass into the heart via the venous system, and then into the lungs through the pulmonary arteries. They reach their peak in 3-5 days and then enter the bronchioles, migrate up the trachea, and are subsequently swallowed. They generally molt to third-stage in either the lungs, trachea or esophagus before reaching the stomach. They remain in the stomach several days where they molt to fourth-stage,

10-13 days following infection. Finally, they migrate to the small intestine, undergo considerable growth, and molt to fifth-stage or immature adults. This stage is reached approximately 19-27 days after infection. The fifth-stage matures and eggs are passed in the feces of the host 4-5 weeks after exposure.

Schacher (1957) investigated the morphology and development of each successive stage of T. canis. He noted that during first-stage the cuticle, and digestive, excretory and nervous systems were formed. Only minor changes occurred in the infective second-stage (average dimensions, 385 μ x 19 μ). Ascarid-type lips appeared and sexual differentiation was initiated during third-stage (0.5-1.5 mm). At fourth-stage (1.5-20 mm), sexual differentiation was essentially complete, accompanied by considerable growth. The fifth-stage features were sexual maturation and growth. Adult males were 4-10 cm and adult females were 5-18 cm in length.

Somatic Migration and Prenatal Infection

In dogs older than three months of age, somatic migration predominates (Sprent, 1958; Webster, 1958). The hatched larvae enter the liver from the intestine via the lymphatic and hepatic portal systems, migrate to the lungs and thence to the heart by way of the pulmonary veins. From the heart they are distributed throughout the somatic tissues via the systemic circulatory system (Levine, 1968).

Sprent (1958) in a series of experiments using adult dogs, orally inoculated them with infective T. canis eggs. Upon autopsy at specific times, he found second-stage larvae in the liver, lungs, kidneys, brain and musculature. No adult worms, however, developed in the intestine. Evidently, the larvae in the somatic tissues never developed beyond the second-stage. Also, Webster (1958) noted that more larvae migrated to the somatic tissues of female than male dogs. These were thought to be the primary source of larvae for prenatal infections (Yutuc, 1949; Sprent, 1958; Webster, 1958; Douglas and Baker, 1959). Earlier, however, it was thought that the pregnant bitch usually ingested infective eggs during pregnancy to cause prenatal infections (Fulleborn, 1921; Schillinger and Cram, 1923; Augustine, 1927). The release of the encapsulated larvae from the somatic tissues of the pregnant bitch which invade the fetuses in utero, was thought by Webster to be stimulated by hormonal changes during pregnancy. The exact route larvae use to penetrate the fetuses is unknown, but the fact that two larvae were recovered from the umbilical cord of a fetus by Scothorn, Koutz and Groves (1965) suggested to them that this organ may be the primary route to infection.

In order to determine when infective larvae enter the fetuses, Douglas and Baker (1959) inoculated eight worm-free bitches at specific time intervals during pregnancy with 20,000 T. canis eggs. By surgically removing the fetuses at various times for examination, they

demonstrated that at least 14 days are required following inoculation before the larvae invade the fetuses in utero. Invasion of the fetuses, however, did not occur before the 42nd day of gestation. These results are supported by Scothorn, et al. (1965) who found upon examination of fetuses from 11 different litters that second-stage larvae invaded the fetuses some time between the 30th and 56th day of pregnancy.

Infective larvae migrate to the liver of the fetuses, where further development is retarded until parturition (Augustine, 1927). Within 30 minutes after birth, however, the larvae migrate to the lungs and molt to third-stage. Evidently, the change-over in circulation of the blood in the heart and lungs is necessary to initiate migration and further development (Scothorn, et al., 1965). By the third day, some of the larvae migrate up the trachea, are swallowed, and molt to fourth-stage. Fifth-stage or immature adults are found in the intestine by the end of the first week (Sprent, 1958), and patent infections are observed 23-40 days following birth (Douglas and Baker, 1959). This prenatal route is the method by which most puppies become infected with T. canis (Nifontov, 1949; Yutuc, 1949; Sprent, 1958; Webster, 1958; Douglas and Baker, 1959; Scothorn, et al., 1965; Wiseman, 1969). In some areas up to 100% prenatal infections occur (Scothorn, et al., 1965).

Studies in Experimental Hosts

In 1921 Fulleborn experimentally produced T. canis infections in guinea pigs. Since then other workers have infected such animals as white mice, rats, rabbits, hamsters (Beaver, 1962), pigs, (Done, Richardson and Gibson, 1960) sheep (Schaeffler, 1960), chickens and pigeons (Galvin, 1964) and monkeys (Beaver, 1962). Viable second-stage larvae have been recovered from the tissues of all these animals. In some cases larvae have been recovered 1-2 years following infection and up to ten years in the rhesus monkey (Beaver, 1969). Most of these animals may be considered as paratenic or transport hosts.

Mice

Many researches (Sprent, 1952, 1953; Smith and Beaver, 1953; Lee, 1960; Oshima, 1961a, 1961b; Borman, unpublished data; Burren, 1968) have investigated the migratory route of T. canis larvae in white mice. Sprent (1952) found upon oral inoculation of mice with infective eggs that the hatched larvae readily disappeared from the intestinal contents, which probably indicated a somatic migration. Further examination revealed larvae in the liver, lungs, kidneys and carcass. He discarded the heart, spleen, uro-reproductive organs, head, skin, and feet before examination.

In 1953, Sprent made additional observations on migratory behavior of T. canis in white mice which had been infected for 1-6

months. This time he assayed the liver, head, brain, and front and hind musculature for larvae. He found larvae from the liver up to four months after infection. After the first few days, they were distributed generally to the brain, head, and front and hind musculature. Even though large numbers were recovered from the brain, no apparent disturbances of locomotion were noted.

Essentially the same results were obtained by Smith and Beaver (1953) who orally inoculated white mice with 200 embryonated eggs. They found larvae in the liver and lungs during the early stage of infection, but later, numerous larvae were found in the brain.

Oshima (1961a) showed that most larvae in mice leave the small and large intestine between 24 hours and 36 hours, respectively, following inoculation. Between the fourth and sixth day, however, an increase in the number of larvae recovered from the intestine was reported. This second invasion of the larvae into the intestinal wall, although slight, was suggested by Oshima as evidence that a small number of larvae were able to take a tracheal route of migration as well as a somatic route. The rest of Oshima's results on the migratory behavior of T. canis in the liver, lungs, kidneys, brain and total carcass are essentially the same as reported by Sprent (1952, 1953).

In 1967, Borman (unpublished data) demonstrated that the somatic migratory route of T. canis in mice was not appreciably affected by inoculating hatched infective second-stage larvae into the

thoracic and peritoneal cavities and cecum, rather than orally inoculating them with embryonated eggs. The larvae seemed to invade the tissues in which they were in contact and then continue on a normal somatic route of migration. Earlier, this same phenomenon of inoculating animals with infective hatched larvae had been effectively demonstrated by Fülleborn (1921).

Borman (unpublished data) also investigated the effects of sex, age and pregnancy on the migratory pattern of T. canis larvae in white mice. He discovered that sex did not alter the migration or affect the number of larvae recovered from infected animals of different sexes. With respect to age, he recovered significantly more larvae from the tissues of 15 day-old mice than from 60 day-old mice. Pregnancy did not seem to appreciably influence migration patterns or infection rates; however, larvae were able to penetrate the uterus and fetuses. The number of larvae invading the fetuses was small compared to the total inoculated into the pregnant females. These results were in contrast to Oshima's (1961b), since he was not able to recover any larvae from fetuses of infected pregnant mice. Borman also found larvae in the mammary glands of lactating females and concluded that nursing mice may become infected through this method.

In 1968 Burren studied the histopathology of the migration of T. canis in mice. His observations on migration were similar to those found by Sprent (1952, 1953), Smith and Beaver (1953), Oshima (1961a)

and Borman (unpublished data). Burren found larvae in the intestinal wall from 1-32 hours, and in the mesenteric lymph nodes and hepatic portal vein 16-48 hours after inoculation. At 16-60 hours, they were numerous in the liver, and from 40-72 hours, larvae were observed in the lungs. Migration into the brain and musculature occurred at two days and persisted for the one-year observation period. Larvae invaded the eyes of only three of 100 mice observed, and none were found in the spleen and kidneys.

Pigs

Since pigs could become naturally infected with T. canis through their eating habits and close association with dogs, Done, Richardson and Gibson (1960) chose them as an experimental animal for investigating the migratory behavior of T. canis. They found that in pigs the migration of larvae into the liver, lungs and kidney reached a peak more slowly and persisted longer than in mice. Other organs or tissues in which larvae were found were the heart, tongue, brain, lumbar spinal cord, and masseter, diaphragm, left fore limb and right fore limb musculature. No larvae, however, were found in the spleen.

Experimental toxocariasis in pigs was characterized by extreme symptoms of nervous disorders affecting locomotion in contradistinction to those found by Sprent (1953) in mice.

Sheep

Schaeffler (1960) experimentally inoculated eight yearling sheep and ten lambs (2-10 days old) with T. canis eggs. Extensive somatic migration was found to occur in the organs and tissues of the lambs by the fourth day of infection, but hardly any migration took place beyond the liver in yearling sheep. From these results, Schaeffler (1960) concluded that age definitely influences the migratory pattern of T. canis in sheep.

Chickens and Pigeons

T. canis infections have been successfully produced in chickens and pigeons by Galvin (1964). He noted that infective larvae remained in either host's tissues up to 142 days following infections. The larvae, however, were found predominately in the liver. Because of the susceptibility of chickens and pigeons for T. canis infections, Galvin (1960), suggested that they might serve as paratenic hosts.

Prevalence of Infection in Dogs

In 1946 Hill reported a 25% infection rate among dogs used at Duke University Hospital for experimantal purposes, whereas Butler and Grundman (1951) found 4.5% of 200 stray dogs infected in Salt Lake City, Utah.

One of the most extensive surveys of helminth infection in dogs was done by Ehrenford (1952 and 1957). Of 1,465 dogs surveyed from the Ohio River Drainage, he found 21.0% infected with T. canis. Of those infected, 32.8% and 9.4% were males and females, respectively. Further analysis according to age and sex, showed that puppies of both sexes had a high incidence of infection and that adult male dogs had a significantly higher infection than adult females. Evidently, the female from 6-36 months develops an increasing immunity to infection, whereas no immunity is experienced in males up to 36 months. Also, seasonal variations of T. canis infections were noted in male dogs with the incidence being greater during the winter months. Females, however, showed a consistently uniform infection throughout the year.

In Knoxville, Tennessee, Ciordia and Jones (1956) found 21% of 68 dogs infected with T. canis, and Vaughn and Jordan (1960) reported a 6.5% incidence rate in an exclusive residential area in New Orleans, where stray dogs were not tolerated. In a follow-up study in New Orleans, Vaughn and Murphy (1962) found 22% of 623 pound dogs infected with T. canis. Of those infected, 42% were immature dogs and 11% were mature dogs. Observation from these two surveys in New Orleans show that stray dogs are more commonly infected than pet dogs.

At the State University of Iowa, 14% of 224 dogs were positive for T. canis infections (Braum and Thayer, 1962). In Hawaii, Ash

(1962) surveyed 96 dogs and found 24% of immature dogs and 4% of adult dogs infected. In southeastern Michigan, Worley (1964) noted a 69.5% infection rate in dogs, and Lillis (1967) discovered 23% of 2,737 dogs infected in a survey in New Jersey. Styles (1967) found 93% of 120 stray dogs under six months of age infected in Mexico City. From autopsy studies of dogs in the same city, Schantz and Biagi (1968) reported 75.6% of young dogs and 6.1% of older dogs infected. In Iran, Sadighian (1969) noted a 34.7% incident rate. Wiseman (1969) reported the infection rates from the following regions: London, 21.3%; Malta, 28.8%; Nigeria, 36.5%; Uganda, 12.5%; Kenya (Nairobi), 5.7%; Kenya (Masai Village), 12.0%; and Tanzania, 28.0%. These surveys carried out in both temperate and tropical regions indicate that T. canis is world wide in distribution.

In 1965 (Anonymous, 1965) it was estimated that some 18 million families in the United States owned approximately 26 million dogs. Surveys conducted in this country indicate that from 5 to 70% of all dogs are infected with T. canis, and thus this constitutes an important public health hazard inasmuch as they are capable reservoir hosts for human infections of visceral larva migrans (Ehrenford, 1956).

MATERIALS AND METHODS

Source and Care of Animals

All gerbils used in this experiment were raised from a single colony, which had been maintained at Brigham Young University, Provo, Utah, for approximately four years. They were housed and cared for in the parasitology animal room. Lighting for the room was provided 14 hours daily by fluorescent lights connected to an automatic timing switch. The temperature and humidity, however, fluctuated according to ambient conditions.

The animals were enclosed in 7" x 14" aluminum cages with mesh wire screen tops. Corn-cob litter (San-i-cel, Paxton Processing Co., Inc., Paxton, Illinois) was used for bedding. Water was supplied by standard laboratory water bottles with metal and glass tube spouts, and a pelleted ration (Purina Laboratory Chow, Ralston Purina Co., St. Louis, Missouri) was fed. The gerbils were watered and fed every other day, and the litter was changed periodically.

Source and Preparation of Inoculum

Unembryonated Toxocara canis eggs were secured from the College of Veterinary Medicine, University of Illinois, Urbana, Illinois, where experimentally infected dogs are maintained. The

unembryonated eggs were placed in 1% formalin, 0.5 cm deep in 90 mm petri dishes treated with a water soluble silicone compound (Siliclad, Clay-Adams, Inc., New York). The petri dishes with the eggs were placed in an incubator (Thelco Model 2, Precision Scientific Co.) for embryonation at 30 C for six weeks to insure maximum development of the infective second-stage larvae. After the eggs were embryonated, they were removed from the petri dishes, stored in 1% formalin in 50 ml screw-capped glass bottles at 4 C until needed. The inoculum was later prepared by concentrating the 1% formalin-egg suspensions to 2150-2250 eggs per 0.5 ml of solution.

Inoculation of Gerbils

The standardization techniques developed by Oshima (1961a) for infecting mice with Toxocara canis embryonated eggs were adopted for this experiment. One exception was that eggs older than 60 days, but less than 120 days were used. All gerbils were slightly anesthetized in an ether-saturated jar prior to oral inoculation with T. canis infective eggs. The inoculum was administered in 0.5 ml dosages by means of a stomach tube constructed from a piece of Intramedic polyethylene tubing (I.D. .034" x O.D. .050", Clay-Adams, Inc., New York) attached to a cut-off 20-gauge hypodermic needle fastened to a 0.5 ml Glaspak disposable syringe (Becton, Dickinson and Co., Rutherford, New Jersey).

Digestion of Tissues

All animals were anesthetized at specific time intervals in an ether-saturated jar, weighed, labeled and placed on an 8" x 12" wax dissecting tray. For the purpose of this study, females less than 55 gm and males less than 60 gm were categorized as juveniles. A 22-gauge hypodermic needle attached to a 5 ml glass syringe coated with Anti-clot (Clinton Laboratories, Los Angeles, California) was used to withdraw 2-3 ml of blood by cardiac puncture from the animals.

After the blood was removed, most of the gerbils were immediately skinned and prepared for tissue digestion. Some animals, however, were placed in the refrigerator (1-24 hours) at 4 C until they could be processed. Only the skin and paws of the gerbils were discarded. The following organs or tissues were excised for digestion: head, brain,eyes, heart, lungs, liver, spleen, stomach and contents, small intestine and contents with associated mesenteries and lymph nodes, large intestine and contents with associated mesenteries and lynph nodes, kidneys, uro-genital tract, front-quarter musculature and hind-quarter musculature. After 72 hours, the entire gastrointestinal tract was assayed intact.

The digestive enzyme solution was prepared by placing 10 gm of pepsin (Pepsin, N. F., Powder, Matheson Coleman and Bell, Norwood, Ohio; East Rutherford, N. J.), 8.5 gm of NaCl and 5 ml of 6 N HCl in

1000 L of distilled water.

The individual tissues were placed in a Waring Blendor (Model 1002, Waring Products Co., Winsted, Conn.) with a minimum of 50 ml of enzyme solution. All tissues were blended at low speed for three seconds with the exception of the head, and the front and hind musculature, which were blended for ten seconds. The blender cup was rinsed with an additional 30-50 ml of solution, after which the blended tissues were poured into labeled 4 oz, loosely capped, glass jars and incubated at 37-39 C for 8-24 hours.

Baermannization

After the tissues were digested, the larvae were recovered by standard baermannizing techniques using 3.5-inch, 58^{o} long-stemmed conical funnels. The stems of the funnels were closed off by a piece of rubber tubing with an attached pinch clamp. One-fourth-inch grid wire screens were placed in the bottoms of the funnels to support the digested tissues. The funnels were filled with 0.85% NaCl solution, and the tissues were then baermannized through two layers of cellulose tissues (Kimwipes, Kimberly-Clark, Neenah, Wis.) for 8-24 hours.

Counting of Larvae

The blood that was withdrawn from each gerbil by cardiac puncture was placed in a 15 ml centrifuge tube and spun two minutes at 2000

x gravity in an International Clinical Centrifuge. Afterwards, an aliquot sample was extracted from the bottom of each tube and assayed for larvae. No larvae, however, were ever recovered from any of the samples.

After the digested tissues were baermannized, 15-18 ml of fluid were collected from each funnel and placed in a 20 ml centrifuge tube. The centrifuge tubes were stored for a minimum of one hour at 4 C to allow the larvae to settle to the bottom of the tubes, after which time an aspirator was used to draw the fluid from the top of the larvae. A calibrated micropipet was used to place the desired amount of the thoroughly mixed sediment on a microslide for examination. A cover-slip was placed on top of the microslide, and the larvae were counted with a Swift binocular compound microscope using a 4X objective. All larvae recovered from the individual tissues and organs were recorded according to age and sex of the gerbils. The numbers of larvae recovered from the front and hind musculature were multiplied by two, since only a front and hind quarter of each animal were assayed.

RESULTS

Thirty-seven adult female and 39 adult male gerbils were each inoculated with 2150-2250 infective T. canis eggs. One animal of each sex was killed each hour up to four hours, and then every four hours thereafter up to 72 hours. Afterwards, paired animals were killed at half-day intervals to day eight, and then on days nine and ten, with the exception that no females were killed on days 6 1/2 and 9. From 10-40 days, pairs were killed at 5-day intervals. Also, 17 juvenile females and eight juvenile males were administered the same dosage of infective eggs as the adults, but were killed at fewer selected intervals, since there were not enough young animals available for each time period.

Distribution and Migratory Route of Larvae

Table 1 shows the average percent distribution of the total infective larvae recovered from the organs and tissues of the adult male and female gerbils with respect to time. The results indicate that the larvae begin hatching in the gastro-intestinal tract between 1-2 hours after inoculation. Evidently, only a few larvae hatched in the stomach, since most of them were observed at this time in either the small or large intestine. Of those larvae recovered the first 28 hours, the

Time Since Infection	Stomach	Small Int.	Large Int.	Liver	Lung	Kidneys	Head	Brain	Eyes	Front Muscle	Hind Muscle
Hour 1											
2		37.0	63.0								
3	5.0	2.0	93.0								
4	4.0	1.0	95.0								
8		2.0	97.0			1.0					
12	2.0	10.0	88.0								
16	2.0	17.0	80.0			1.0					
20	8.0	7.0	73.0	10.0	1.0	1.0					
24			61.0	35.0		4.0					
28	2.0	4.0	52.0	42.0							
32	1.0	7.0	31.0	61.0							
36		11.0		86.0	0.5				0.5	0.5	0.5
40		1.0	1.0	98.0							
44		1.0	3.0	96.0							
48			0.5	99.0	0.5						
52			2.0	97.0						1.0	
56				100.0							
60	0.2		0.4	98.0	0.2	1.0	0.2				
64	0.3	0.3		97.0	0.5	0.2	1.0			0.6	
68			1.0	96.0	0.5		0.5			2.0	
72			3.4	69.0	15.0	3.7	1.5	1.2	0.5	3.7	1.0
Days 3 1/2			1.0	68.0	6.7	12.3	2.0	1.9	0.5	3.2	3.8
4			2.0	75.0	8.4	4.0	3.0	1.2		4.0	2.0
4 1/2			0.7	18.0	10.0	13.0	8.0	10.0	0.4	28.0	10.7
5				33.0	2.0	20.0	13.5	5.0	0.5	23.0	2.5
5 1/2				7.7	7.0	40.0	10.0	4.7	1.7	22.0	4.1
6				44.0		13.5	18.0	2.0	0.4	14.5	5.6
6 1/2				2.9	2.0	15.0	16.8	6.4	1.4	37.0	17.5
7				36.0			16.0	1.2	0.7	34.0	11.5
7 1/2			0.4	0.4	1.5	6.6	13.2	11.8	0.6	38.0	27.0
8				5.0		14.0	26.0	16.0		14.0	25.0
9				10.3		63.0	15.5	3.4	2.6	5.2	
10				2.4	4.8	12.0	12.9	15.0	0.6	39.0	13.8
15				2.7	1.8	9.8	19.5	23.0		28.0	21.0

majority were found in the large intestine, but by 36 hours most had disappeared from that organ. After the larvae had hatched, they presumably penetrated into or through the intestinal wall, and subsequently either invaded other parts of the body, or were picked up by the circulatory system and were carried to other body organs. As early as ten hours after infection, larvae were detected in the liver, and by 56 hours all of the larvae that were recovered were in that organ. After about three days 69% of the larvae recovered were still in the liver; however, at that time 15% were found in the lungs, thereby suggesting a larval migratory pattern in gerbils similar to that demonstrated for other hosts. After the larvae once passed through the lungs, they were presumably disseminated throughout most of the parts of the body, since at about 4 1/2 days, larvae were recovered for the first time in moderate numbers from several other organs. At about 5 1/2 days the peak recovery (40%) was from the kidneys, and at about 7 1/2 days it occurred in the front musculature (38%). At the same approximate time, 27% of the larvae recovered were from the hind musculature. At approximately eight days, larvae were noted in significant numbers in the head (26%) and also the brain (16%). After that time it was no longer possible to detect a pattern of migration by the larvae. They were ultimately found in all organs and tissues assayed at least at one time period during the project, indicating a generalized somatic migration throughout the entire body.

Although only small numbers of larvae were recovered from the eyes of the gerbils, it was notable that some larvae were found there as early as three days after infection and fairly regularly thereafter, as long as the gerbils were examined.

Effect of Sex of Host on Migratory Route of Larvae

All of the larvae recovered from the adult and juvenile gerbils were recorded according to sex of the host. Since there were not sufficient juvenile gerbils available, however, for paired observations with adult gerbils at each selected examination period, only the data from the adult gerbils were used in the assessment of the effect of sex of the host on the larval migratory pattern. Tables 2 through 6 show the total number of larvae recovered from different organs and tissues from each adult male or female gerbil killed at the specified intervals listed previously. Figures 1 through 12 illustrate this same information graphically, with the exception that the results for the heart, spleen and gastro-intestinal tract were not included, since so few larvae were recovered from those sites. Figure 13 shows the total percent of larvae recovered from all organs assayed from all adult gerbils killed in this study. Table 7 shows this same information for the adult gerbils, as well as the comparable data for the juveniles.

While considerable variation existed in the number of larvae

Table 2. Total larvae recovered from the stomach, small intestine and large intestine of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection	Stomach			Small Intestine			Large Intestine		
	Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour 1	0	0	0	0	0	0	0	0	0
2	0	0	0	8	8	8	6	21	14
3	0	22	11	4	4	4	172	240	206
4	17	0	8	0	4	2	270	164	217
8	0	0	0	0	0	0	475	9	242
12	7	0	4	31	5	18	212	116	164
16	10	0	5	42	30	36	196	120	158
20	0	33	16	0	28	14	216	65	141
24	0	0	0	0	0	0	10	120	65
28	0	12	6	6	19	12	84	196	140
32	6	0	3	32	0	16	150	0	75
36	0	0	0	39	30	34	0	0	0
40	0	0	0	4	5	4	4	8	6
44	0	0	0	0	4	2	7	32	20
48	0	0	0	0	0	0	0	4	2
52	0	0	0	0	0	0	0	29	14
56	0	0	0	0	0	0	0	0	0
60	0	3	2	0	0	0	6	0	3
64	0	3	2	3	0	2	0	0	0
68	0	0	0	0	0	0	4	2	3
72	0	0	0	0	0	0	0	40	20

Table 3. Total larvae recovered from the liver, lungs and kidneys of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection	Liver			Lungs			Kidneys		
	Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour 1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	4	2
12	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	4	0	2
20	0	39	20	3	0	2	4	0	2
24	60	15	68	0	0	0	9	0	4
28	164	64	114	0	0	0	0	0	0
32	182	117	145	0	0	0	0	0	0
36	202	342	272	2	0	1	0	0	0
40	490	308	399	0	0	0	0	0	0
44	462	596	529	0	0	0	0	0	0
48	785	40	412	4	0	2	0	0	0
52	459	1050	754	0	0	0	0	0	0
56	1270	432	851	0	0	0	0	0	0
60	440	450	445	2	0	1	11	0	6
64	497	302	400	0	3	2	0	2	1
68	401	330	366	3	0	2	0	0	0
72	292	496	394	66	108	87	30	12	21
Days 3 1/2	105	324	214	32	9	20	33	44	38
4	290	395	342	54	23	38	4	34	19
4 1/2	49	182	116	91	33	62	78	94	86
5	170	56	113	0	12	6	0	132	66
5 1/2	19	26	22	14	27	20	118	115	116
6	120	225	172	0	0	0	93	12	52
6 1/2	14	-	14	7	-	7	72	-	36
7	28	174	101	0	0	0	0	0	0
7 1/2	0	3	2	8	5	5	20	36	28
8	3	4	4	0	0	0	21	0	10
9	12	-	12	0	-	0	74	-	74
10	12	7	10	15	23	19	28	70	49
15	12	3	8	4	6	5	26	-	26
20	48	40	44	0	3	2	24	2	13
25	12	142	77	0	0	0	0	0	0
30	324	35	180	0	2	1	0	56	28
35	7	54	36	0	3	2	3	2	2
40	24	40	32	0	0	0	5	25	15

Table 4. Total larvae recovered from the head, brain and eyes of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection	Head			Brain			Eyes		
	Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour									
1	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	0	0	0
8	0	0	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0	0	0
16	0	0	0	0	0	0	0	0	0
20	0	0	0	0	0	0	0	0	0
24	0	0	0	0	0	0	0	0	0
28	0	0	0	0	0	0	0	0	0
32	0	0	0	0	0	0	0	0	0
36	0	0	0	0	0	0	0	3	2
40	0	0	0	0	0	0	0	0	0
44	0	0	0	0	0	0	0	0	0
48	0	0	0	0	0	0	0	0	0
52	0	0	0	0	0	0	0	0	0
56	0	0	0	0	0	0	0	0	0
60	0	3	2	2	0	1	0	0	0
64	3	4	4	0	0	0	0	0	0
68	3	0	2	0	0	0	0	0	0
72	0	17	8	0	14	7	2	3	2
Days									
3 1/2	0	12	6	12	0	6	3	0	2
4	28	0	14	-	11	11	0	0	0
4 1/2	30	75	52	108	19	64	5	0	2
5	0	91	46	0	33	16	0	3	2
5 1/2	29	28	28	0	27	14	5	5	5
6	96	48	72	16	0	8	3	0	2
6 1/2	81	0	40	30	-	30	7	-	4
7	78	12	45	0	7	4	0	4	2
7 1/2	40	72	56	36	64	50	3	2	2
8	27	12	20	18	6	12	0	0	0
9	18	-	18	4	-	4	3	-	3
10	45	56	50	56	61	58	5	0	2
15	57	53	55	81	47	64	0	0	0
20	12	9	10	32	120	76	0	2	1
25	14	3	8	6	0	3	0	4	4
30	0	55	28	0	87	44	2	3	2
35	18	0	9	8	4	6	0	0	0
40	33	64	48	52	68	60	2	2	2

Table 5. Total larvae recovered from the front musculature, hind musculature and heart of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection		Front Musculature			Hind Musculature			Heart		
		Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour	1	0	0	0	0	0	0	0	0	0
	2	0	0	0	0	0	0	0	0	0
	3	0	0	0	0	0	0	0	0	0
	4	0	0	0	0	0	0	0	0	0
	8	0	0	0	0	0	0	0	0	0
	12	0	0	0	0	0	0	-	0	0
	16	0	0	0	0	0	0	0	0	0
	20	0	0	0	0	0	0	0	0	0
	24	0	0	0	0	0	0	0	0	0
	28	0	0	0	0	0	0	0	0	0
	32	0	0	0	0	0	0	0	0	0
	36	0	4	2	0	4	2	0	2	1
	40	0	0	0	0	0	0	0	0	0
	44	0	0	0	0	0	0	0	0	0
	48	0	0	0	0	0	0	0	0	0
	52	8	0	4	0	0	0	0	0	0
	56	0	0	0	0	0	0	0	0	0
	60	0	0	0	0	0	0	0	0	0
	64	0	6	3	0	0	0	1	1	1
	68	8	6	7	0	0	0	0	0	0
	72	36	6	21	0	10	5	0	0	0
Days	3 1/2	0	20	10	16	6	11	4	0	2
	4	24	14	19	12	6	9	0	2	1
	4 1/2	196	162	179	90	48	69	14	3	8
	5	0	156	78	0	16	8	0	6	3
	5 1/2	52	76	64	16	8	12	0	0	0
	6	30	84	57	16	28	22	0	0	0
	6 1/2	182	-	182	84	-	84	3	-	2
	7	136	56	96	64	0	32	0	0	0
	7 1/2	168	156	162	130	102	116	0	2	1
	8	8	12	10	28	10	19	0	0	0
	9	6	-	6	0	-	0	0	-	0
	10	182	128	155	42	66	54	0	0	0
	15	34	6	20	42	12	27	0	0	0
	20	12	68	40	12	20	16	0	0	0
	25	74	0	74	66	0	33	0	0	0
	30	6	210	108	28	54	41	0	3	2
	35	72	84	78	26	90	58	0	0	0
	40	180	184	182	90	78	84	0	2	1

Table 6. Total larvae recovered from the uro-genital tract, spleen and gastro-intestinal tract of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection	Uro-genital Tract		Spleen			Gastro-intestinal Tract		
	Male	Fem.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour 1	0	0	0	0	0			
2	0	0	0	0	0			
3	0	0	0	0	0			
4	0	0	0	0	0			
8	0	0	0	0	0			
12	0	0	0	0	0			
16	0	0	0	0	0			
20	0	0	0	0	0			
24	0	0	0	0	0			
28	0	0	0	0	0			
32	0	0	0	0	0			
36	0	0	0	3	2			
40	0	0	0	0	0			
44	0	0	0	0	0			
48	0	0	0	0	0			
52	4	0	0	0	0			
56	0	0	0	0	0			
60	0	0	0	0	0			
64	0	0	0	0	0			
68	0	1	0	0	0			
72	3	0	0	0	0			
Days 3 1/2	0	0	0	0	0	0	6	3
4	11	6	0	0	0	19	0	10
4 1/2	23	0	0	4	2	9	0	4
5	0	0	0	0	0	0	0	0
5 1/2	10	0	0	0	0	0	0	0
6	14	0	0	0	0	0	0	0
6 1/2	0	-	2	-	2	0	0	0
7	12	0	0	0	0	0	0	0
7 1/2	0	2	0	0	0	0	3	2
8	0	0	0	0	0	0	0	0
9	0	-	0	-	0	0	0	0
10	3	0	0	0	0	0	0	0
15	3	0	0	2	1	0	0	0
20	8	0	0	0	0	0	0	0
25	0	0	0	0	0	2	0	1
30	0	0	0	0	0	0	0	0
35	2	0	0	0	0	0	0	0
40	0	0	0	0	0	0	0	0

Table 7. Total percentage of larvae recovered from adult male and female and juvenile male and female gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection		Adult Gerbils			Juvenile Gerbils		
		Male	Female	Ave.	Male	Female	Ave.
Hour	1	0	0	0			
	2	.6	.8	.7			
	3	8.0	12.1	10.0			
	4	13.3	7.8	10.6			
	8	22.0	.6	11.3		13.5	13.5
	12	11.3	5.5	8.4			
	16	11.0	6.8	8.9	36.0		36.0
	20	10.1	7.5	8.8		2.0	2.0
	24	3.7	6.1	4.9		2.7	2.7
	28	11.3	13.2	12.2			
	32	16.8	5.3	11.1	23.4	51.3	37.4
	36	12.2	17.5	14.4			
	40	22.7	14.6	18.8		33.0	33.0
	44	21.3	28.7	25.0			
	48	35.0	1.9	18.5	37.1		37.1
	52	21.4	49.2	35.3			
	56	57.7	19.6	38.6		7.5	7.5
	60	20.9	20.6	20.8			
	64	22.9	14.6	18.8	22.5		22.5
	68	19.0	15.4	17.2			
	72	19.4	32.1	25.8		24.0	24.0
Days	3 1/2	9.6	18.9	14.2	7.0	40.9	23.8
	4	19.7	23.5	16.6		17.1	17.1
	4 1/2	30.0	28.4	29.2			
	5	7.9	23.7	15.8		5.3	5.3
	5 1/2	12.0	14.4	13.2			
	6	18.1	18.5	18.3	8.3		8.3
	6 1/2	21.9	-	21.9			
	7	14.3	11.8	13.0		16.5	16.5
	7 1/2	19.0	20.5	19.8			
	8	4.9	2.1	3.5		21.4	21.4
	9	40.2	-	40.2			
	10	18.0	18.7	18.4		17.4	17.4
	15	12.9	13.3	13.1	30.0		30.0
	20	7.5	12.3	9.9		36.9	36.9
	25	7.8	6.9	7.4		67.0	67.0
	30	16.8	23.5	20.2			
	35	5.6	4.3	5.0			
	40	18.0	21.5	19.8	10.7	13.0	11.8

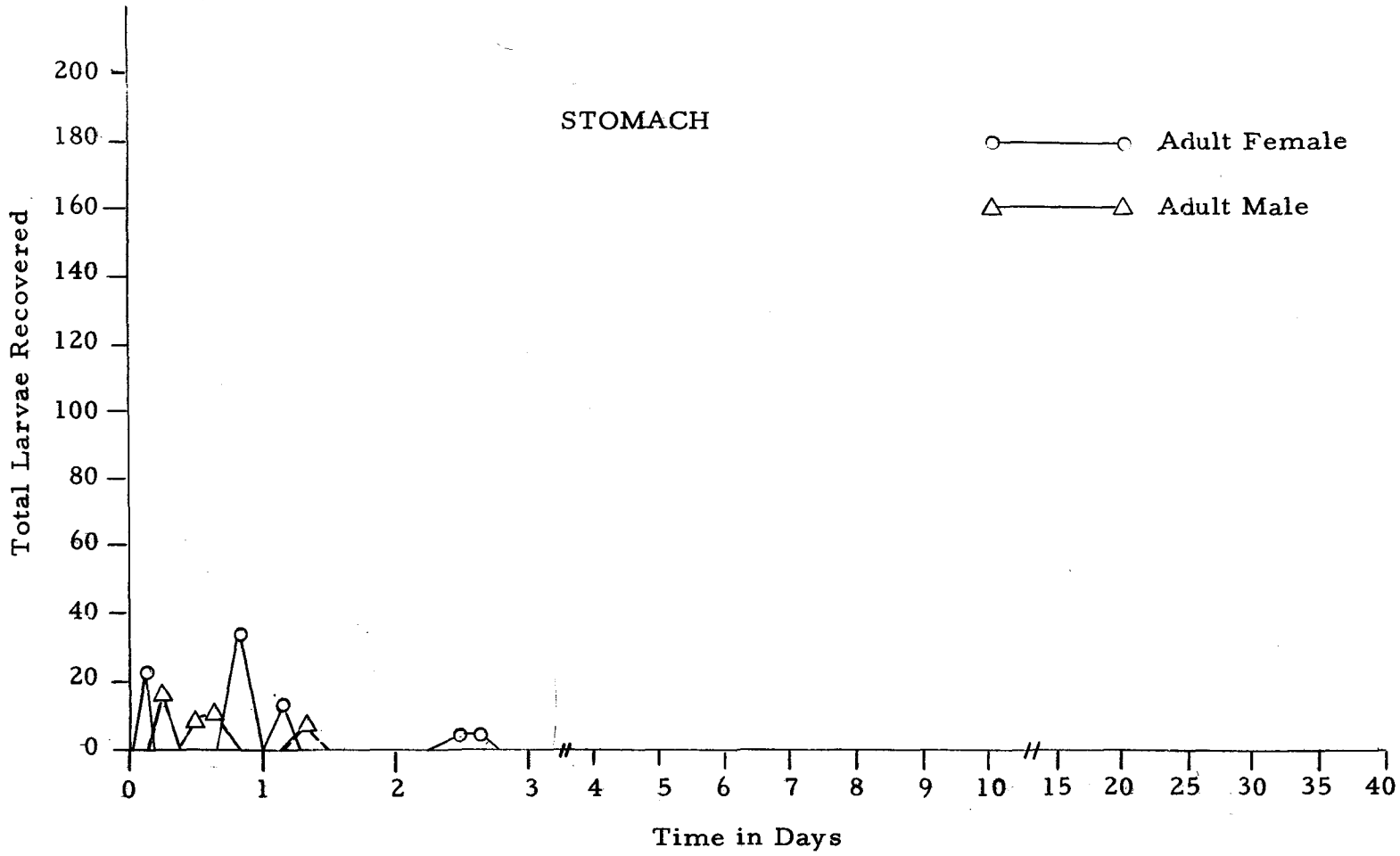

Fig. 1. Total larvae recovered from the stomach of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

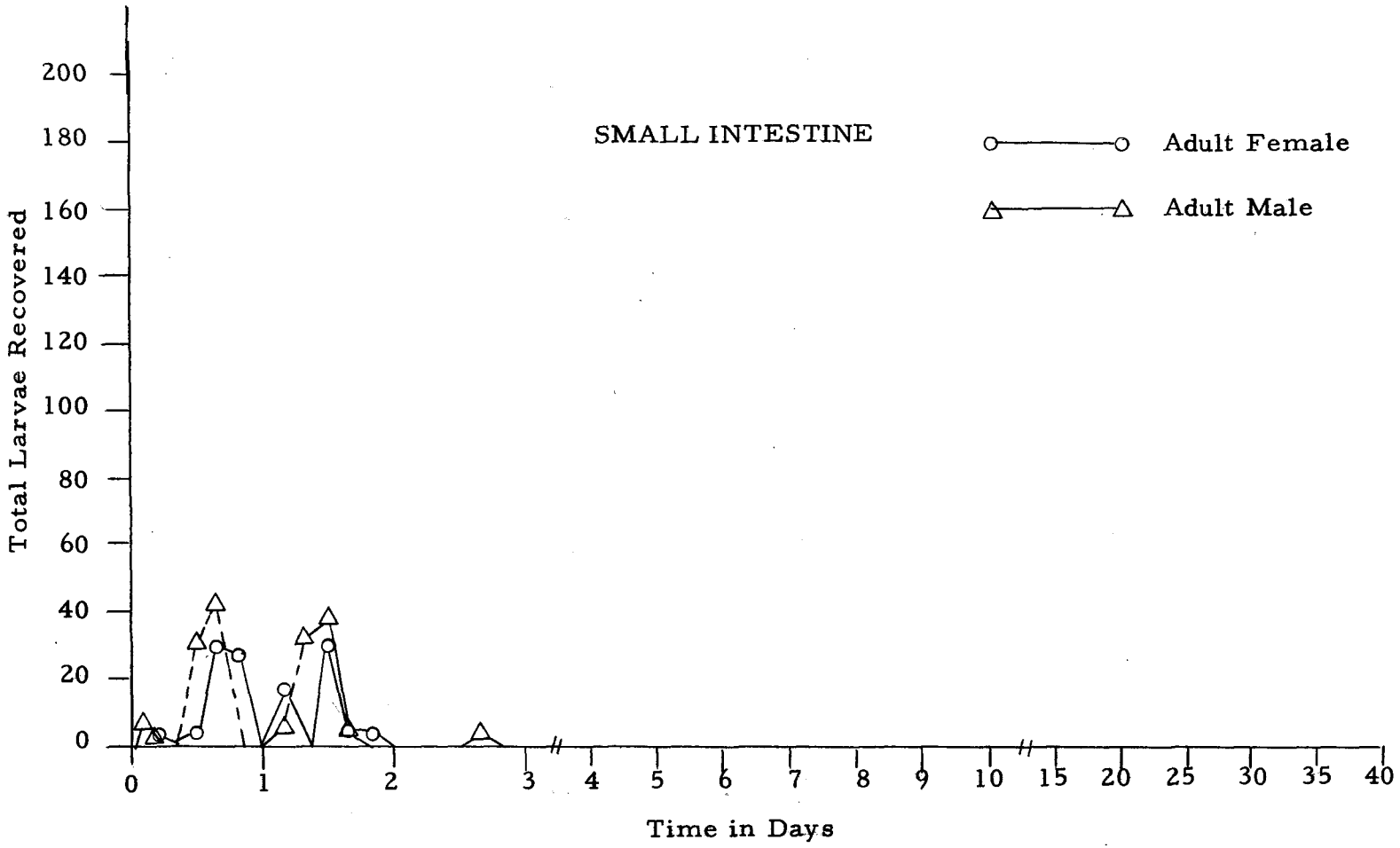

Fig. 2. Total larvae recovered from the small intestine of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

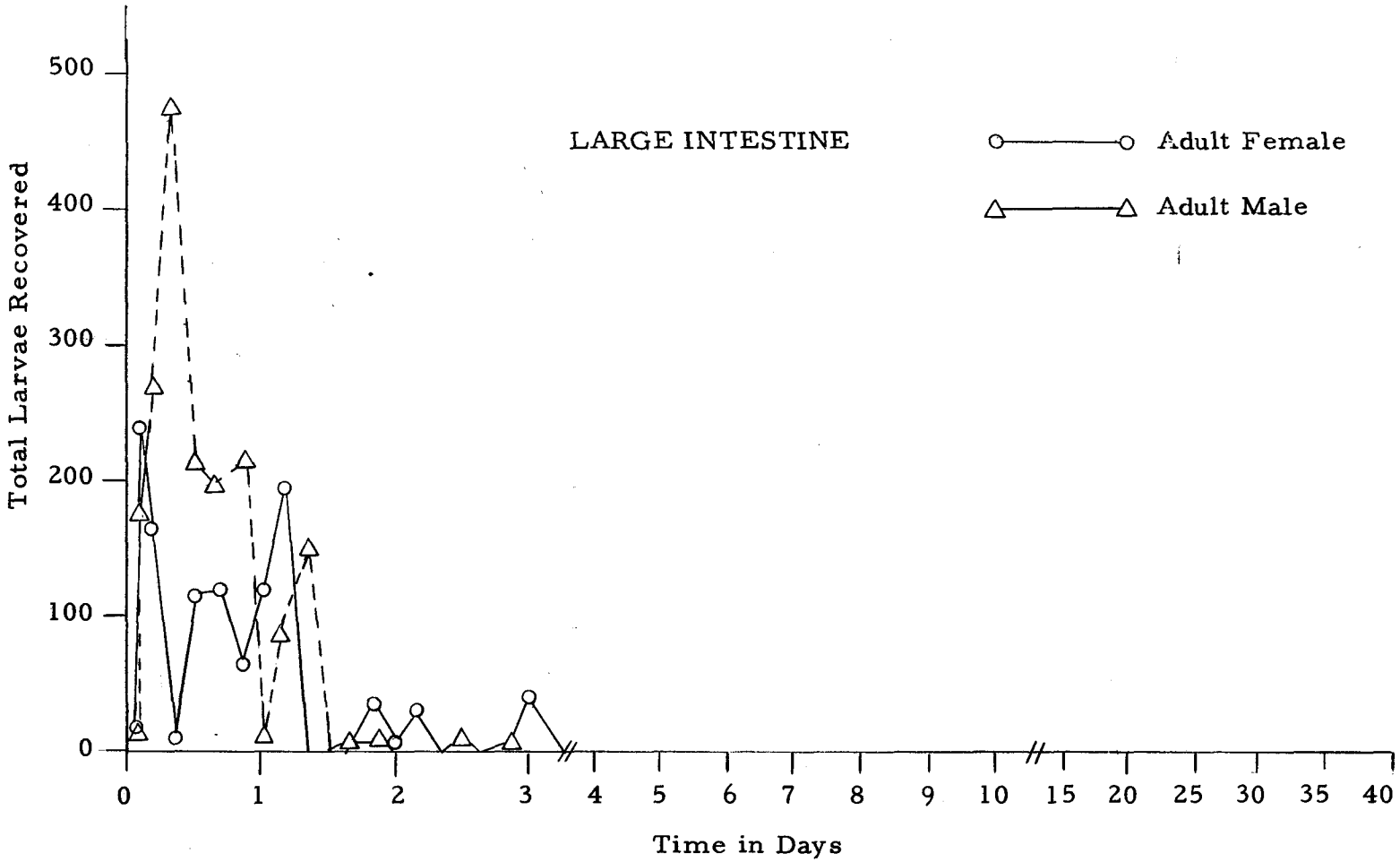

Fig. 3. Total larvae recovered from the large intestine of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

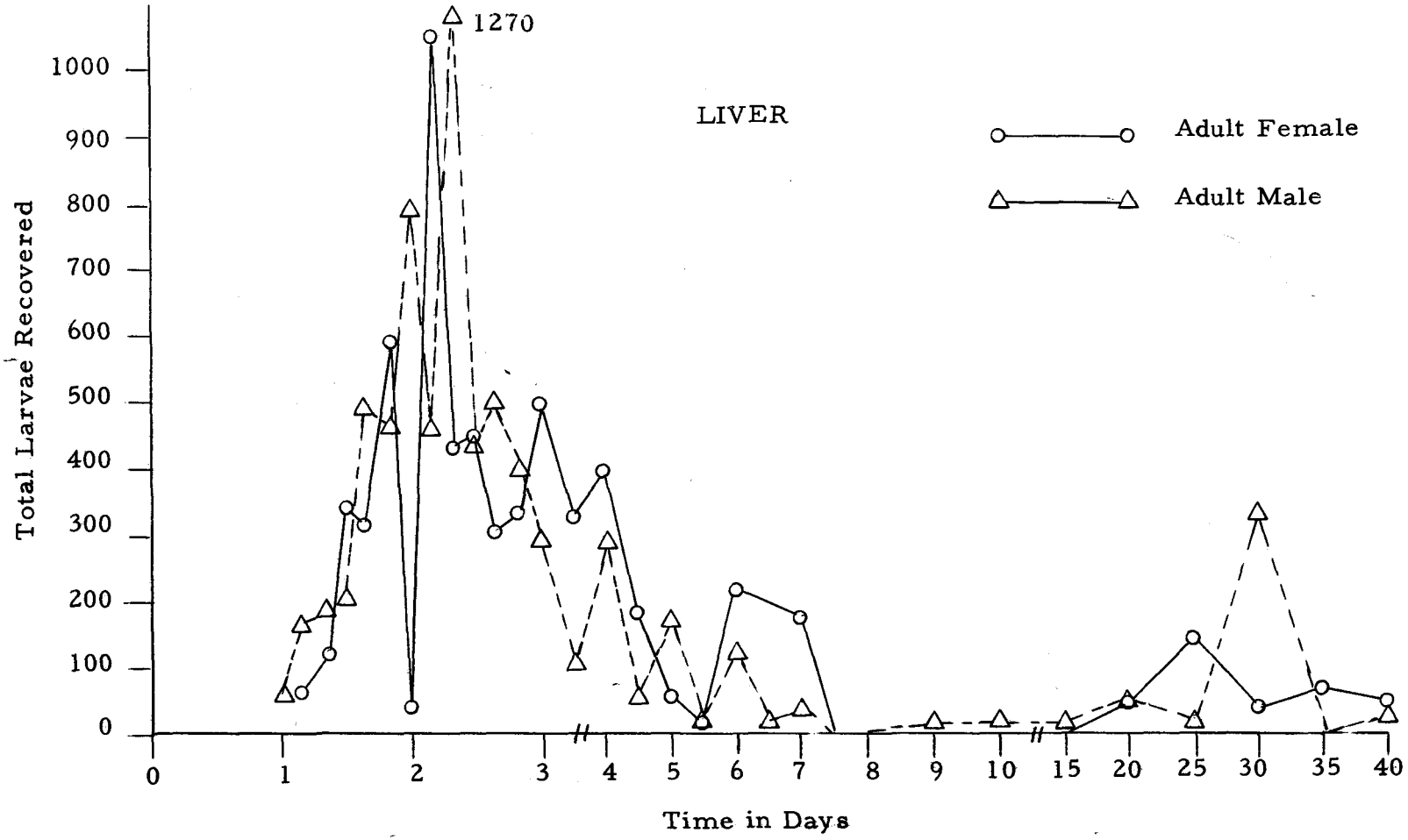

Fig. 4. Total larvae recovered from the liver of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

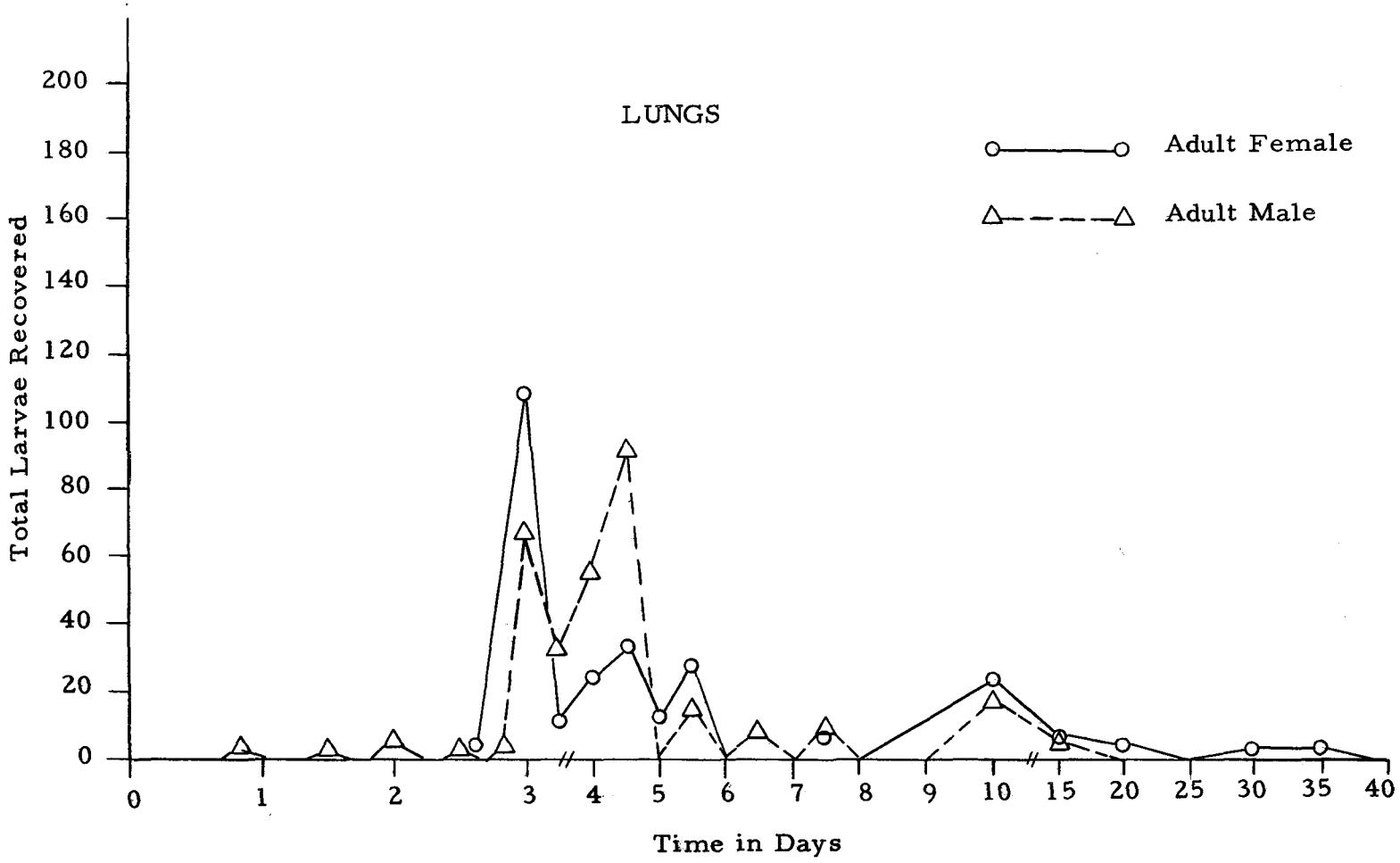

Fig. 5. Total larvae recovered from the lungs of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

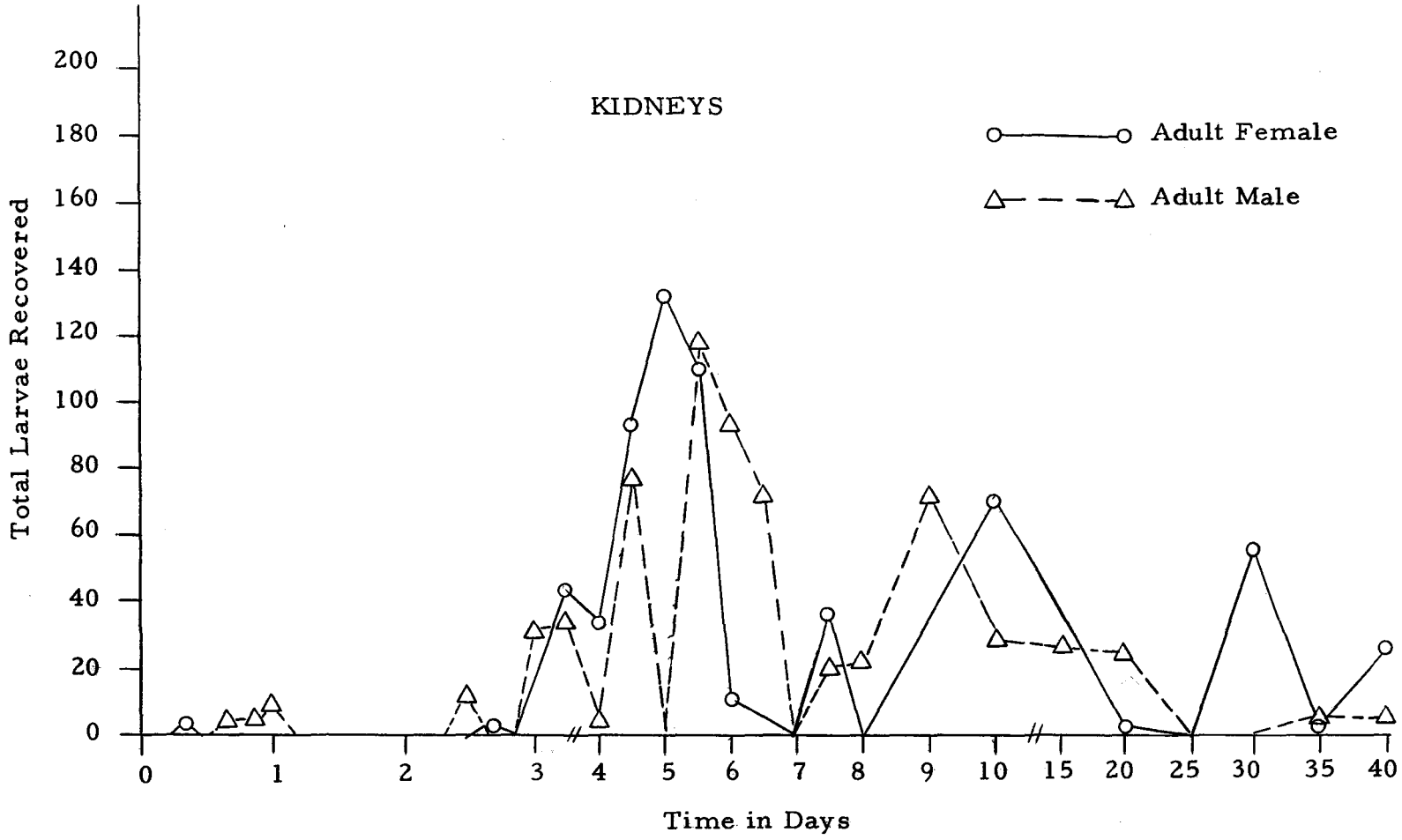

Fig. 6. Total larvae recovered from the kidneys of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

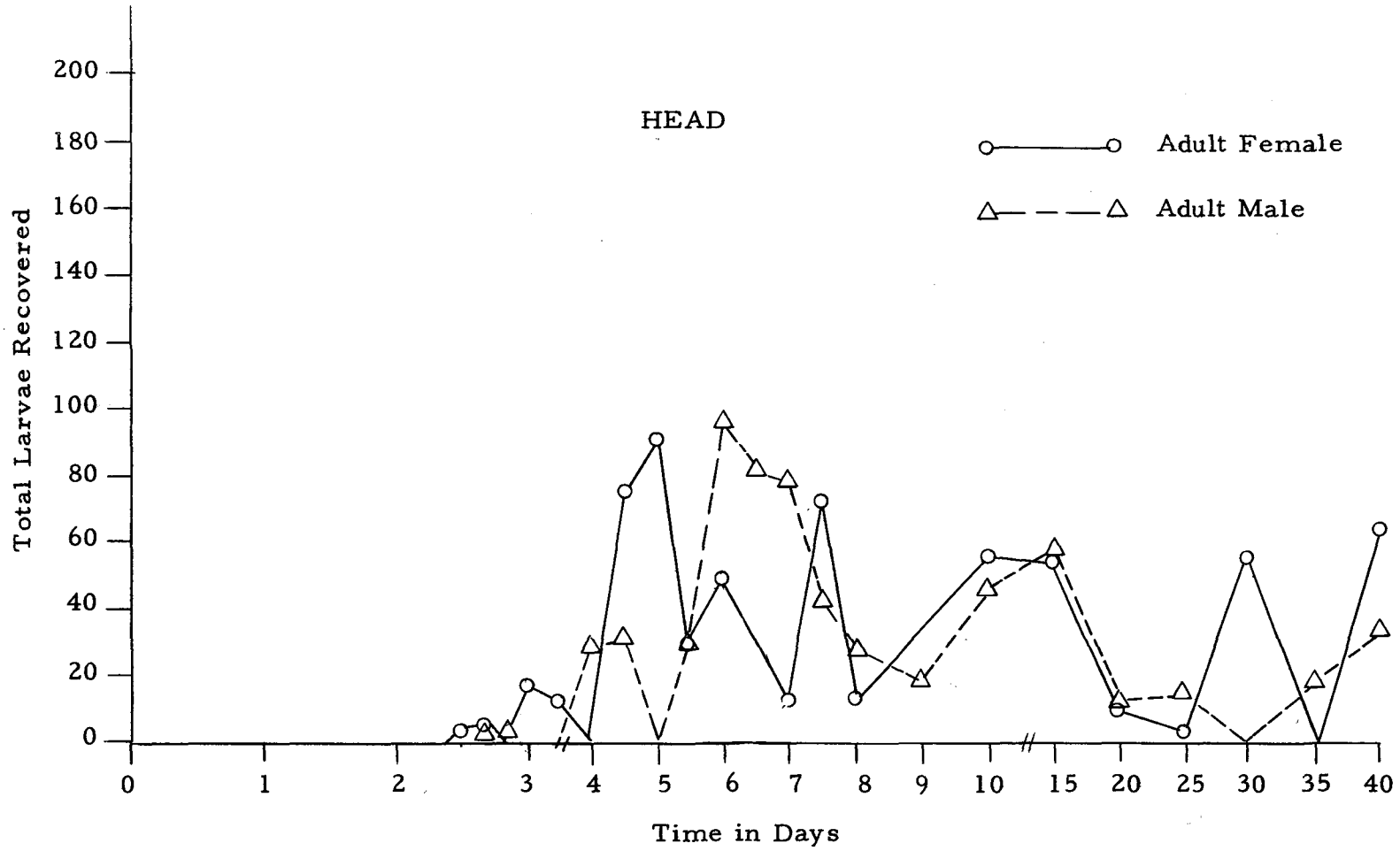

Fig. 7. Total larvae recovered from the head of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

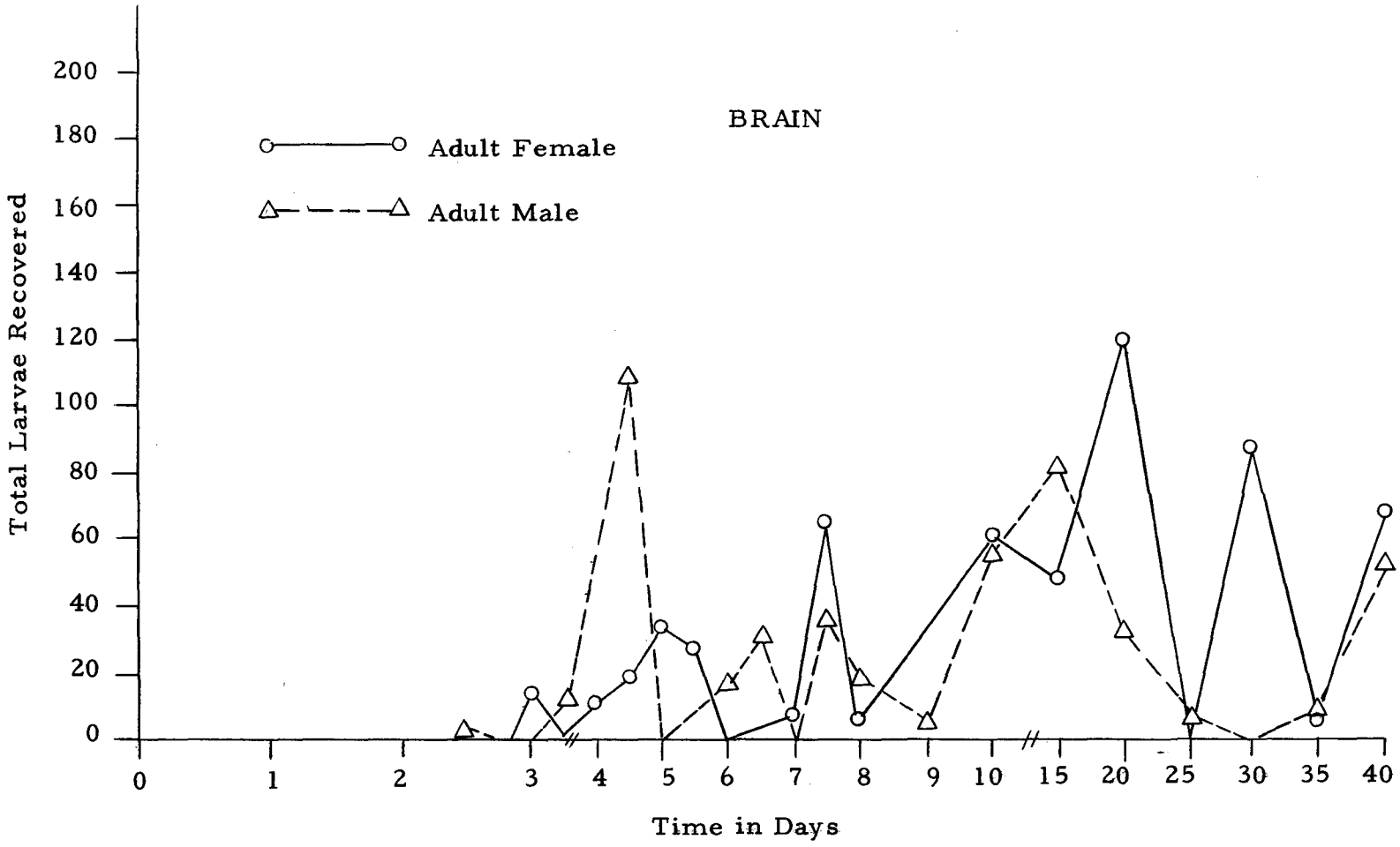

Fig. 8. Total larvae recovered from the brain of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

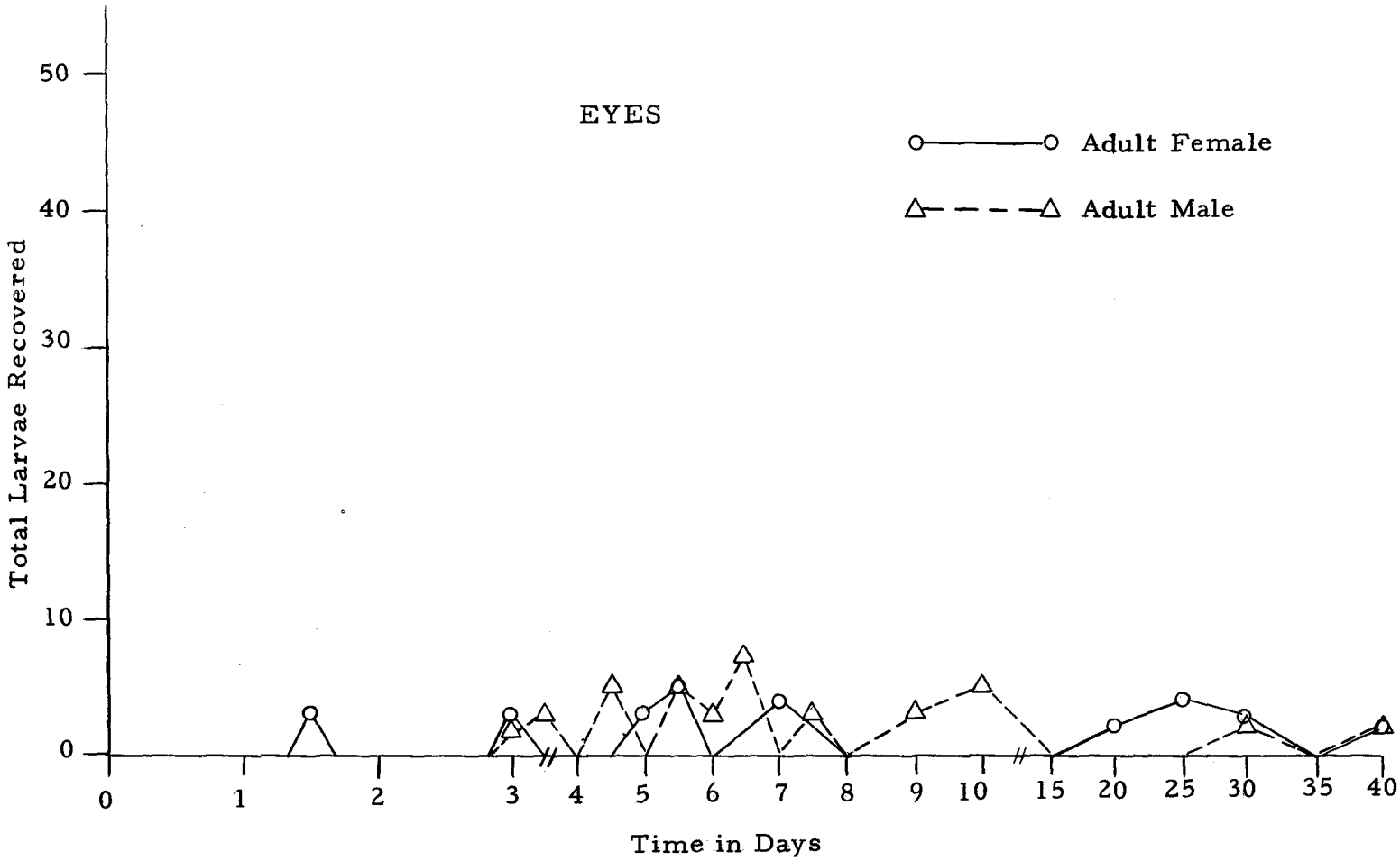

Fig. 9. Total larvae recovered from the eyes of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

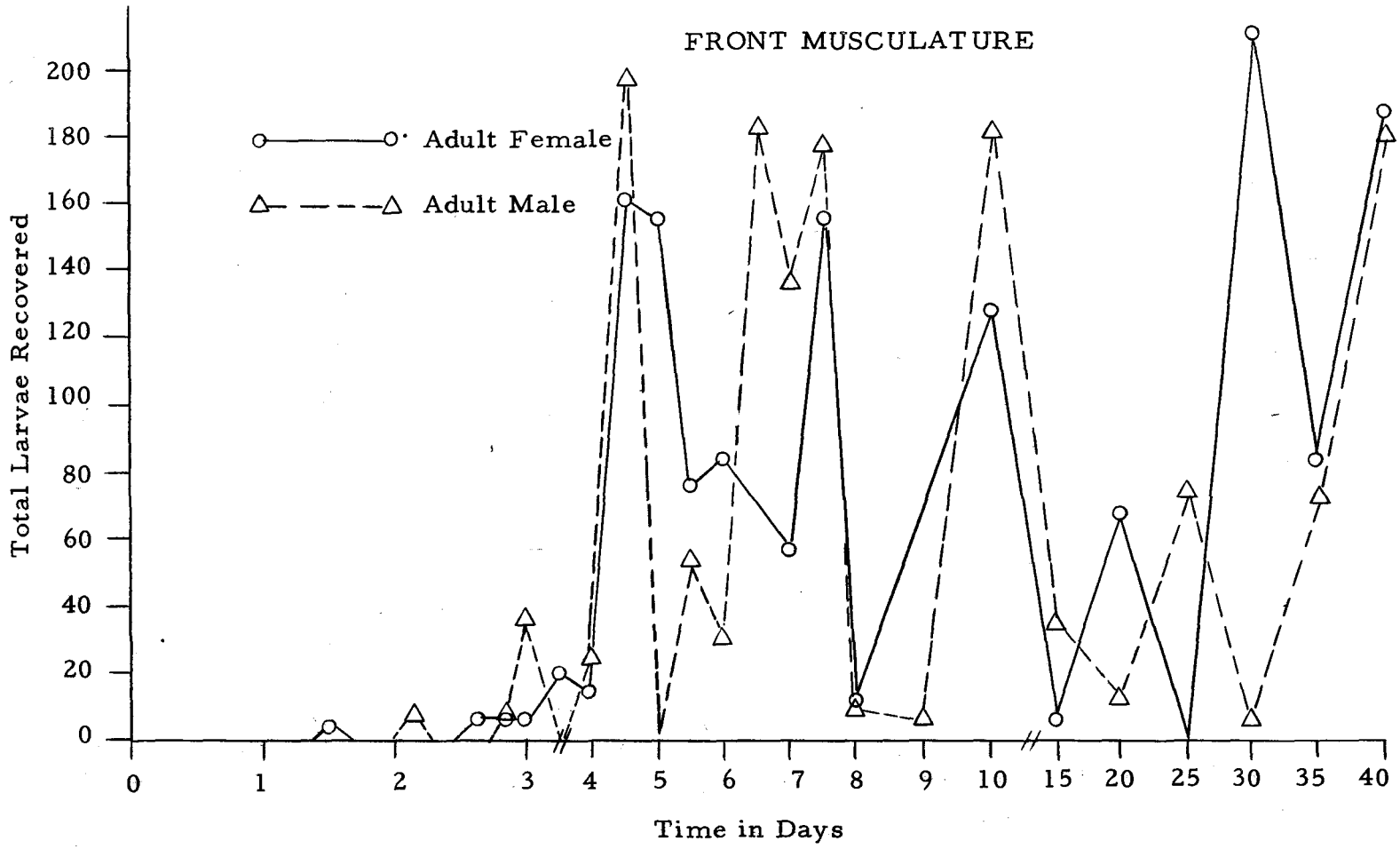

Fig. 10. Total larvae recovered from the front musculature of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

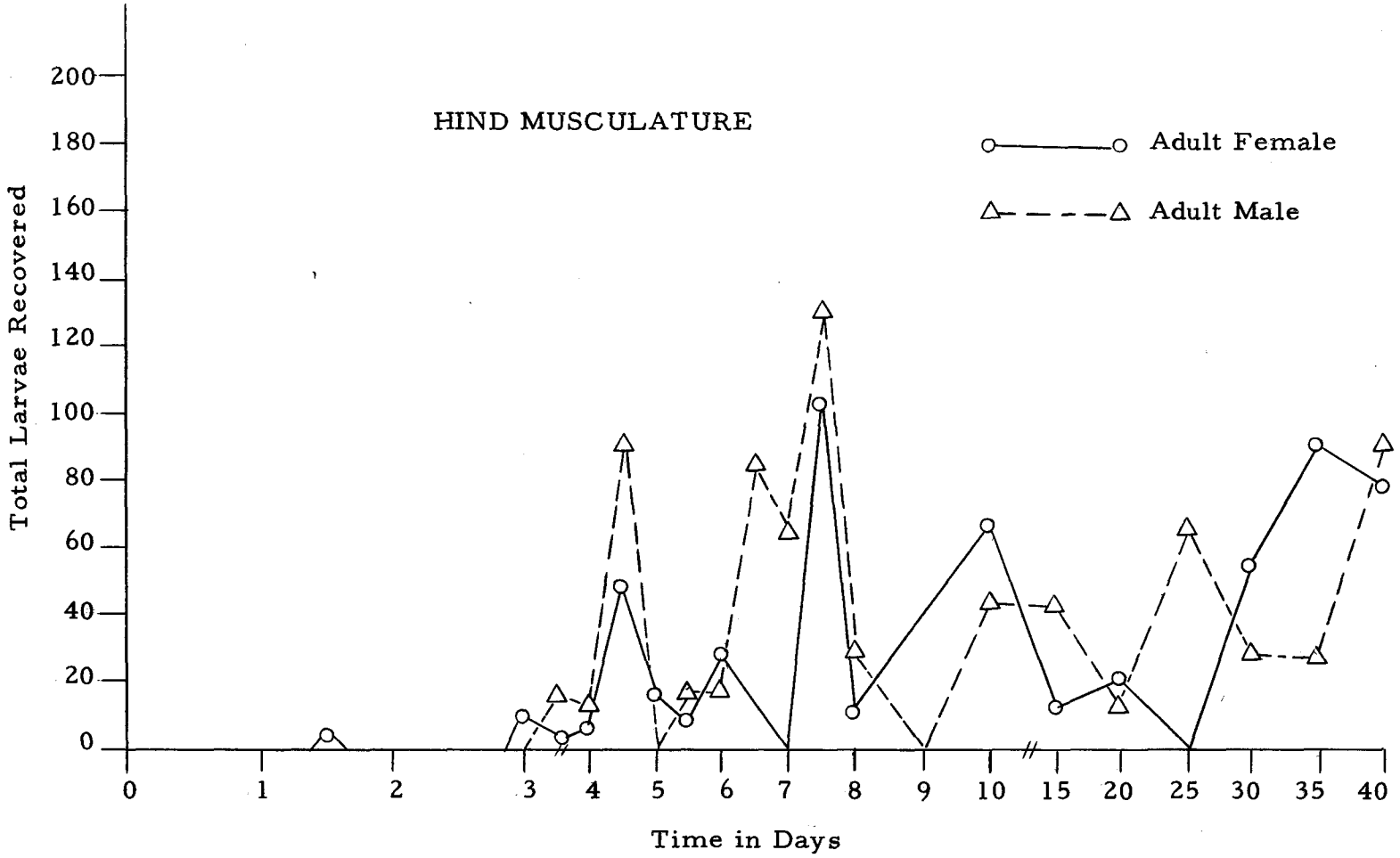

Fig. 11. Total larvae recovered from the hind musculature of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

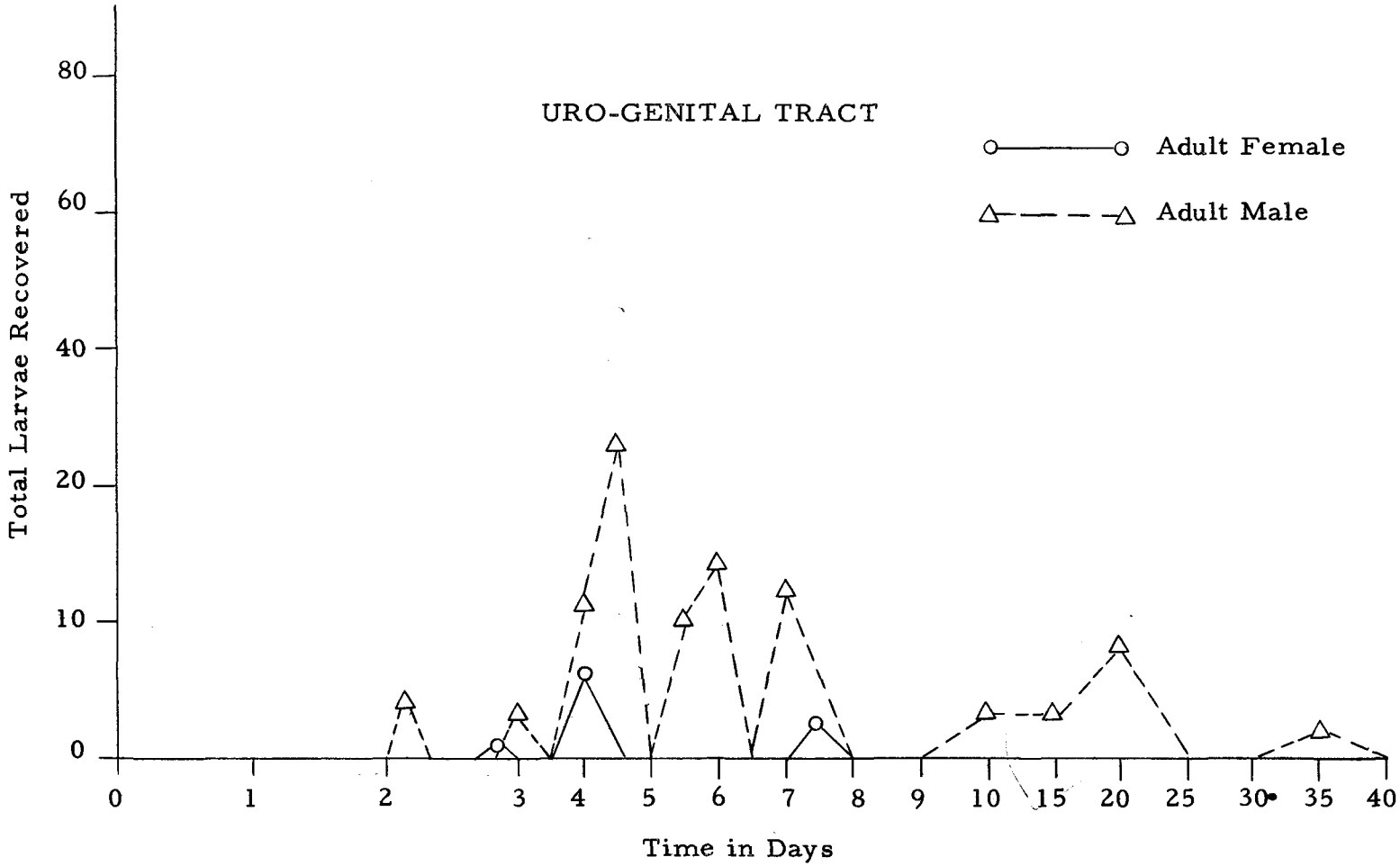

Fig. 12. Total larvae recovered from the uro-genital tract of adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

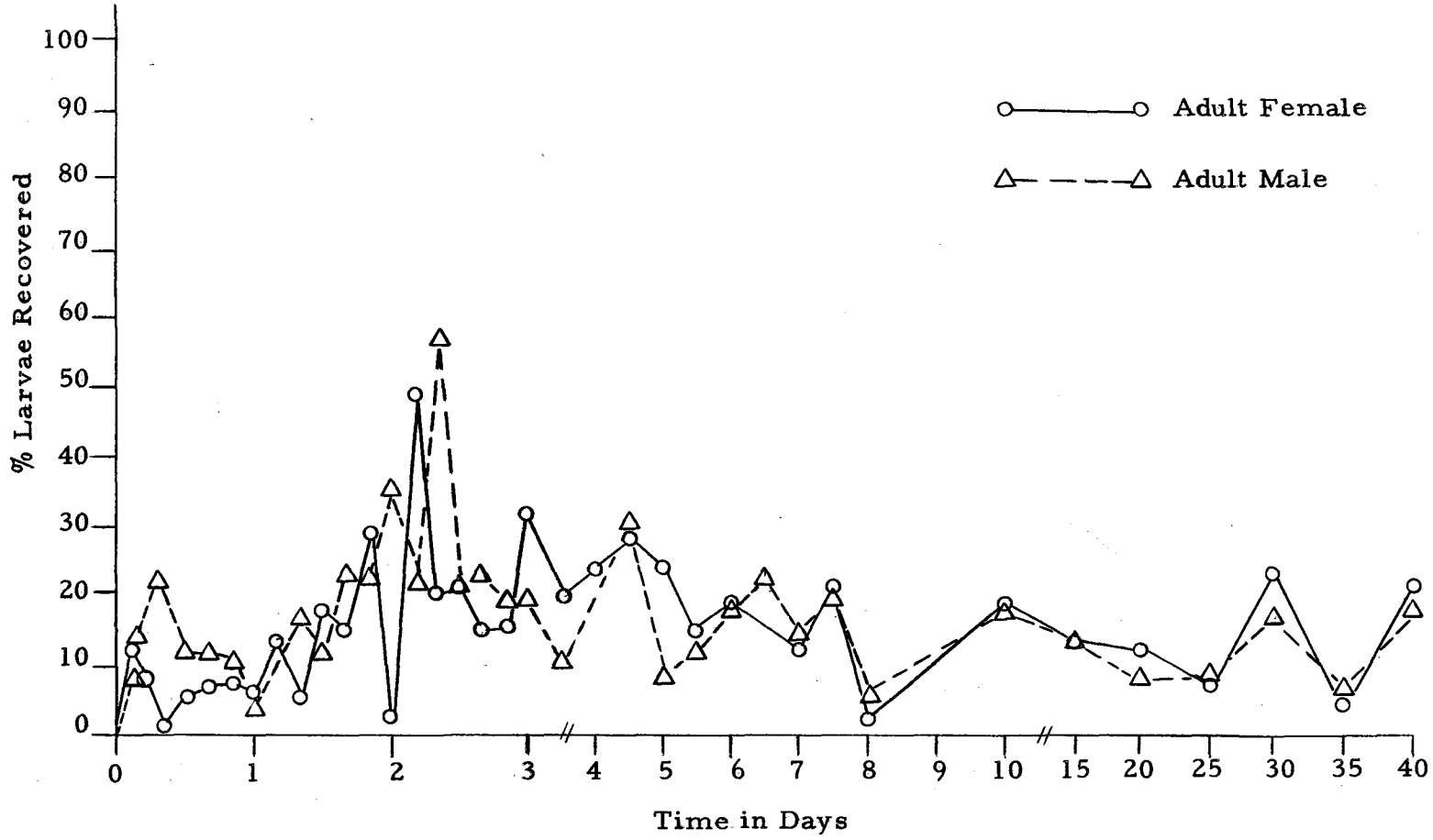

Fig. 13. Percentages of larvae recovered from adult male and female gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

recovered from the different organs and tissues assayed from both male and female adult gerbils, it can be seen that, in general, the sex of the host had no appreciable effect upon the migratory pattern of the larvae. One probable exception was noted from the results on the recovery of larvae from the uro-genital tracts. Larvae were recovered from this site from only three adult female gerbils, while ten adult male gerbils had larvae in these tissues. Also only a total of nine larvae were recovered from the females, whereas 93 were recovered from the males. Such a difference in number of positive hosts and also in number of total larvae recovered, strongly suggests that the sex of the host does influence larval migration to the uro-genital tract.

Effect of Age of Host on the Migratory Route of Larvae

Tables 8 through 12 show the total number of larvae recovered from different organs and tissues from each juvenile male and female gerbil killed in this study. Table 13 shows the individual and average weight of all gerbils used. Figures 14 through 24 compare the difference in total numbers of larvae recovered from each different organ and tissue assayed (with the exception of the heart, spleen, and uro-genital and gastro-intestinal tracts) from the juvenile gerbils, with the average number of larvae recovered from adult gerbils. Table 7 and Figure 25 illustrate the difference in the total average percent recovery of larvae inoculated in juvenile gerbils against the total average

Table 8. Total larvae recovered from the stomach, small intestine and large intestine of juvenile gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection	Stomach			Small Intestine			Large Intestine		
	Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour 1									
2									
3									
4									
8		3	3		0	0		288	288
12									
16	0		0	0		0	765		765
20		0	0		0	0		8	8
24		0	0		0	0		33	33
28									
32	0	9	4	24	11	18	168	396	282
36									
40		6	6		3	3		213	213
44									
48	0		0	7		7	0		0
52									
56		0	0		0	0		0	0
60									
64	3		3	2		2	0		0
68									
72		3	3		0	0		8	8

Table 9. Total larvae recovered from the liver, lungs and kidneys of juvenile gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection		Liver			Lungs			Kidneys		
		Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour	1									
	2									
	3									
	4									
	8		0	0		0	0		0	0
	12									
	16	9		9	0		0	0		0
	20		35	35		0	0		0	0
	24		0	0		4	4		0	0
	28									
	32	310	704	507	0	3	2	0	3	2
	36									
	40		480	480		5	5		3	3
	44									
	48	768		768	0		0	3		3
	52									
	56		150	150		8	8		0	0
	60									
	64	455		455	0		0	0		0
	68									
	72		452	452		36	36		9	9
Days	3 1/2	68	840	454	0	24	12	0	3	2
	4		31	31		63	63		40	40
	4 1/2									
	5		36	36		0	0		0	0
	5 1/2									
	6	15		15	11		11	26		26
	6 1/2									
	7		4	4		0	0		0	0
	7 1/2									
	8		56	56		7	7		26	26
	9		22	22		0	0		0	0
	10		19	19		8	8		0	0
	15	38		38	3		3	0		0
	20		32	32		2	2		5	5
	25		214	214		2	2		0	0
	30									
	35									
	40	8	19	14	2	2	2	8	0	4

Table 10. Total larvae recovered from the head, brain and eyes of juvenile gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection		Head			Brain			Eyes		
		Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour	1									
	2									
	3									
	4									
	8		0	0		0	0		0	0
	12									
	16	0		0	0		0	0		0
	20		0	0		0	0		0	0
	24		20	20		0	0		0	0
	28									
	32	0	0	0	0	0	0	0	0	0
	36									
	40		0	0		0	0		0	0
	44									
	48	0		0	0		0	0		0
	52									
	56		8	8		0	0		2	2
	60									
	64	10		10	3		3	0		0
	68									
	72		0	0		6	6		2	2
Days	3 1/2	0	0	0	0	0	0	0	0	0
	4		46	46		10	10		2	2
	4 1/2									
	5		41	41		0	0		2	2
	5 1/2									
	6	45		45	12		12	0		0
	6 1/2		0	0						
	7		72	72		84	84		4	4
	7 1/2		0	0						
	8		144	144		52	52		6	6
	9		16	16		7	7		4	4
	10		55	55		112	112		0	0
	15	0		0	12		12	0		0
	20		96	96		177	177		13	13
	25		127	127		125	125		2	2
	30									
	35									
	40	35	13	24	8	38	23	2	0	1

Table 11. Total larvae recovered from the front musculature, hind musculature and heart of juvenile gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection		Front Musculature			Hind Musculature			Heart		
		Male	Fem.	Ave.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour	1									
	2									
	3									
	4									
	8		0	0		0	0		-	
	12									0
	16	0		0	0		0	0		0
	20		0	0		0	0		0	0
	24		0	0		0	0		0	0
	28									
	32	0	0	0	0	0	0	0	0	0
	36									
	40		0	0		0	0		0	0
	44									
	48	20		20	0		0	4		4
	52									
	56		0	0		0	0		0	0
	60									
	64	10		10	0		0	0		0
	68									
	72		0	0		6	6		0	0
Days	3 1/2	36	10	23	42	18	30	0	0	0
	4		102	102		40	40		0	0
	4 1/2									
	5		20	20		16	16		0	0
	5 1/2									
	6	6		6	56		56	-		
	6 1/2									
	7		148	148		42	42		0	0
	7 1/2									
	8		92	92		76	76	2		2
	9		66	66		0	0		0	0
	10		148	148		32	32		0	0
	15	626		626	-		-	0		0
	20		348	348		154	154		0	0
	25		746	746		288	288		0	0
	30									
	35									
	40	126	112	119	42	88	65	0	0	0

Table 12. Total larvae recovered from the uro-genital tract, spleen and gastro-intestinal tract of juvenile gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

Time Since Infection		Uro-genital Tract		Spleen			Gastro-intestinal Tract		
		Male	Fem.	Male	Fem.	Ave.	Male	Fem.	Ave.
Hour	1								
	2								
	3								
	4								
	8		0		0	0			
	12								
	16	0		0		0			
	20		0		0	0			
	24		0		0	0			
	28								
	32	0	0	0	0	0			
	36								
	40		0		0	0			
	44	0				0			
	48			0		0			
	52								
	56		0		0	0			
	60								
	64	0		0		0			
	68								
	72		0		0	0			
Days	3 1/2	0	0	0	0	0	1	5	3
	4		0		0	0	1		0
	4 1/2								
	5		0		0	0	0		0
	5 1/2								
	6	8		0		0		0	0
	6 1/2								
	7		0		0	0	0		0
	7 1/2								
	8		0		0	0	0		0
	9		0		0	0	3		3
	10		0		0	0			
	15	0		0		0	0		0
	20		0		0	0		2	2
	25		0		0	0		2	2
	30								
	35								
	40	0	0	0	0	0	0	8	4

Table 13. Individual and average weights of all gerbils used in the experiment.

Time Since Infection		Weight (Gm) Adult Gerbils			Weight (Gm) Juvenile Gerbils		
		Male	Female	Ave.	Male	Female	Ave.
Hour	4	81.0	76.0	78.5			
	8	67.9	57.6	62.8		36.1	36.1
	12	69.8	62.9	66.4			
	16	73.0	75.5	74.2	54.7		54.7
	20	70.4	67.4	68.9		52.7	52.7
	24	73.0	87.3	80.1		32.0	32.0
	28	70.2	56.3	63.2			
	32	78.6	78.8	78.7	37.1	53.6	45.4
	36	70.4	73.3	71.8			
	40	66.4	60.0	63.2		55.0	55.0
	44	66.5	66.0	66.3			
	48	72.0	65.5	68.8	49.4		49.4
	52	80.7	60.6	70.6			
	56	78.2	67.7	73.0		51.0	51.0
	60	68.8	70.4	69.6			
	64	88.5	64.7	76.6	48.1		48.1
	68	60.0	59.2	59.6			
	72	76.3	60.7	68.5		50.0	50.0
Days	3 1/2	74.1	97.8	80.9	29.0	52.7	40.8
	4	93.5	68.2	80.9		52.1	52.1
	4 1/2	67.5	63.8	65.6			
	5	87.4	107.8	97.6		52.6	52.6
	5 1/2	80.1	59.0	69.6			
	6	80.2	77.8	79.0	57.8		57.8
	6 1/2	72.2	-	72.2			
	7	77.1	60.6	68.9		27.2	27.2
	7 1/2	79.0	65.1	72.0			
	8	97.6	79.1	88.4		52.0	52.0
	9	75.5	-	75.5		43.7	43.7
	10	93.8	117.8	105.8		55.5	55.5
	15	78.4	107.8	93.1	36.1		36.1
	20	80.1	55.1	67.6		40.7	40.7
	25	59.8	63.8	61.3		35.6	35.6
	30	83.9	62.4	73.2			
	35	78.4	69.3	73.8			
	40	80.6	64.5	72.6	46.7	45.9	46.8

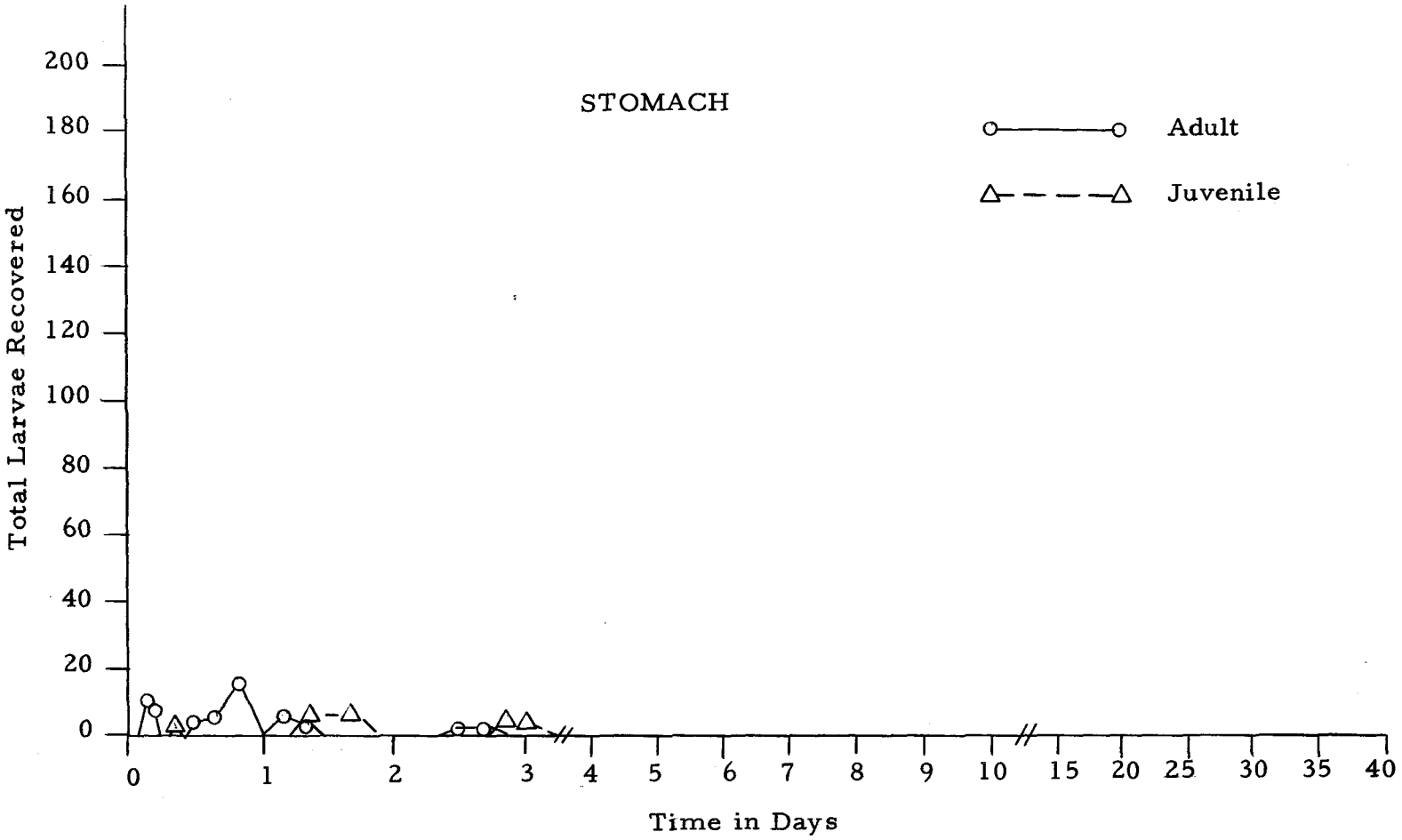

Fig. 14. Total larvae recovered from the stomach of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

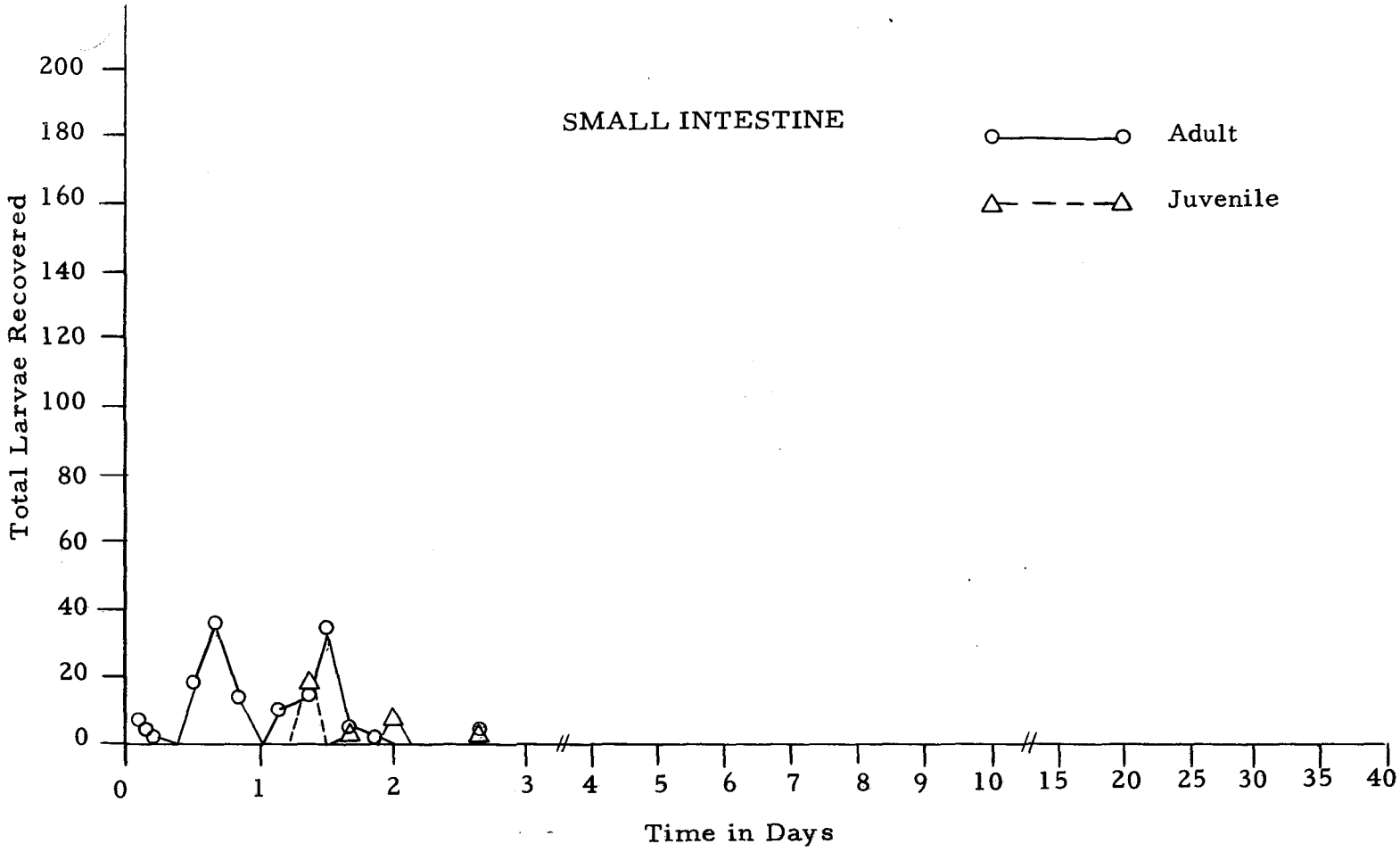

Fig. 15. Total larvae recovered from the small intestine of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

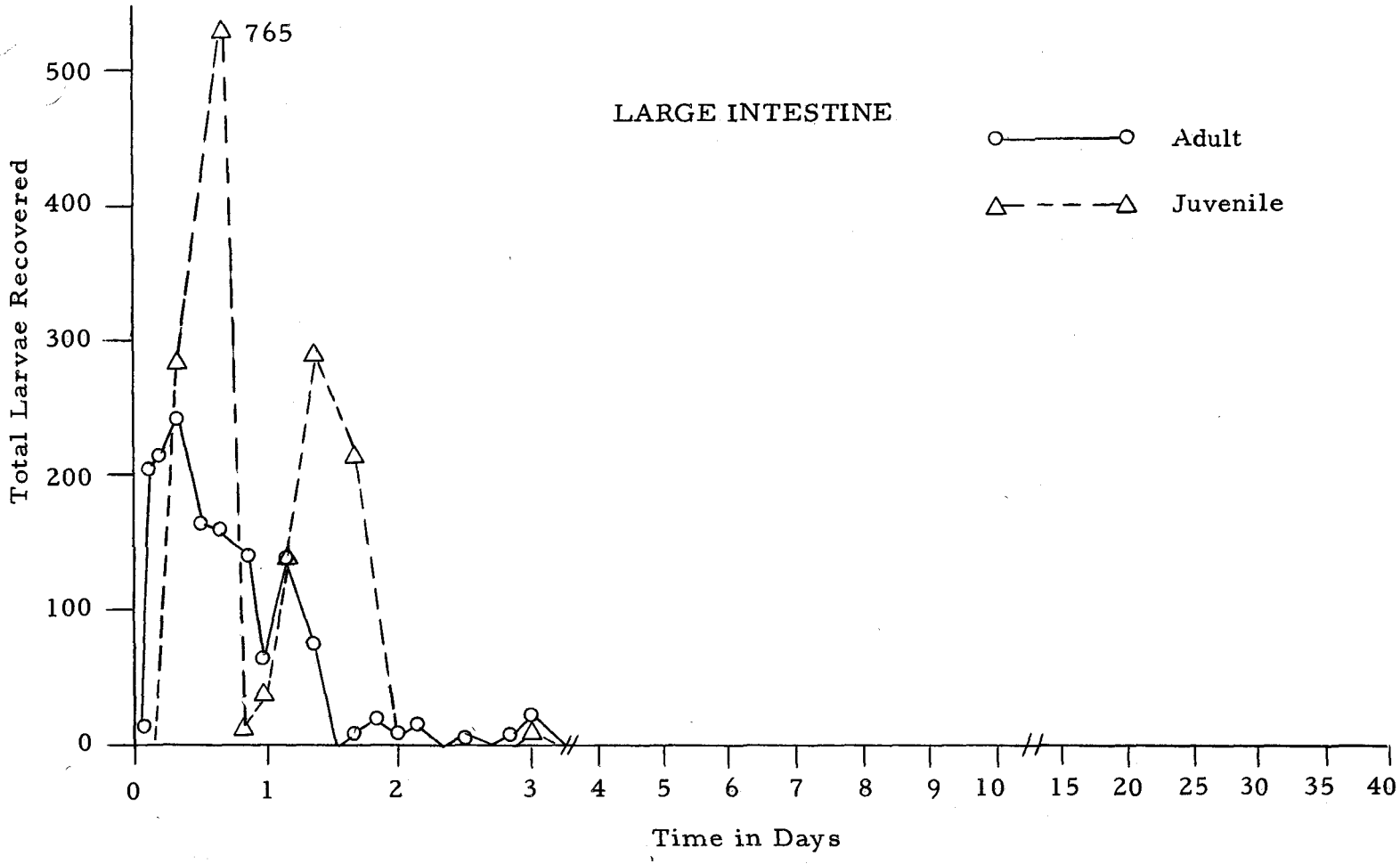

Fig. 16. Total larvae recovered from the large intestine of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

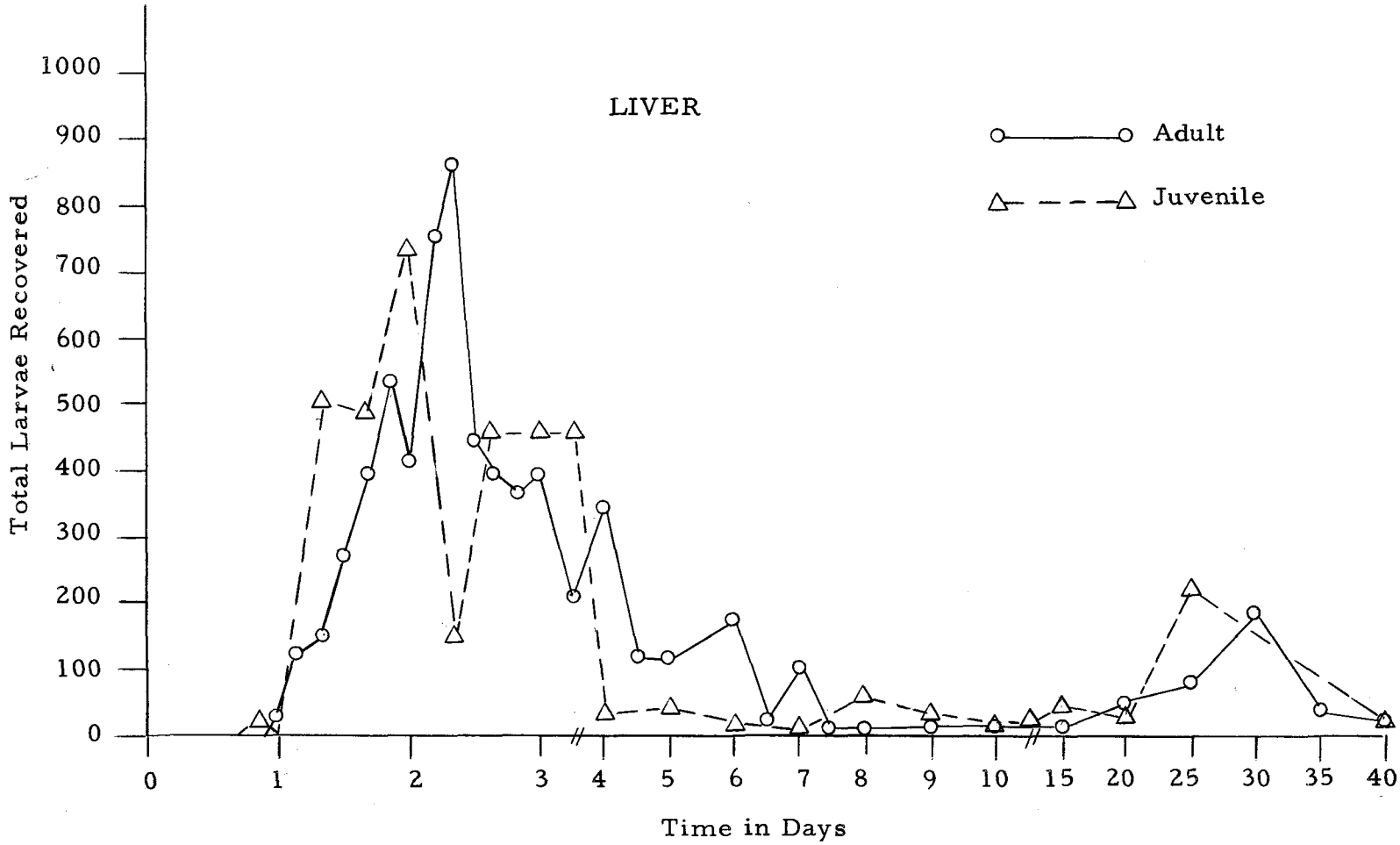

Fig. 17. Total larvae recovered from the liver of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

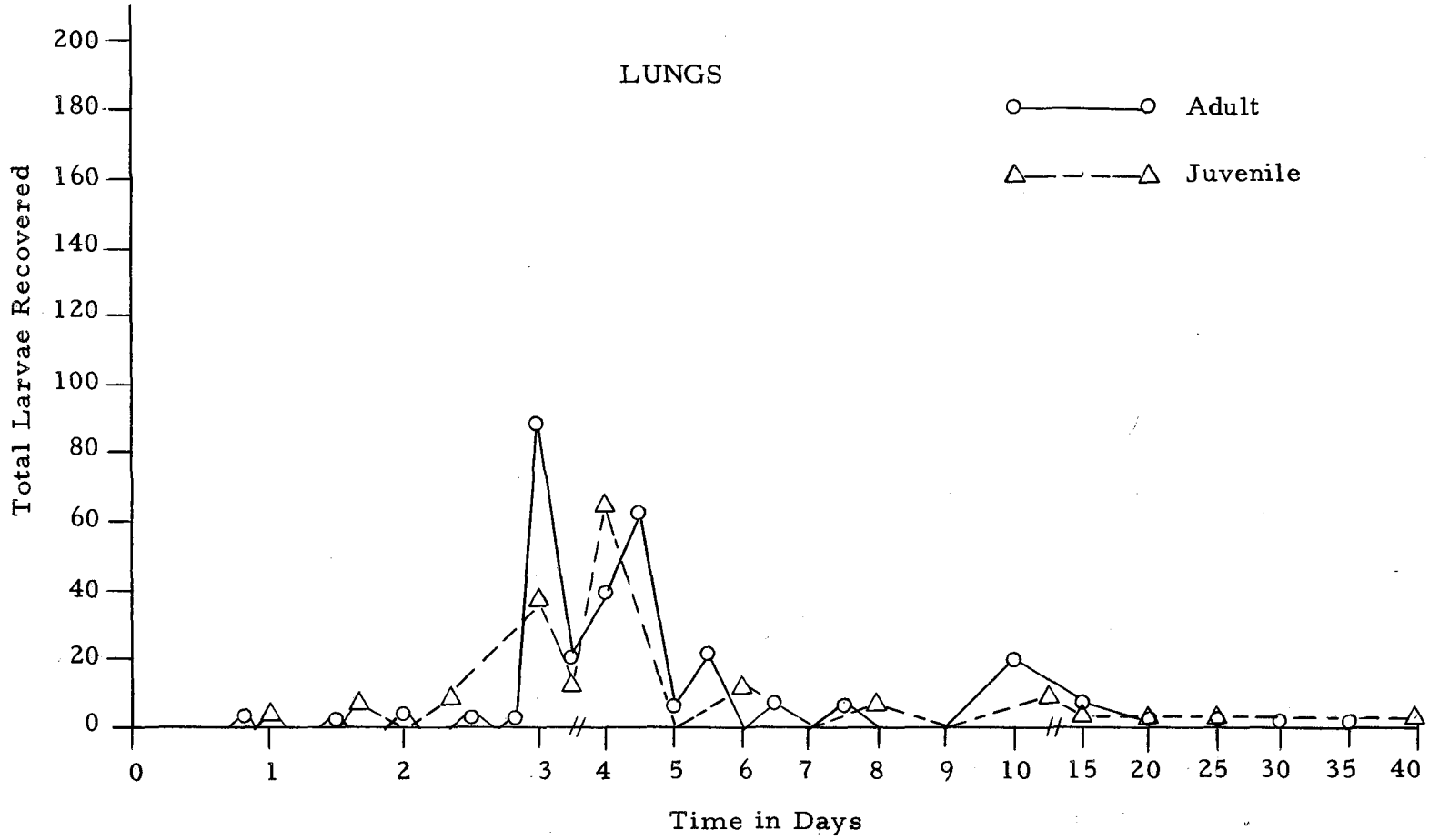

Fig. 18. Total larvae recovered from the lungs of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

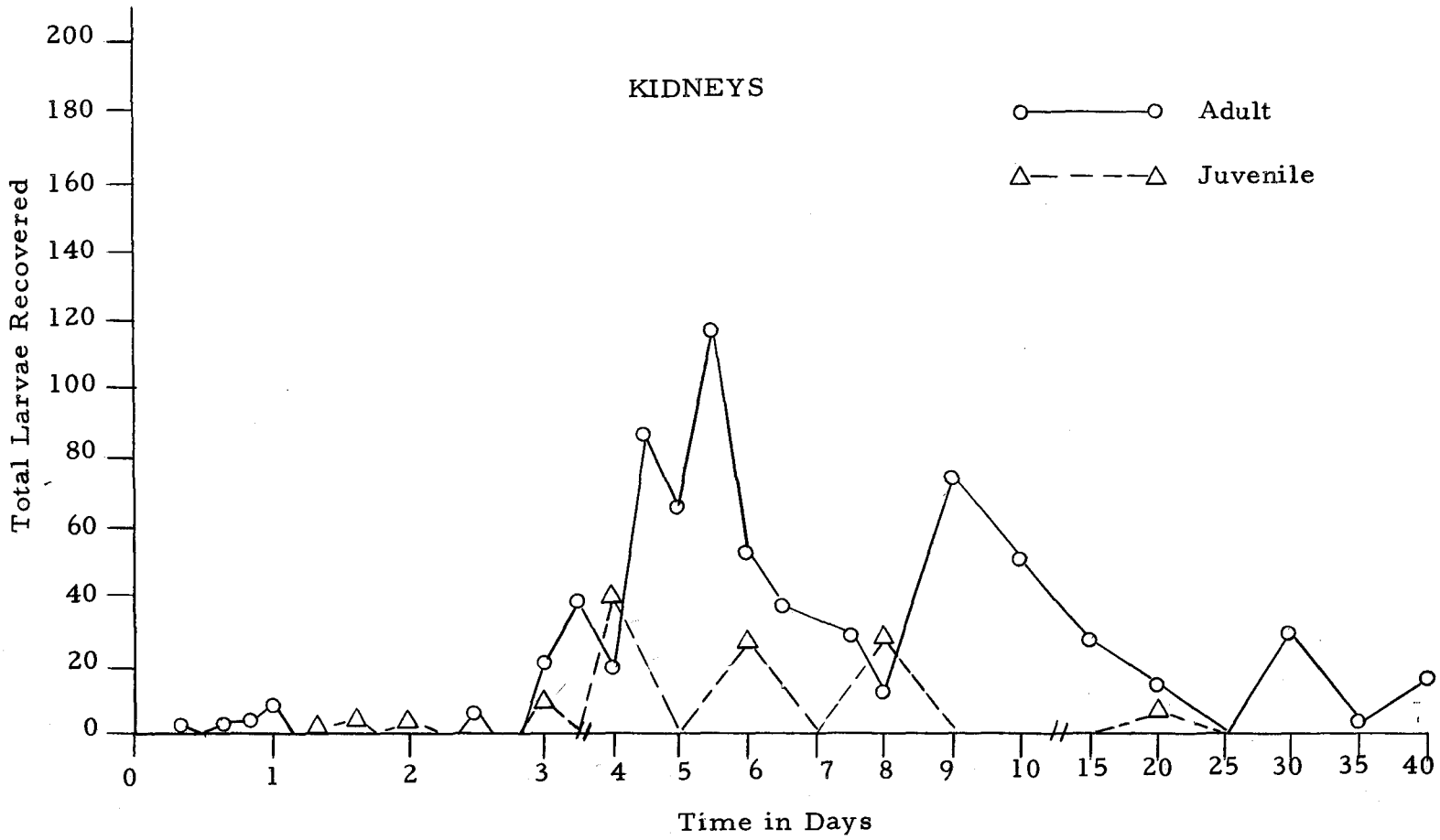

Fig. 19. Total larvae recovered from the kidneys of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

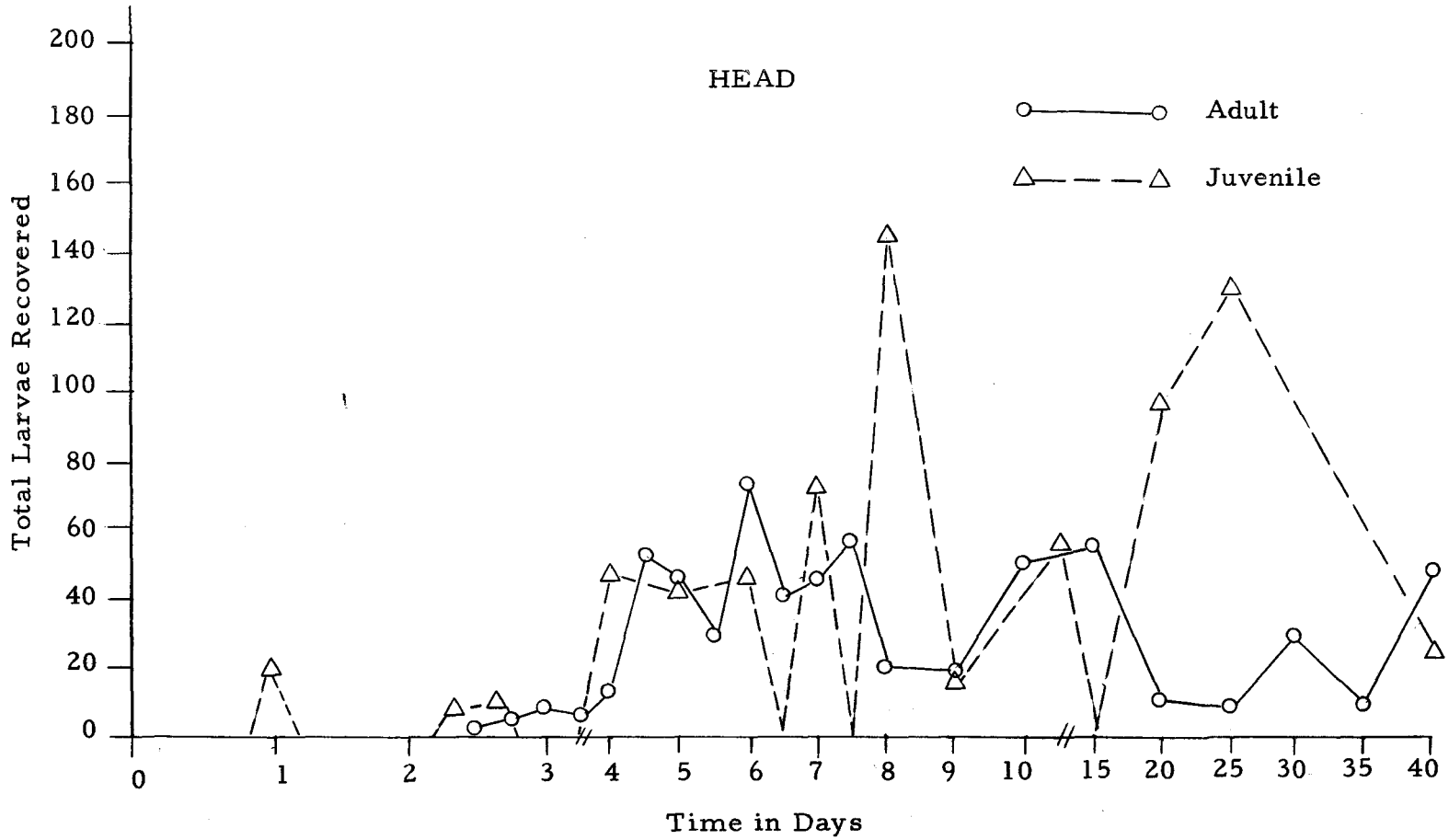

Fig. 20. Total larvae recovered from the head of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

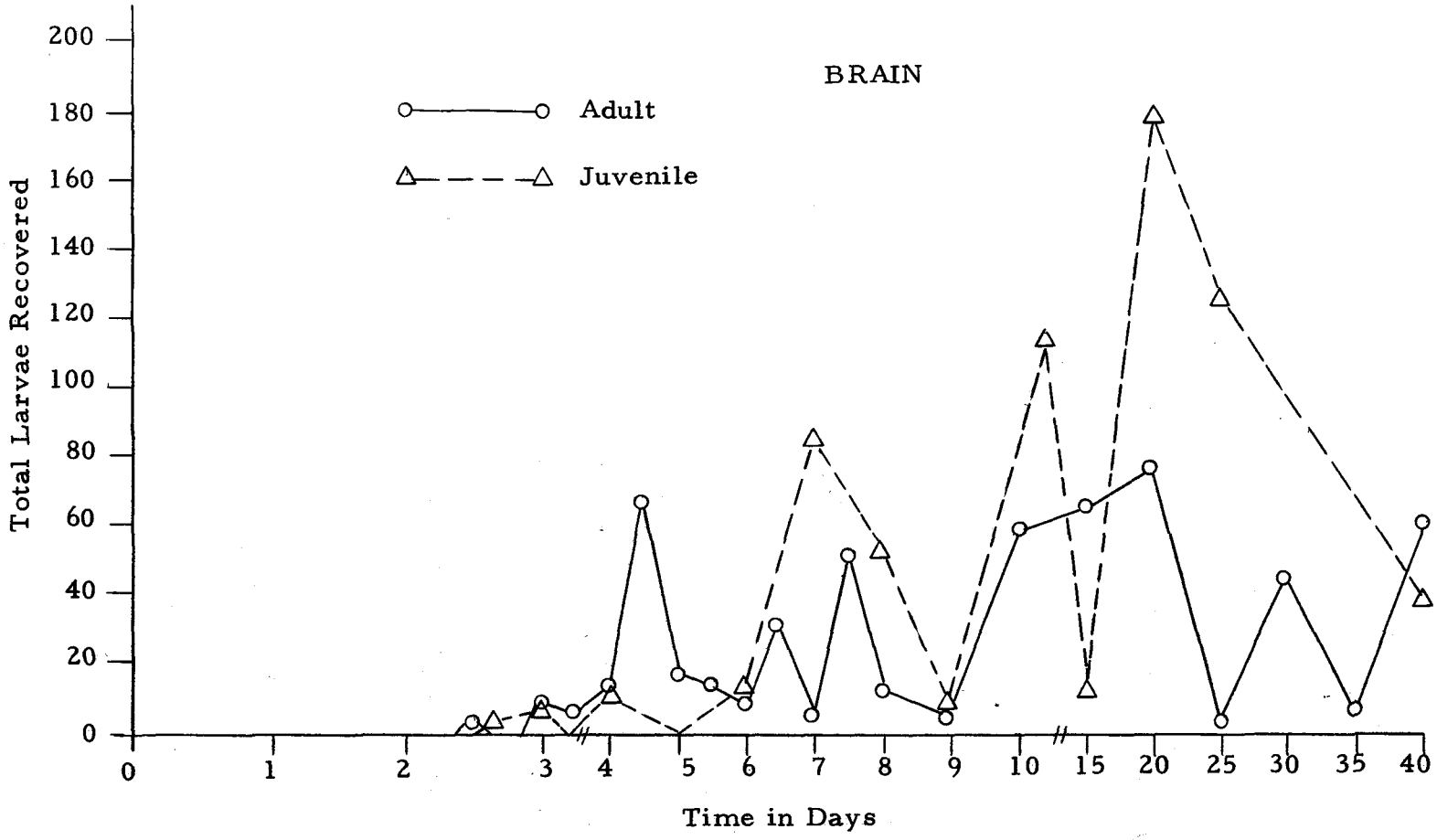

Fig. 21. Total larvae recovered from the brain of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

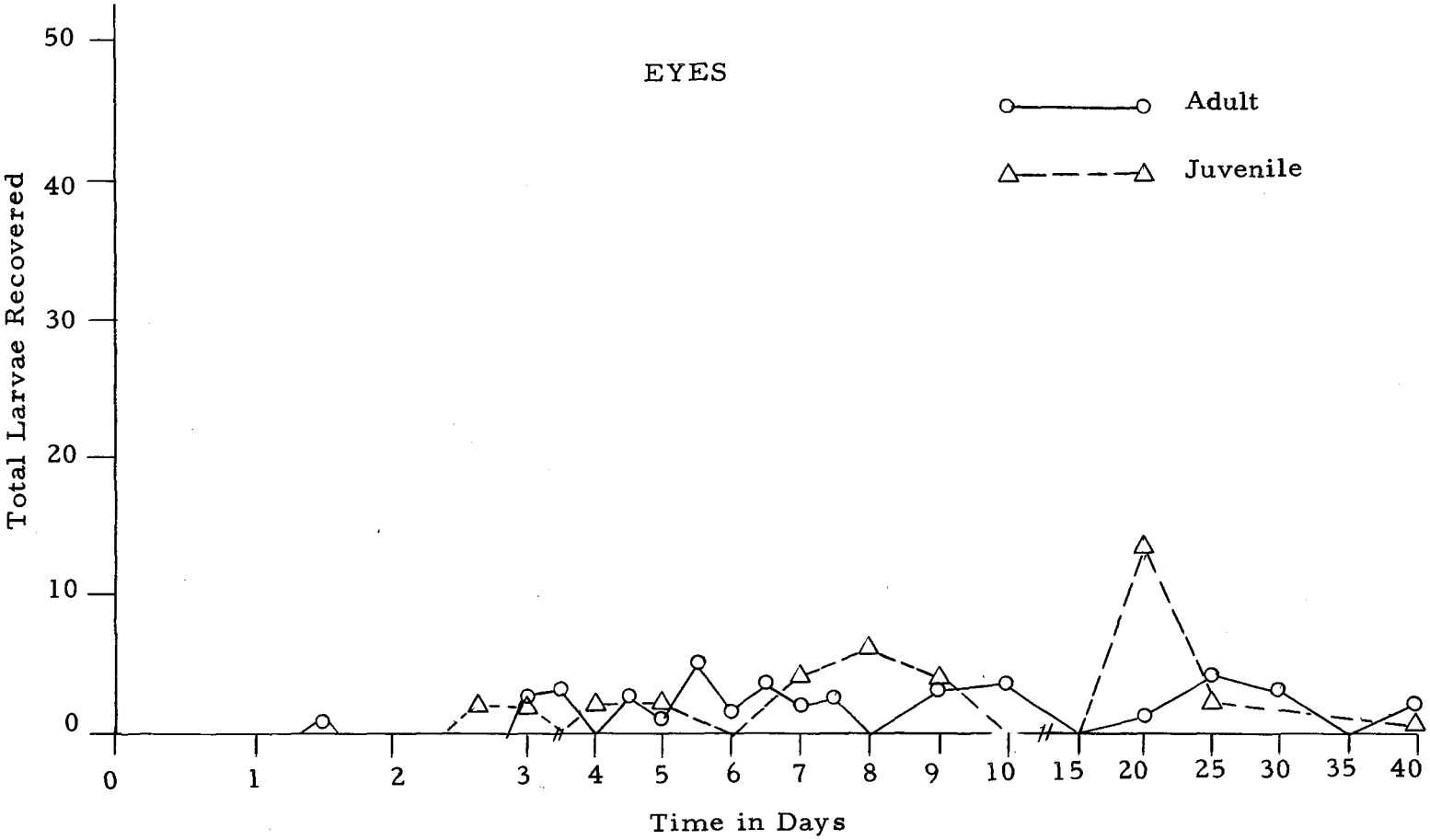

Fig. 22. Total larvae recovered from the eyes of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

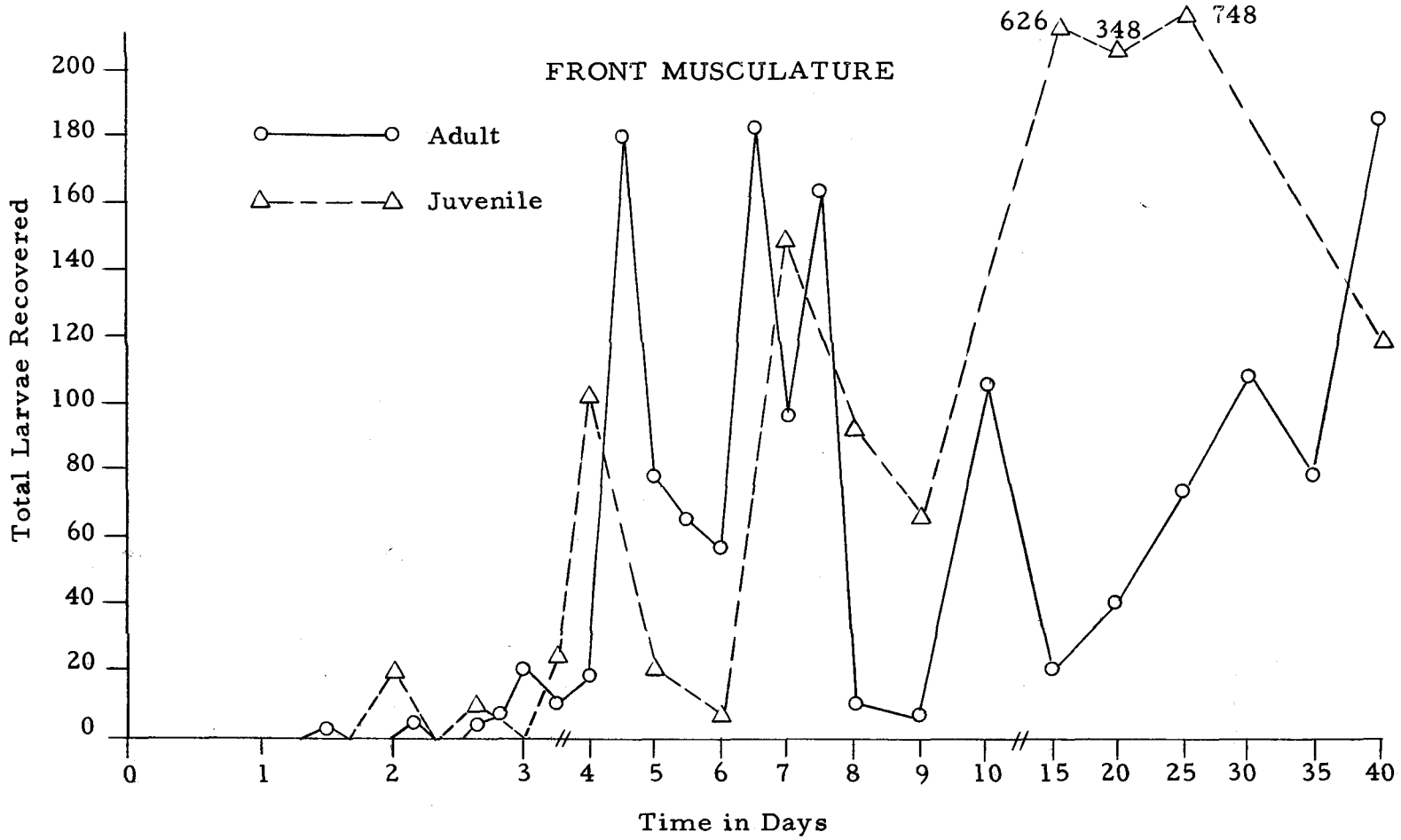

Fig. 23. Total larvae recovered from the front musculature of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

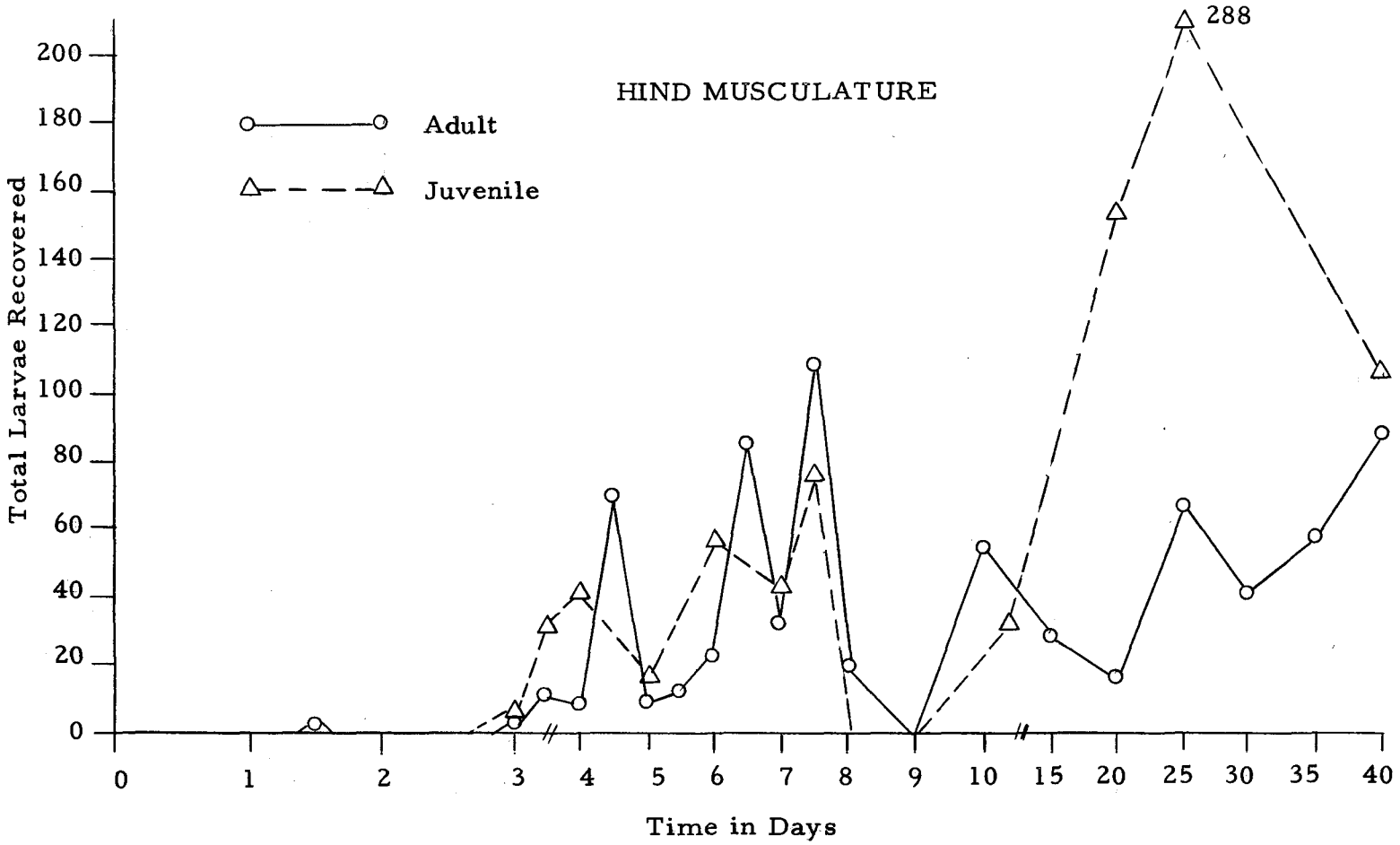

Fig. 24. Total larvae recovered from the hind musculature of juvenile gerbils versus the average number recovered from adult gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

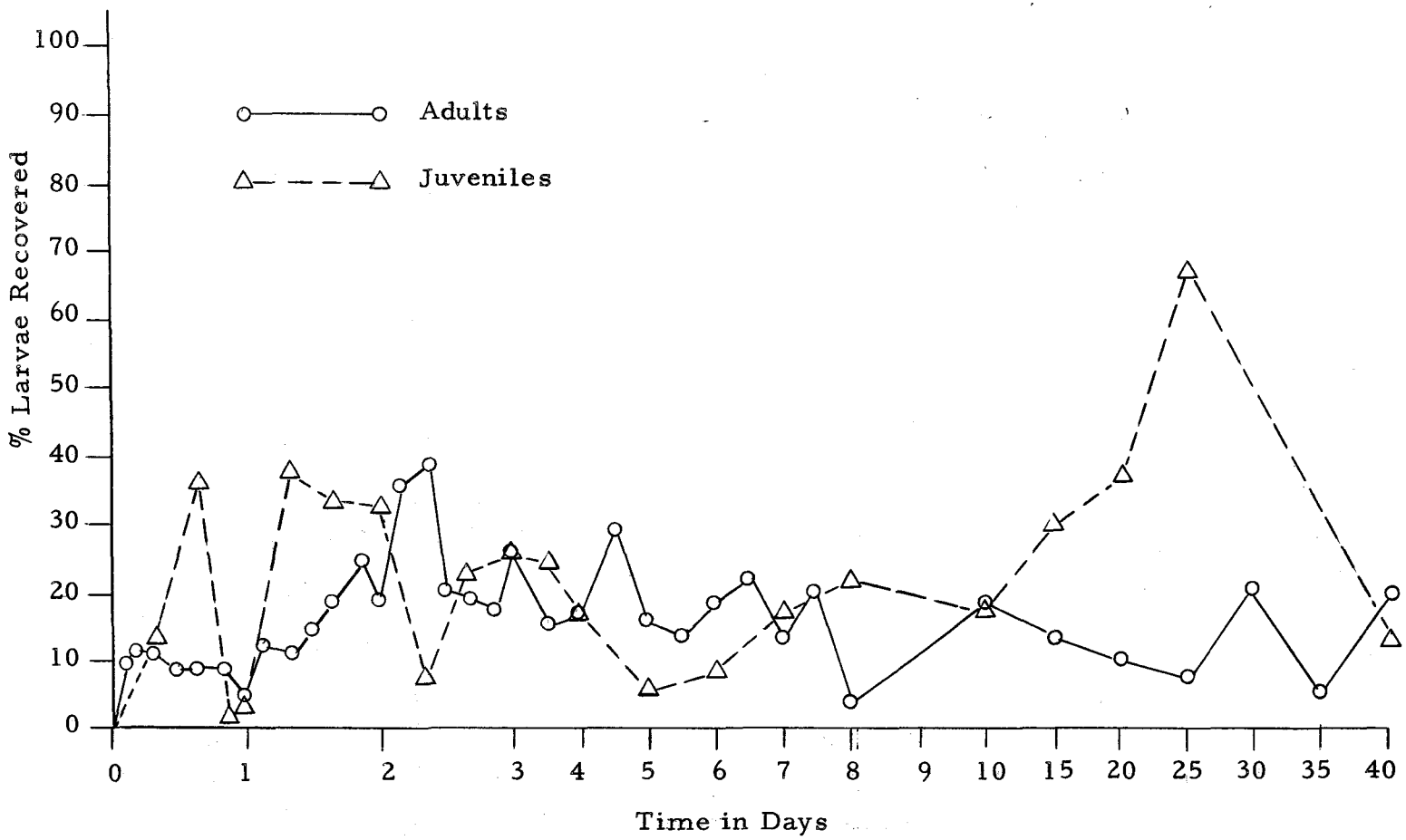

Fig. 25. Average percentages of larvae recovered from adult gerbils versus the average percentages of larvae recovered from juvenile gerbils inoculated with 2150-2250 embryonated Toxocara canis eggs.

percent recovery from all adult gerbils.

In general, it was noted that there were no consistent differences in the number of larvae recovered from the different organs and tissues assayed from the two age groups. Slight differences, however, which may be significant, were noted, in that considerably more larvae were recovered from the kidneys of the adults than from the juvenile gerbils, while it appeared that more larvae were recovered from the juveniles from the areas of the front and hind musculature, and possibly from the head and brain regions. With respect to the total number of larvae recovered at any one time interval, the only possible difference was that consistently more larvae were recovered from the juveniles than from the adults between the 15th and 25th day after infection. As stated earlier, the small numbers of juveniles available for this study, precludes any definitive interpretation of the effect of age of host on the migratory route of the infective larvae, although the findings herein reported suggest that age may influence the migratory pattern of the larvae at least in the head, brain, kidneys, and front and hind musculature.

DISCUSSION

The migratory route of T. canis in gerbils was found to occur similarly to that reported for other experimental animals, in which larval migration has been studied. However, there was no evidence in the current study to suggest that a second invasion of the intestinal tract (tracheal migration) existed in gerbils, as reported in mice by Oshima (1961) and suggested by Burren (1968). Evidently, larval migration in gerbils is only somatic in nature.

One of the most significant findings in the present study was that larvae were recovered from the eyes as soon as three days after infection and frequently thereafter for the entire duration of the experiment. Burren (1968) found larvae in the eyes of only three of 100 mice which he examined. No other author has reported finding larvae in the eyes of experimental animals, although larvae have frequently been diagnosed in the eyes of humans, especially in young children that have become infected through ingesting soil containing embryonated T. canis eggs (Beaver, et al., 1952; Beaver, 1958, 1962, 1969).

Sprent (1952) was able to recover larvae from the kidneys of infected mice and suggested that those organs were of major importance in the migratory route of T. canis. Burren (1968) did not find any larvae in the kidneys or spleen of mice which he infected, however. Done,

et al. (1960) were able to recover larvae from the kidneys, but not from the spleen of experimentally infected pigs. In the present study only a few larvae were recovered from the spleens of gerbils, whereas the kidneys were found to be an important organ in the migratory route, since 40% of all larvae recovered 5 1/2 days after infection were found in those organs. The subsequent concentration of larvae in the front and hind musculature and head and brain regions does not differ significantly from somatic migrations demonstrated for other ascarids; however, the fact that 42% of all larvae recovered on the eighth day were near the brain seemed to be a significant point. Locomotor difficulties associated with larval migrans is undoubtedly correlated with invasion of larvae into the central nervous system, but to date this has not been studied extensively in experimental toxocariasis. It appears that T. canis larvae are somewhat erratic in their migratory behavior in gerbils, and are capable of invading any organ or tissue of the body.

In the present study it was also determined that the sex of the gerbil did not have any appreciable effect upon the total numbers of larvae recovered from the entire animal, but did exert an influence on the numbers recovered from the uro-genital tract. Ten adult male gerbils had a total of 93 larvae recovered from the uro-genital tract, whereas three adult female gerbils yielded only a total of nine larvae from this site. The fact, however, that larvae were found in the

uro-genital tract of females, suggests that this organ may be a source of infection for the young in utero. Borman (unpublished data) was also able to recover infective larvae from the fetuses of pregnant mice; although, he noted no other appreciable influence of sex on the migratory pattern of infective larvae. Oshima (1961), was not able to recover any larvae from fetuses of infected pregnant mice. The fact that Borman baermannized the digested tissues he was examining, whereas Oshima centrifuged the digestive sediment only before microscopic examination, may be a partial explanation for the discrepancy in their work.

Although sufficient numbers of juvenile gerbils were not available for a comprehensive study of the effects of age of host on the migratory behavior of T. canis, the results herein reported suggest that age may influence the larval migration in some body sites. It was noted that there were more larvae in the kidneys of adult gerbils killed, whereas more larvae were recovered from the juveniles from the front and hind musculature and possibly from the head and brain regions. There was no significant effect noted in the total numbers of larvae recovered from the entire animal between the two age groups, except the limited data available did indicate that somewhat more larvae were recovered from juveniles 15-25 days after infection, than from the adults. Recovery rates at all other times were similar.

Borman (unpublished data) found that age was an influential

factor on larval migration in infected mice, since he was able to recover considerably more larvae from 15-day old than from 60-day old mice. Schaeffler (1960) also found that the age of sheep definitely affected the migratory behavior of larvae, since he found that the larvae underwent a more extensive somatic migration in younger animals than in older animals. He recovered more larvae from the internal organs, brain and musculature of the young lambs, whereas he found that in older animals more larvae tended to concentrate in the liver. Apparently, the age of the sheep did not affect the total numbers of larvae recovered from all tissues he examined, but rather influenced only the migratory behavior of the larvae. This observation was essentially the same as was noted in the present study with gerbils.

A noticeably greater percentage of larvae was recovered from the gerbils killed 44-56 hours than at other time intervals. This increased recovery rate at these times was probably due to the fact that 96-100% of the larvae recovered from the gerbils at those times were found in the liver. Since the liver tissue is digested readily, the larvae were undoubtedly more easily recovered in baermannization. Also there would have been less error introduced through the recovery techniques, since at that time practically all of the larvae were present in only one tissue sample. From 60 hours to about eight days the percentage of recovered larvae declined gradually. An explanation for this may be that as some of the larvae leave the liver and

migrate to the somatic tissues they die in transit or while becoming encapsulated in the tissues or both. Such a decline was also noted by Done, et al. (1960) in their studies on experimentally infected pigs. A further difficulty in the recovery would also prevail after the larvae had been generally distributed throughout the entire body, since the chance of losing them in several samples during digestion or baermannization would be increased.

In general, the migratory pattern of larvae in gerbils is not markedly different from that studied this extensively in any other experimental animal. Since gerbils are so easily maintained in the laboratory, and since it is now evident that they are an ideal experimental host for T. canis infections, the possibility exists to use these animals for additional studies related to "visceral larva migrans."

SUMMARY

The migratory route of infective T. canis larvae in gerbils and the effect of sex of host on this migration were determined by orally inoculating 76 adult gerbils with infective embryonated eggs. Limited studies were also done on the effect of age of host on the larval migratory pattern using some paired observations from 25 juvenile gerbils. Male and female gerbils were killed at specific time intervals, and the following organs and tissues were assayed for the presence of larvae: head, brain, eyes, heart, lungs, liver, spleen, stomach and contents, small intestine and contents with associated mesenteries and lymph nodes, large intestine and contents with associated mesenteries and lymph nodes, kidneys, uro-genital tract, front-quarter musculature, and hind-quarter musculature. The organs and tissues were blended in 1% pepsin at pH 1.8 and then incubated 8-24 hours at 37-39 C. Larvae were recovered from the digested tissues by baermannizing them 8-24 hours, after which time they were collected into centrifuge tubes and refrigerated at least one hour at 4 C to allow settling of the larvae. An aspirator was used to draw the fluid from the top of the tube, and the remaining sediment was measured and assayed for larvae by aliquot counts.

Larvae were first recovered from the gastro-intestinal tract at

two hours; however, by 36 hours most had presumably either penetrated the gastro-intestinal wall and invaded other parts of the body, or else they were picked up by the circulatory system and carried to other body organs. At ten hours larvae were detected in the liver, and at 56 hours all of the larvae recovered were found in that organ. At 72 hours 15% of the larvae were found in the lungs, thereby suggesting a typical ascarid-type migration for T. canis in gerbils. At 4 1/2 days larvae were detected for the first time in moderate numbers in several other body organs, indicating that they had successfully passed through the lungs and had been disseminated generally throughout the entire body. At 5 1/2 days the peak larval recovery was from the kidney, and at 7 1/2 days the peak occurred in the front and hind musculature. At approximately eight days significant numbers of larvae were recovered from the head and brain regions. After that time a general migratory pattern was no longer distinguishable, and larvae were ultimately detected at least once in all tissues and organs assayed in this study.

The sex of the host was probably an influential factor on migration of the larvae only in the region of the uro-genital tract, since considerably more larvae were recovered from that site in the male gerbils than in the females. With respect to the effect of age of host, it was noted that more larvae were recovered from the kidneys of

adult gerbils than from juveniles, while the younger animals yielded more larvae from the front and hind musculature, and possibly from the head and brain regions.

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THE MIGRATORY ROUTE OF *TOXOCARA CANIS* IN GERBILS

An Abstract

Presented to the

Department of Zoology and Entomology

Brigham Young University

In Partial Fulfillment

of the Requirements for the Degree

Master of Science

by

Thomas C. Baker

August 1969

This abstract by Thomas C. Baker is accepted in its present form by the Department of Zoology and Entomology of Brigham Young University as satisfying the abstract requirement for the degree of Master of Science.

ABSTRACT

Infective embryonated eggs of Toxocara canis, the common round worm of dogs and other carnivores, and the causative agent of "visceral larva migrans" in humans, were injected into 76 adult and 25 juvenile Mongolian gerbils, in order to obtain information on the larval migratory pattern in these experimental hosts. At specific intervals thereafter, paired adults or juveniles were killed and 14 different tissues and organs were enzymatically digested and then baermannized to determine larval recovery.

The majority of infective embryonated eggs hatched in the gastrointestinal tract within the first few hours, afterwhich the larvae showed peak concentrations in the liver on the second day, followed by progressively increasing numbers in the lungs on the third day, kidneys at 5 1/2 days, front and hind musculature at 7 1/2 days, and finally in the head and brain regions at about eight days. Larvae were recovered at least once from all of the tissues and organs examined, thereby suggesting a generalized somatic migration throughout the entire body.

The results obtained from paired observations on either male and female adult gerbils, or with juvenile gerbils, suggest that neither sex nor age of the gerbils markedly influence the migratory pattern of the T. canis larvae in these hosts.