BIOLOGICAL AND BIOCHEMICAL INVESTIGATIONS ON THE NEMATODE, SYNGAMUS TRACHEA (MONTAGU, 1811) CHAPIN, 1925

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Russell Francis Krueger

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BIOLOGICAL AND BIOCHEMICAL INVESTIGATIONS ON THE NEMATODE, *SYNGAMUS TRACHEA* (MONTAGU, 1811) CHAPIN, 1925

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AN ABSTRACT

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ABSTRACT

The purpose of this investigation was to study parasitism as it is represented in the life cycle of the nematode, Syngamus trachea, and concurrently to establish biochemical characteristics of this species with special emphasis on the role of respiration and carbohydrate metabolism.

Oral, intravenous, intraperitoneal, and subcutaneous routes of inoculation of chickens and turkeys were tried. No infection was obtained with intraperitoneal and subcutaneous inoculation. A previously untried route of infection, intravenous inoculation of suspensions of larvae, proved to be superior to established methods because of the ease of controlling the degree and intensity of infection. Syngamus was recovered from the trachea as early as eight days following intravenous inoculation and had a $14\frac{1}{2}$ day prepatent period. Oral inoculation resulted in gapeworms in the trachea on the ninth day and a prepatent period of 15 days.

After approximately two weeks of age chickens became more difficult to infect and after three to four weeks of age became refractory to gapeworm infection. Turkeys as old as 186 days could easily be infected. The intensity of gapeworm infections in turkeys began to decline after 24 days and by 37 days infections were negligible. Gapeworms were recovered from turkeys as long as 82 days after inoculation.

The red pigmented pseudocoelomic fluid of Syngamus appears to be an iron porphyrin compound which is probably a hemoglobin. This hemoglobin is not identical with that of the host's hemoglobin. These hemoglobins are compared and differences noted.

Wet and dried weight values covering the life span of Syngamus in the trachea of turkeys are given. The percent dried weight of paired Syngamus was 26.2% ± 2.9%.

The metabolic activities of Syngamus — aerobic oxygen consumption and carbon dioxide liberation, endogenous and exogenous carbohydrate utilization, carbohydrate content, butyric acid utilization, and anaerobic carbon dioxide liberation — were most pronounced in the youngest and smallest gapeworms. These metabolic activities all gradually decreased as the weight of the gapeworms increased. There appeared to be a relationship of metabolic activity to egg production.

Respiration studies were conducted by standard manometric techniques. The rate of oxygen utilization of paired Syngamus decreased from 18.54 to 4.08 microliter per mg dried weight per hour as the worms aged and increased in weight. The rate of oxygen consumption was decreased by such inhibitors as azide, cyanide, 2,4-dinitrophenol, fluoride, iodoacetate, and malonate. Inhibition by fluoride and malonate took place only in a calcium free substrate. Oxygen utilization by Syngamus was also decreased following gassing with carbon monoxide and was further decreased when gapeworms

were maintained in darkness. The respiratory quotient (RQ) in a phosphate buffered substrate, with or without 0.005 M glucose, was approximately 0.87. In a substrate containing 0.065 M butyric acid the RQ for Syngamus was 0.708. A RQ of approximately 1.0 was obtained with an acetone powder prepared from Syngamus in a substrate containing 0.005 M glucose.

The range of the polysaccharide content in Syngamus was 0.33% to 0.76% and the rate of endogenous polysaccharide utilization was about 0.35% wet weight of Syngamus per 24 hours. Exogenous glucose utilization decreased with increased size of gapeworms from 3.89 to 0.23 mg per gram wet weight per hour. There was a pronounced increase in the rate of exogenous glucose utilization when gapeworms were maintained in vitro for ten days.

TABLE OF CONTENTS

INTRODUCTION

Studies in parasitology are no longer confined to areas of morphology, taxonomy, and ecology, but rather have been extended to include biochemical and physiological aspects of parasitism. One must recognize and appreciate the past contributions which serve as a foundation for the field of parasitology, and at the same time realize that a constant evolution is taking place.

In this present day a perusal of the literature on biochemical studies of parasites will reveal a real dearth of information. The parasitic protozoa have been less neglected than the parasitic metazoa in this respect. In an attempt to gain insight into the physiology of the parasitic metazoa, the nematode, Syngamus trachea (Montagu, 1811) Chapin, 1925, was chosen for biological and biochemical investigation.

Syngamus trachea has as its habitat the trachea of birds. Frequently the bird experiences difficulty in breathing which results in gasping or gaping, thereby serving as basis for its common name, the gapeworm. It has a characteristic red color and presents a forked appearance by reason of the perpetual syngamy of the male and female nematodes. The very location and nature of Syngamus are reason enough for selecting this worm for investigation.

In such an unusual habitat, the trachea, Syngamus trachea

is exposed to an abundance of oxygen. Oxygen is also available to parasitic helminths which live in the blood, lungs, and swim bladder. Most helminths are found in an environment poor in oxygen, such as the lumen of the intestinal tract; others, although few in number, exist in body tissues where oxygen is available only by way of the host's circulatory system. von Brand (1946) calls attention to this situation and states that there is practically nothing known about parasitic worms that live in environments rich in oxygen. von Brand (1952) also raised the question concerning the relationship of carbohydrate metabolism of such parasitic invertebrates having a free excess of oxygen and the free-living forms. This question and the status of these parasitic forms remains unanswered.

Syngamus trachea is a member of the large superfamily of nematodes, Strongyloidea. Some of the representatives of this superfamily are of vast medical and veterinary importance. Most of these Strongyloidea, as do most parasitic nematodes, inhabit the lumen of the intestinal tract. Here they attach themselves by strong, pronounced buccal capsules and suck blood while the teeth or cutting plates in the buccal capsules tear off bits of mucosa. At the same time, the parasite is subject to a wide variety of intestinal contents. For such parasites it is difficult to ascertain the nutritional or environmental relationships in the presence of such a heterogeneous assortment of nutrients, wastes, and metabolic products of assorted biota, as well as enzymes and secretions of the

intestinal tract. Syngamus trachea possesses similar morphological and physiological attributes as other Strongyloidea but inhabits a quite simple, constant, and easily defined environment. Its nutrition in the trachea of the bird is limited to constituents of the blood, lymph, and tracheal mucosa (Clapham, 1935, 1939; Rogers, 1940; and Wehr, 1940). With data obtained from an investigation of Syngamus one can feel more assured that it represents characteristics of the parasite. Such information might then be compared with data obtained on species from highly variable environments. The data obtained and techniques developed might be applicable to particular members of the Strongyloidea which are of medical and economic importance.

The aim of this investigation was to analyze parasitism as it is represented in the life cycle of Syngamus trachea, and concurrently to determine biochemical characteristics of this species, placing special emphasis on respiration and the role of carbohydrate metabolism.

HISTORICAL REVIEW

I. Biological

The earliest published account of *Syngamus trachea* was by Dr. Andrew Wiesenthal (1797), a physician from Baltimore, Maryland. The English naturalist, George Montagu (1811) first described the parasite on a scientific level. He named it *Fasciola trachea*. von Siebold (1836) gave a better descriptive account of the parasite and also changed the scientific name to *Syngamus trachealis*. Chapin (1925) pointed out that the species name was incorrect according to the International Code of Zoological Nomenclature and therefore he changed the species name back to the original, resulting in the name it is known by today. Other early studies on the parasite were by Dujardin (1845) and Cobbold (1861).

The first positive contribution on the life cycle of *Syngamus trachea* was by Ehlers (1872). He concluded that transmission to a new host occurred by ingestion of eggs containing mature larvae. Mégnin (1880, 1881a, 1881b, 1882) and Railliet (1901) arrived at the same conclusion unaware of Ehlers' earlier work. The findings of these early workers were confirmed by later investigators (Ortlepp, 1923; Leiper, 1926; Lerche, 1928; Szidat, 1928; Taylor, 1928; Rice, 1929; Morgan and Clapham, 1934; Wehr, 1937, 1939; and Olivier, 1944). It was observed by Mégnin (1880) and Theobald (1899-1900) that

the eggs of Syngamus, if kept moist, remained viable for about one year. Mégnin (1880), Ortlepp (1923), and Lerche (1928) suggested that development in the egg to an infective larva occurs within one week. Walker (1886) reported that it took much longer.

Years later Wehr (1937) redescribed the larval stages of Syngamus. He found that the larvae moult twice and are infective as third stage larvae.

In addition to direct transmission by ingestion of Syngamus eggs containing infective larvae, a Franklinville, New York, physician, Dr. H. D. Walker (1886), demonstrated that the earthworm can become infected with a larval form of Syngamus and thus serve as a vehicle for transmission to the bird. The importance of Walker's findings were subverted by Salmon (1886, 1899) who cast doubt on certain aspects of his work. However, the role played by the earthworm as a transport host was confirmed by other investigators (Garman, 1897; Ranson, 1916; Waite, 1920; Clapham, 1934; Morgan and Clapham, 1934; Taylor, 1935; Ryzhikov, 1941). These investigators not only established the role of the earthworm in transmission, but they also incriminated other invertebrates such as slugs, snails, and insects. Taylor (1935, 1938) found that the larvae may remain viable in the earthworm up to four and one-half years. Madsen (1952) believes there is much evidence in favor of an invertebrate host acting as an intermediary in most natural infections. The infection is more difficult to establish without an intermediate host suggesting that the

larvae are subjected to some physiological stress which seems to be eliminated when an intermediate host is used (Clapham, 1938).

Once larvae are ingested by the bird their fate from intestine to lungs is unknown. Ortlepp (1923), Wehr (1937), Clapham (1939), and Guilford and Herrick (1954) postulate that the route of migration from the digestive tract to the lungs is through the blood stream. They base their conclusions on finding third stage infective larvae approximately twenty-four hours after ingestion in various portions of the body such as the lungs, liver, air sac, and postcaval vein. Although their observations indicate that the blood stream is a likely route of migration this postulate has not been satisfactorily answered (Biester and Devries, 1944; Morgan and Hawkins, 1953).

Wehr (1937) states that the fourth stage larvae attaches to the female fourth stage larvae in the lungs sometime between the third and seventh day after infection and then migrate as attached pairs to the trachea. Guilford and Herrick (1954) agree with Wehr that the paired gapeworms reach the trachea on the ninth day after infection, but Walker (1886) believed that only six to seven days were required. A wide range of time has been given for the prepatent period of Syngamus. Fourteen to 25 days have been cited (Walker, 1886; Ortlepp, 1923; Lerche, 1928; Wetzel and Quittek, 1940). Syngamus has been reported as living in a chicken for as long as 147 days and 224 days in a turkey (Wehr, 1939).

There appears to be a distinct difference in susceptibility of chickens and turkeys to infection with Syngamus trachea. Olivier (1944) in summarizing his investigation and the observations of others on species susceptibility suggested that turkeys are more susceptible than chickens. He also concluded that young turkeys are more susceptible than young chickens of the same age. Wehr (1939) was in agreement with these observations and in addition thought that chickens lost their infections more readily and became refractive to infection at an earlier age. Madsen (1952) stated that this difference in susceptibility "in this respect seems to have been somewhat overstressed", inasmuch as he and others, Theobald (1899-1900), Klee (1903), Waite (1920), Ranson (1921), Morgan (1931), Crawford (1940), and Olivier (1943) have encountered gapeworms in adult chickens. It was suggested by Ranson (1921) that age, stress, and debilitation might be the reasons for finding this infection in chickens. Clapham (1933, 1934) was able to demonstrate that Vitamin A and general mineral deficiencies produced sufficient physiological changes in ten week-old chickens that they could be infected.

The investigations of Olivier (1943, 1944) using turkeys and chickens, and Guilford and Herrick (1954) using pheasants, showed that an acquired immunity develops in birds infected with Syngamus trachea. Immunity was demonstrated by challenge doses of infective larvae.

Madsen (1952) concluded that a number of "natural hosts for gapeworm exists among the passerine birds and among

gallinaceous birds, the partridge and turkey". He discusses and summarizes the distribution and epidemiology of Syngamus trachea infections throughout the world. In conclusion he states, "the epidemiology of the gapeworm is thus very complicated and much work remains to be done".

II. Biochemical

There have been no biochemical investigations of the gapeworm, Syngamus trachea. Inroads have been made into some few biochemical aspects of other nematodes.

There is surprisingly little information on an essential basic biochemical characteristic, the dry weight of nematodes. Too frequently this information, which is essential for the determination of respiratory values, does not appear in publications. Values for less than a dozen nematodes are available for consideration. Information on dry weight has been largely confined to the two large nematodes, Ascaris lumbricoides (Weinland, 1901; Flury, 1912; Gurtner, 1948) and Parascaris equorum (Schimmelpfennig, 1903; Flury, 1912). Only a few investigators have included such values for the smaller nematodes (von Brand, 1938, 1952; Bueding, 1949; Lazarus, 1950).

A second basic consideration of parasitic nematodes is that of glycogen content. It is the most frequent carbohydrate determination made on nematodes. Unquestionably glycogen is the polysaccharide stored in the parasitic nematodes. Baldwin and King (1942) found the glycogen present in Ascaris

lumbricoides to be approximately the same as that in mammals. The distribution and amount of glycogen found in several species of nematodes are summarized by von Brand (1952). Most of the glycogen present in nematodes in localized in the subcuticle, muscles, ovaries, and eggs.

One must admit the existence of simple sugars in parasites because it is in these forms that assimulation and utilization occurs. Reducing sugars have been identified in Ascaris by Rogers (1945), in Litomosoides carinii by Bueding (1949), and in Parascaris by Faurê-Fremiet (1913).

The investigations on various aspects of carbohydrate metabolism and aerobic and anaerobic gas exchange have been conducted on less than two dozen nematodes. Some of the larval forms of nematodes which have been studied with regard to metabolic processes associated with carbohydrate utilization and respiration are: Eustrongylides ignotus (von Brand, 1942, 1945; von Brand and Simpson, 1944), Neoaplectana glaseri (Rogers, 1948; Massey and Rogers, 1949, 1950), Nippostrongylus muris (Rogers, 1948), Strongyloides papillosus (Costello, 1957), Trichinella spiralis (Stannard, McCoy, and Latchford, 1938; Goldberg, 1956). Some phases of intermediate carbohydrate metabolism of Strongyloides ratti have been studied by Jones et al., (1955a, 1955b).

The carbohydrate metabolism and utilization, and aerobic and anaerobic respiration of adult nematodes have been investigated in the following species: Ascaridia galli, Nematodirus spp., Neoaplectana glaseri, Nippostrongylus

muris (Rogers, 1948; Massey and Rogers, 1949, 1950); Ascaris lumbricoides (Adam, 1932; von Brand, 1934; Krüger, 1936; Laser, 1944); Dracunculus insignis (Beuding and Oliver-Gonzáles, 1950); Haemonchus contortus (Rogers, 1948); Heterakis spumosa, Ostertagia circumcincta, Strongylus equinus, Strongylus vulgaris, Syphacia obvelata (Lazarus, 1950); Litomosoides carinii (Bueding, 1949); and Nematodirus spp. (Massey and Rogers, 1949, 1950). A few other nematodes which are not included above have been investigated for various specific biochemical characteristics. Of all the nematodes, Ascaris lumbricoides has received the major attention from numerous investigators.

From knowledge that is available on carbohydrate metabolism of a few nematodes, one might assume that the well known processes of the Meyerhof-Embden system and Krebs cycle sequences take place in parasitic nematodes as it does in mammalian tissues. von Brand (1950) suggests that the problems which arise concerning the metabolism of carbohydrates in nematodes are linked with the character of respiration for individual species.

Hemoglobin in nematodes was first demonstrated and reported by Keilin (1925). He recovered it from the muscle and perivisceral fluid of Ascaris lumbricoides. At that time he raised the question of its physiologic significance. The functional nature of such a respiratory accessory still has not been settled (Davey, 1938; Wharton, 1941; Davenport, 1949; Rogers, 1949a).

Rogers (1940) in a study on the hematological nature of the material present in the intestine of nematodes and trematodes used Syngamus trachea as one of the representatives of nematodes which ingest hemoglobin. He found hematin present in the black-pigmented ingesta. However, he was unable to extract a hemoglobin. In his discussion on the nature of the ingesta of the species studied he states that the body fluid of S. trachea contains hemoglobin. There were no data or references cited for such a fact, and von Brand (1952) did not include S. trachea in the list of nematodes for which hemoglobin has been identified.

It is apparent from the foregoing review that further investigation is necessary in order to explain certain aspects of the life cycle of Syngamus trachea. This, coupled with a biochemical investigation, will not only elucidate the nature of Syngamus but may also be applicable to other parasitic nematodes.

MATERIALS AND METHODS

I. Biological

A. Studies on the life cycle.

The nematode was originally obtained in the larval form encysted in a collection of heterogeneous earthworms. These earthworms were secured from the pheasant rearing enclosures on the Michigan Department of Conservation Game Farm, Mason, Michigan.

Approximately a dozen earthworms were fed to each of seven 21 day old White Leghorn chickens. The birds were sacrificed 17 days later. The tracheae were removed and examined for the paired, adult Syngamus. Only one pair was recovered from the seven chickens and it was identified as Syngamus trachea. The female worm was mature and passing typical eggs. The uteri were removed from the worm and ground in a small amount of tap water using a pestle and mortar. This suspension of eggs and debris was filtered through several layers of cheesecloth and placed in a petri dish. Enough water was added to the suspension to obtain a depth of about one cm. The petri dish was left uncovered at room temperature for twelve days. Evaporated water was replaced by adding tap water daily, and the suspension was agitated slightly by gentle rotation of the dish. On the twelfth day larvae and the associated debris were loosened

from the bottom of the dish by means of a rubber-tipped rod. The embryonated eggs and freed larvae were washed several times in tap water. Much of the debris was removed by this step. The embryonated eggs and larvae, suspended in a small amount of tap water, were added to a petri dish filled with soil which contained ten medium size (four to five cm) earthworms, Allolobrophora caliginosus. Fourteen days later these earthworms were fed to a ten day old Leghorn chicken. The bird was sacrificed 17 days later. The trachea was removed and examined for the presence of Syngamus. The specimens recovered were identified as S. trachea. These gapeworms served as parent stock for the investigation.

The initial procedure used for obtaining infective larvae was modified slightly. The changes consisted of increasing the incubation time of the eggs to 14 days and the exposure time of the earthworms to larvae to 21 days. This schedule was maintained throughout the investigation when the earthworm was used.

B. Infection of chickens and turkeys.

A number of routes of inoculation were used. The oral route of inoculation was studied in infections established by passage of laboratory prepared suspensions of larvae directly into the crop by means of a plastic tube, and the forced feeding of earthworms which contained the infective larvae. Food was withheld from the birds six to eight hours prior to per os inoculation. Suspensions of larvae were also

administered intraperitoneally, intravenously, and subcutaneously. The medial wing vein (venae profunda humeri) was used for intravenous inoculation. Usually the number of larvae given were determined by counting aliquants of the suspensions containing larvae. Suitable dilutions were then made in order to place the inoculum in a volume of one ml.

In order to determine the time required for the appearance of gapeworms in the trachea and the prepatent period of the worm, turkeys were inoculated intravenously with larvae and orally with earthworms. Beginning on the sixth day following inoculation two birds were sacrificed each day for 19 days and observations were made immediately following death.

C. Acquisition, care, and management of turkeys and chickens.

The chickens and turkeys used in this investigation were obtained as day old birds. The Poultry Husbandry Department of Michigan State University supplied the White Leghorn chickens and some Broad-breasted Bronze turkeys. Broad-breasted Bronze turkeys were also obtained from Janssen Farm's Hatcheries, Zeeland, Michigan. A majority of the birds were hens although no attempt was made to select one sex or the other. New Hampshire cockerels were obtained from Delamarter's Hatchery, East Lansing, Michigan.

All the birds were kept in electrically heated brooders at suitable temperatures until they were four weeks old. After this they were housed either in standard wire bottom poultry cages or in isolation rooms in which wood shavings

were used as litter. Infected birds were kept separate from uninfected birds.

The feed used throughout these studies contained no antibiotics or other medication. The chick starter mash and turkey starter crumbles used were manufactured by A. E. Staley Manufacturing Co., Decature, Illinois and Valley City Milling Co., Portland, Michigan. Feed and water were supplied ad libitum.

D. Care and maintenance of earthworms.

The colony of earthworms, Allolobrophora caliginosus, used as transport hosts for the larvae of Syngamus trachea was established from a single identified specimen obtained in the vicinity of East Lansing, Michigan. This specimen was placed in soil which had been autoclaved for 45 minutes at 121° C. To this soil scraps of organic material (vegetables, fruit, oatmeal, coffee grounds, etc.) were added. Moisture as well as additional organic materials were added to this worm bed as the need demanded.

II. Biochemical

A. Recovery of Syngamus trachea.

The biochemical investigations were conducted on Syngamus trachea recovered from infected turkeys. Turkeys were infected intravenously with the third stage infective larvae, thereby insuring knowledge of the exact age of the gapeworms. Where at all possible every pair of gapeworms used for any one biochemical determination was obtained from the same turkey. This minimized possible variation which might occur in gapeworms obtained from different birds.

In the trachea the male gapeworm is found permanently attached to the female. In separating the pair one member is usually destroyed; therefore, because of this inseparable nature, all investigations were conducted on paired Syngamus.

The turkeys were killed by cutting the jugular veins and piercing the brain. The trachea was removed immediately. The entire length of the trachea was opened and the paired Syngamus removed with a moistened camel's hair brush. The anterior end of the male gapeworm usually was embedded in the mucosa, and was loosened with a fine probe. The gapeworms were placed in cold (3° C) 0.9% saline and left there until all were removed from the trachea. They were then washed in six to eight changes of saline (3° C) followed by immersion for 15 minutes in saline warmed to 37° C. Antibiotics were added to the warmed saline to obtain a final concentration of 400 units penicillin and 0.4 mg dihydrostreptomycin per ml. After five minutes in the solution

containing antibiotics the gapeworms were wahed in four changes of saline (3° C). The entire procedure required 30 to 45 minutes. The gapeworms were then held in saline at 3° C until used.

B. Determination of differences in body substance of Syngamus trachea.

To determine the wet (live) weight of Syngamus the gapeworms were removed from the cold saline solution with a camel's hair brush and placed on Whatman No. 1 filter paper to remove excess moisture. They were then transferred to tared containers and weighed to the fourth decimal place on an analytical balance. Dry weights were determined by drying to constant weight in a hot air oven (100° C) for 10 to 12 hours.

Total carbohydrate and polysaccharide determinations were performed on pairs of gapeworms. The procedure used was similar to that of Bueding (1949). Polysaccharides were determined according to the methods of Good, Kramer, and Somogyi (1933) and Somogyi (1945). Total carbohydrates were determined after deproteination (Nelson, 1944) using the spectrophotometric method of Somogyi (1945). Values obtained by the Somogyi method are expressed in mg percent glucose.

C. Identification and characterization of a red pigment in the pseudocoelomic fluid of Syngamus trachea.

To obtain the red fluid present in the pseudocoelom of Syngamus ten large (approximately 3 mg wet weight each)

female gapeworms were removed from the final cold saline rinse with a camel's hair brush and blotted dry on Whatman No. 1 filter paper. They were then transferred to the inside rim of a 12 ml conical, graduated centrifuge tube. The tube contained ten ml of dilute ammonium hydroxide (eight ml concentrated NH_4OH in one liter distilled water) or 0.1 N hydrochloric acid for collection of the pseudocoelomic fluid. The female gapeworms were oriented on the wall of the tube in such a way that the posterior ends came within five mm of the fluid while the anterior ends were quite close to the rim of the tube. With a fine probe the tips of the posterior ends of the gapeworms were pierced. A cotton plug was inserted into the tube in such a way as to secure the gapeworms. The tube was centrifuged at 750 rpm for ten minutes in a size 2 International Centrifuge.

A second method for the collection of the red pseudocoelomic fluid consisted of placing the pierced gapeworms directly into a conical centrifuge tube containing distilled water or 0.9% saline and centrifuging at 1600 rpm for ten minutes. The fluid containing the red pigment was then removed with a pipette. This method proved to be as reliable as the first for the collection of pseudocoelomic fluid.

The identification and characterization of the red pigment in pseudocoelomic fluid was by means of absorption curves obtained with a Bausch and Lomb Spectronic 20 colorimeter. Absorption curves were determined on pseudocoelomic fluid of Syngamus and on similarly treated samples of turkey

blood. Treatments and procedures used on the paired samples were discussed or suggested by Hawk, Oser, and Summerson (1947) and Hunter (1951).

The absorption spectrum of oxyhemoglobin was demonstrated on samples taken in dilute ammonium hydroxide. The tube containing the sample was shaken vigorously to aerate the solution and absorption curves were then determined.

Reduced hemoglobin absorption curves were secured under four different conditions. (1) Two to three drops of 10% sodium hydrosulfite were added to a dilute NH_4OH solution containing the pigmented pseudocoelomic fluid and gently heated for 20 minutes to a temperature of not more than 55° C. Absorption curves were determined at pH 7 and pH 8. The pH of all solutions was determined with a Beckman pH meter and adjusted through the use of acetic acid. (2) The second condition was made by the addition of two to three drops of 10% sodium hydrosulfite to the samples, followed by evacuation and heating (not higher than 55° C) for 20 minutes. The colorimeter tube was sealed with a rubber stopper and a vacuum was drawn and maintained during heating. After an absorption curve was determined the tube was cooled, unstoppered, shaken vigorously, and a second absorption curve was determined on the aerated sample. (3) A third sample was placed in a colorimeter tube and sealed with a rubber stopper. The tube was evacuated and a vacuum was maintained while it was gently heated (not higher than 55° C) for one hour. Absorption curves were obtained on this sample while

warm; and again after it was cooled to room temperature, unstoppered, and shaken to permit aeration. (4) A final condition was established by heating the sample (not higher than 55° C) and passing a constant flow of nitrogen (95% N_2 - 5% CO_2) into the solution. The excess gas was removed by maintaining a negative pressure above the sample. After one hour of such treatment an absorption curve was determined on the sample while warm, and another absorption curve was obtained after cooling, unstoppering, and shaking the sample.

The methemoglobin absorption spectrum was determined on samples collected in ammonium hydroxide solution. Two to three drops of saturated potassium ferricyanide were added, and the solution was adjusted to the desired hydrogen ion concentration. Absorption curves were obtained at pH 6, 7, and 8.

Acid hematin was determined by using dilute hydrochloric acid according to the method of Cohen and Smith (1919). Acid hematoporphyrin resulted when two to three drops of concentrated sulfuric acid were added to a sample collected in dilute ammonium hydroxide (Hunter, 1951).

Carboxyhemoglobin was demonstrated by gassing with carbon monoxide (95% CO - 5% O_2) for 30 minutes. Two methods were used. Carbon monoxide was bubbled through a dilute ammonium hydroxide solution containing pseudocoelomic fluid and then absorption curves were determined. In the second method carbon monoxide was bubbled through a saline solution containing whole gapeworms. The pseudocoelomic fluid was

then released into the saline by piercing the worms and centrifugation. Absorption curves were determined on this solution.

D. Quantitative investigation of endogenous and exogenous carbohydrate utilization by Syngamus trachea.

The endogenous rate of carbohydrate utilization was established by determining the decrease in total carbohydrates and polysaccharide content which resulted when Syngamus was maintained in vitro for 24 hours at 37° C.

Gapeworms were divided into three groups. The first group served as the controls, and total carbohydrates and polysaccharides were determined immediately with this group using methods described above. A second group was placed in the substrate used for respiration studies. The composition of this substrate is described later. One hundred units penicillin and 0.1 mg dihydrostreptomycin were added to each ml of the substrate. The substrate of the third group contained antibiotics as in the second group plus 0.005 M glucose.

Groups II and III were placed in 25 ml of the proper substrate in a standard petri dish and incubated at 37° C for 24 hours. Following incubation the gapeworms were washed six times in saline as a preparatory step for the determination of total carbohydrates and polysaccharides. These determinations were made following the same procedure used for the control group.

The exogenous rate of carbohydrate utilization was obtained by determining the rate of glucose uptake from a

known substrate. The amount of glucose was measured spectrophotometrically by the method of Somogyi (1945). The rate of glucose uptake was investigated under two conditions. Paired gapeworms were maintained in 20 x 150 mm screw-cap culture tubes in 2 ml of the respiration substrate (described later) containing 0.005 M glucose. Antibiotics were added to the substrate to obtain 100 units penicillin, 0.1 mg dihydrostreptomycin, and 100 units nystatin per ml. The tubes were placed in a horizontal position in a 37° C incubator. The substrate was changed daily, and the amount of glucose in the substrate that was removed was determined. The gapeworms were maintained under these conditions for ten days.

Sterility tests were performed on the substrates used for both endogenous and exogenous carbohydrate studies. Brain-heart infusion broth and N.I.H. thioglycolate were used for sterility tests.

The second condition under which the exogenous carbohydrate utilization by Syngamus was examined occurred while the respiration of Syngamus trachea (in toto) was studied with the Warburg apparatus. Glucose concentrations of the substrate from reaction vessels containing parasites were compared with the glucose concentrations of the substrate in the thermobarometric flasks at the end of the determination. The differences between glucose concentrations represented glucose uptake by the parasite.

E. Characterization of metabolism of Syngamus trachea.

The nature of the metabolism of Syngamus trachea was determined by investigation of (1) aerobic respiration, (2) anaerobic respiration, (3) the relationship of carbohydrates to respiration, and (4) the effects of various metabolic inhibitors.

The studies on respiration were conducted in a Precision Warburg 20-unit respirometer at a shaking rate of 120 per minute and a temperature of 37° C. Standard manometric procedures were followed (Umbreit et al., 1949). Oxygen uptake was determined by the direct method. A continuous estimate of carbon dioxide was obtained by subtracting oxygen uptake from the value obtained in a paired vessel without potassium hydroxide. Thus the quantity of carbon dioxide liberated could be followed over a long period of time and comparative relationships noted. Anaerobic respiration values and estimates of carbon dioxide used in respiratory quotients were obtained by making a correction for retension of CO_2 in the buffered substrate. Using paired vessels, 0.2 ml 1 N sulfuric acid was emptied from the sidearm into one reaction vessel at the beginning and into the second vessel at the end of the determination. A total volume of three ml substrate was used in each reaction vessel. These vessels had an approximate capacity of 15 ml. The common atmosphere for manometric work on Syngamus was air. Gas atmospheres of nitrogen (95% N_2 - 5% CO_2) and carbon monoxide (95% CO - 5% O_2) were secured through gassing by means of a 10-phase gassing manifold

while the vessels where in the bath. Twenty minutes of continuous nitrogen flow and 40 to 60 minutes of carbon monoxide flow were the gassing periods.

The substrate used throughout the investigation was selected following preliminary trial and error. It consisted of the basic components and proportions used for metabolism studies of schistosomes by Bueding (1950). The only modification was a lowering of the phosphate concentration. As used in this investigation it consisted of: 0.137 M sodium chloride, 0.0085 M potassium chloride, 0.005 M magnesium chloride, 0.0003 M calcium chloride, and 0.03 M sodium phosphate. A pH of 7.54 for the solution was obtained through the use of appropriate amounts of monobasic and dibasic sodium phosphate and unless noted otherwise contained 0.005 M glucose. The pH of 7.54 is that of turkey blood and the amount of glucose used is within the range of glucose concentration in birds (Spector, 1956). Twenty to 60 mg wet weight of gapeworms per flask was found most suitable for manometric investigation. Only paired gapeworms were used. A brei of the paired Syngamus was prepared as outlined in Umbreit et al. (1949). Gas exchange (O_2 consumption and CO_2 liberation) was calculated and expressed in terms of microliters per mg dry weight of parasite per hour, except when brei or an acetone powder (Green et al., 1937) of the parasite were used. These calculations were based on a minimum period of two hours.

The compounds used as inhibitors were introduced from

the sidearm of the reaction vessel, or were placed directly into the substrate. Comparisons were made between paired flasks with and without the inhibitors. The method of Robbie (1946) was used to control cyanide concentrations. In the investigation of malonate and fluoride as inhibitors calcium was omitted from the substrate because Massey and Rogers (1950) found greater inhibition when a calcium free substrate was used. Studies using carbon monoxide were conducted both in artificial light and in the dark. Darkness was obtained by wrapping the reaction vessels in aluminum foil. Other inhibitors used were azide, iodoacetate, and dinitrophenol.

To remove endogenous nutrients an acetone powder of Syngamus was prepared according to the method of Green et al. (1937). The activity of the incompletely defined enzyme system which occurs in the acetone powder on a 0.005 M glucose substrate was studied by standard manometric methods.

Butyric acid was substituted for glucose in the substrate to demonstrate utilization of fatty acids. The concentration of butyric acid used necessitated neutralization of the substrate with sodium hydroxide.

The chemicals used in this investigation were of U.S.P. or A.C.S. quality. Specific organic reagents were products of Eastman Organic Chemical Division of Eastman Kodak Co., Rochester, New York. Antibiotics were purchased from E. R. Squibb and Sons, New York, N. Y.

RESULTS

I. Biological

A comparison of the routes of inoculation of chickens revealed that infection could be established through intravenous inoculation of infective larvae and forced feeding of earthworms containing encysted infective larvae (Table 1). Per os and subcutaneous inoculation of infective larvae produced no Syngamus infection. It appeared to be more difficult to infect chickens after ten to fourteen days of age. Chickens older than three weeks of age could not be infected by inoculation of earthworms containing larvae and chickens more than 30 days of age could not be infected by intravenous inoculation of larvae. Earthworms infected under natural conditions, in the pheasant rearing areas of the Mason Game Farm, were no better source of inoculum for older chickens.

There was great variation in the degree of Syngamus infections in chickens inoculated with the same number of earthworms. This can be noted in Table 1. Within a group or between groups of earthworm which were similarly infected and maintained, the number of pairs of Syngamus recovered per chicken fluctuated widely. A more uniform degree of infection of chickens was obtained by intravenous inoculation of infective larvae.

The earliest appearance of Syngamus in chickens was in a single bird which died eight and one-half days after

TABLE 1

COMPARISON OF THE DEGREE OF INFECTION OF CHICKENS WITH *SYNGAMUS TRACHEA* OBTAINED BY DIFFERENT ROUTES OF INOCULATION

Route	Inoculum	CHICKENS Age in Days	CHICKENS Number Inoculated	Duration of Infection in Days	*Syngamus trachea* Pairs per Chicken	CHICKENS No. Infected/ No. Inoculated
ORAL						
1	earthworm	1	9	8½ to 12	14,16,26,27,35,41, 42,48	9/9
1	"	1	2	8	0	0/2
1	"	1	3	30	4,4,7	3/3
6	"	10	1	17	3	1/1
10	"	10	1	17	19	1/1
1	"	17	32	12	1,1,1,1,1,2,2,2,2, 2,4,5,7	13/32
10	"	18	1	16	2	1/1
	Earthworms*	21	7	17	1	1/7
8	earthworms	30	2	14	0	0/2
	Earthworms*	42	9	14	0	0/9
2	earthworms	42	3	12	0	0/3
1	ml larvae**	1	2	12	0	0/2
SUBCUTANEOUS						
1	ml larvae	17	6	12	0	0/6

(continued on next page)

TABLE 1

(continued)

Route	Inoculum	CHICKENS Age in Days	CHICKENS Number Inoculated	Duration of Infection in Days	*Syngamus trachea* Pairs per Chicken	CHICKENS No. Infected/ No. Inoculated
INTRAVENOUS						
1.0 ml	larvae	10	2	12	20,29	2/2
1.0 ml	"	14	3	12	22,20,29	3/3
1.0 ml	"	30	6	12	1	1/6
1.0 ml	"	40	3	12	0	0/3
1.0 ml	"	48	2	12	0	0/2
0.5 ml	"	72	1	19	0	0/1
1.0 ml	"	150	1	12	0	0/1
1.0 ml	"	200	3	12	0	0/3

* Earthworms obtained from State Game Farm at Mason, Michigan.

** Suspension of unknown number of infective *Syngamus* larvae.

inoculation with one earthworm. Paired gapeworms were recovered from the trachea of this chicken just above the bifurcation. In turkeys inoculated intravenously with infective larvae, gapeworms were recovered on the eighth day after inoculation; but when earthworms were used, gapeworms were recovered on the ninth day.

Table 2 presents the results obtained from various routes of inoculation of Syngamus trachea in turkeys. Turkeys were infected with suspensions of larvae given intravenously or per os and by forced feeding of infective earthworms. There was no consistent intensity of infection when the earthworm was used as a transport host for the infective larvae. Suspensions given intravenously resulted in a greater degree and intensity of infection than an equal suspension given orally. There appeared to be no difficulty infecting turkeys as old as 186 days.

Intraperitoneal and subcutaneous inoculation of turkeys with suspensions of larvae produced no Syngamus infections. Likewise, larvae held 48 hours at 37° C or obtained from eggs of gapeworms maintained in vitro at 37° C were not infective when inoculated intravenously into turkeys.

The prepatent period of Syngamus trachea in turkeys was 14½ days when the infection was established by intravenous inoculation of larvae. When infections were established by feeding earthworms it was 15 days before viable eggs were recovered.

Gapeworms were recovered from turkeys as long as 82 days

TABLE 2

COMPARISON OF THE DEGREE OF INFECTION OF TURKEYS WITH *SYNGAMUS TRACHEA* OBTAINED BY DIFFERENT ROUTES OF INOCULATION

Route	Inoculum	TURKEYS Age in Days	TURKEYS Number Inoculated	Duration of Infection in Days	*Syngamus trachea* Pairs per Turkey	TURKEYS No. Infected/ No. Inoculated
ORAL						
	2 earthworms	2	11	12	3,3,3,7,7,8,12,12, 14,18,19	11/11
	2 "	3	1	24	3	1/1
	5 "	3	5	24	1,2,3,4	4/5
	4 "	9	10	14	1,7,8,8,11,11,12,17, 26,39	10/10
	1 "	17	80	15	1,1,2,2,4	5/80
	8 "	18	1	12	74	1/1
	3 "	25	20	17	1,1,1,1,1,2,2	7/20
	1 "	28	10	30	1,2,2,3,4,5,6,8,10	9/10
	5 "	30	8	12	1,1,1,2,2,2	6/8
	20 "	107	1	19	13	1/1
	25 "	107	1	37	17	1/1
ORAL						
	2.0 ml larvae*	3	1	24	0	0/1
	0.5 ml "	72	1	19	3	1/1
	1.0 ml "	72	1	19	1	1/1
INTRAPERITONEAL						
	1.0 ml larvae	2	3	24	0	0/3

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TABLE 2

(continued)

Route	Inoculum	TURKEYS Age in Days	TURKEYS Number Inoculated	Duration of Infection in Days	*Syngamus trachea* Pairs per Turkey	TURKEYS No. Infected/ No. Inoculated
SUBCUTANEOUS						
	1.0 ml larvae	3	2	14	0	0/2
INTRAVENOUS						
	0.2 ml larvae	3	1	24	5	1/1
	0.3 ml "	3	1	24	13	1/1
	0.25 ml "	7	1	11	20	1/1
	0.5 ml "	17	2	12	11	1/2
	1.0 ml "	30	2	7	0	0/2
	1.0 ml "	30	2	8	2,5	2/2
	1.0 ml "	30	6	12	17,18,19	3/6
	1.0 ml[a] "	30	3	12	0	0/3
	1.0 ml[b] "	30	3	12	0	0/3
	0.5 ml "	72	1	19	28	1/1

* Suspensions of unknown number of infective *Syngamus* larvae.

[a] Infective larvae held in 37° C incubator for 48 hours.

[b] Eggs from *S. trachea* maintained in 37° incubator.

after inoculation.

When turkeys were inoculated intravenously with suspensions containing known numbers of larvae the ratio of paired Syngamus recovered to larvae given was obtained. These ratios appear in Table 3 along with the number of larvae given, number of paired Syngamus recovered, and duration of infection. In noting the age of the turkeys infected and number of worms recovered it again appears that turkeys of a wide range of ages can be infected equally well.

The highest ratio of pairs of Syngamus recovered to larvae given intravenously was 0.1875. A total (sum of days 13 and 14) of 1048 paired Syngamus were recovered when 5610 larvae were given; and this, when reduced to smaller numbers represents three Syngamus recovered when eight larvae were given, or three paired gapeworms for 16 larvae. When the duration of infection is considered with the ratio of Syngamus recovered it can be noted that after 24 days there is a consistent and rapid decrease in number of gapeworms. This consistent decrease in gapeworms continued until after 37 days only negligible infections remained.

It is apparent from inspection of the data (Table 3) on the number of larvae given turkeys compared to the number of gapeworms recovered that a direct relationship exists. If the numbers of larvae given intravenously are grouped for infections of 24 days duration or less, the relationship shown in Figure 1 exists for paired Syngamus per larvae given. Such a linear relationship was better demonstrated under more

TABLE 3

COMPARISON OF PAIRED *SYNGAMUS TRACHEA* RECOVERED FROM TURKEYS TO NUMBER OF INFECTIVE LARVAE GIVEN INTRAVENOUSLY

Duration of Infection in Days	Ratio*	*Syngamus trachea* Pairs per Turkey	No. Infective Larvae given Intravenously	TURKEYS Number Inoculated	TURKEYS Age Range in Days
9	.0625	66	1060	1	61
10	.1350	1 -- 132[a]	24 -- 1060[a]	16	46, 50
11	.1500	460	3054	1	68
12	.1205	70, 108	600, 875	2	110, 182
13	.1875	87, 97, 140, 263	600, 875, 875, 780	4	47, 164, 182
14	.1875	72, 98, 116, 175	500, 600, 600, 780	4	47, 106, 164
15	.1505	64, 222	600, 1300	2	116, 186
16	.1500	96, 99	600, 700	2	116, 120
17	.0942	41, 70, 364	600, 1350, 3054	3	74, 77, 110
18	.1170	70	600	1	116
19	.0520	45	875	1	182
20	.0879	74, 106, 139	600, 780, 2250	3	47, 81, 110
23	.1514	48,78,104,158,232	395,425,987,987,1300	5	80,83,85,86
24	.1380	36, 395	1300, 1800	2	74, 186
26	.0407	20,31,48,51,81	296,395,495,2250,2250	5	74,80,81,85
27	.0460	140	3054	1	77
28	.0562	27, 147	1300, 1800	2	79, 186
30	.0590	29	494	1	85
32	.0460	23	494	1	85
34	.0050	1, 3, 7	650, 700, 780	3	47

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TABLE 3

(continued)

Duration of Infection in Days	Ratio*	*Syngamus trachea* Pairs per Turkey	No. Infective Larvae given Intravenously	TURKEYS Number Inoculated	TURKEYS Age Range in Days
35	.0460	32	700	1	147
37	.0090	3, 16	900, 1300	2	58, 186
38	.0000	0	700, 3054	2	68, 147
39	.0020	5	1300, 1820	2	186
44	.0000	0	987	1	83
47	.0000	0	850	1	147
82	.0070	0, 1, 10	3500,3500,8700	3	105

* The ratio is the pairs of *Syngamus trachea* recovered to the number of infective larvae given.

[a] Of the 16 turkeys inoculated 15 were infected and from one to 132 gapeworms were recovered. The inoculum used contained between 24 and 1060 infective larvae.

uniform conditions of experimentation and is presented in Figure 2. The consistency of number of pairs of Syngamus recovered to the number of larvae inoculated intravenously is apparent in Figure 2. Only the two lowest levels of inocula resulted in any pronounced fluctuation from a straight linear relationship.

An extreme increase in the size of Syngamus was noted during the first two to three weeks after its appearance in the trachea of the turkey. This increase in growth during a period from the 11th through the 37th day following intravenous infection is shown in Figure 3. Not only was there a progressive linear increase in average dry weight of paired Syngamus on successive days throughout this period, but there was also an accompanying linear increase in average wet weight.

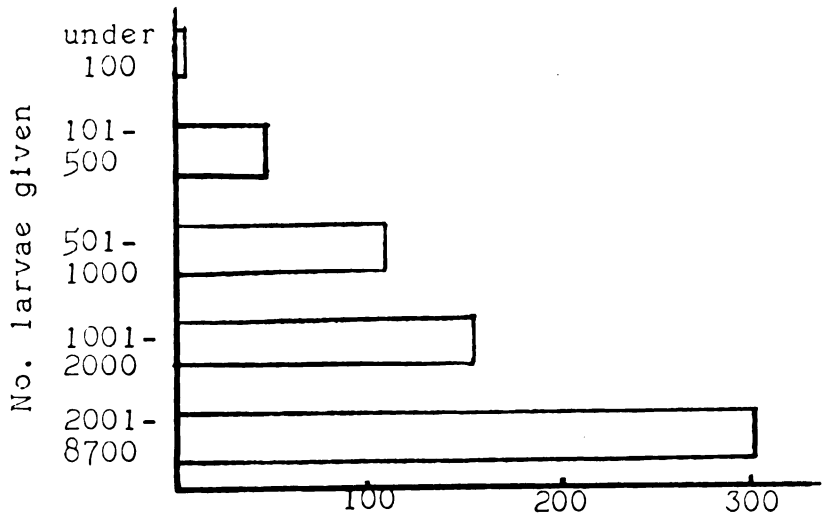


Figure 1. Relationship of pairs of Syngamus trachea recovered from turkeys with infections of less than 25 days duration to the number of larvae used as intravenous inoculum.

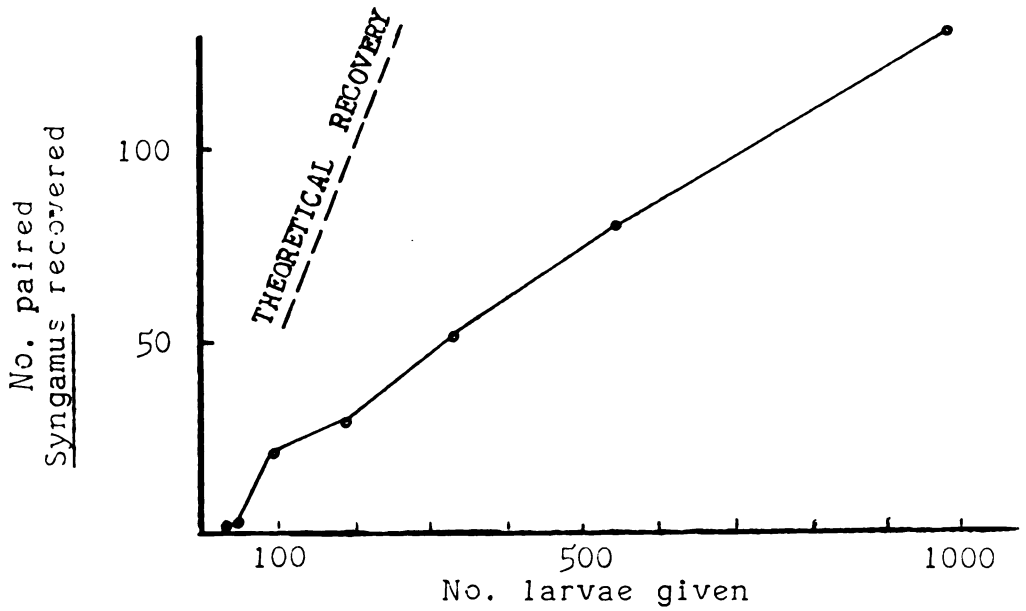


Figure 2. Relationship of number of larvae inoculated intravenously to paired Syngamus trachea recovered from turkeys after ten days*.

*three turkeys, each 46 days of age, used for each level.

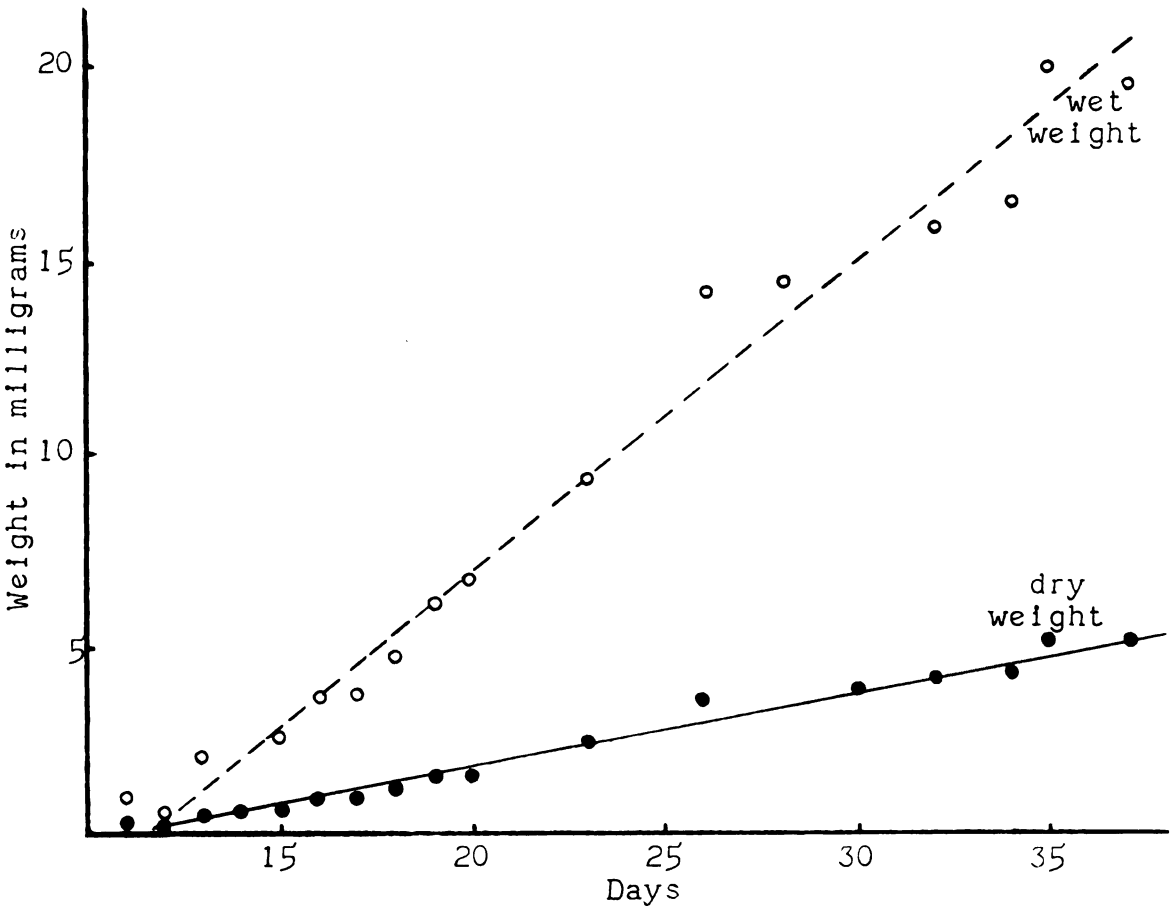


Figure 3. Relationship of average dry weight and wet weight of paired Syngamus trachea to duration of time present in turkey.

II. Biochemical

The relative water content was constant for all sizes of gapeworms used in this investigation. The ratios of dry weight to wet weight for this size range are presented in Table 4. The dried substance of Syngamus appears to be about 26.2% (average ratio 0.262 ± 0.029) of the weight of the living parasite. The majority of the ratios closely approach this mean, but a range of 0.210 to 0.332 was obtained.

The total carbohydrate and polysaccharide contents of paired Syngamus of different sizes and ages were determined. The relationship of percent total carbohydrate and polysaccharide to the wet weight of gapeworms of various ages and sizes appears in Figure 4. The smallest pairs of gapeworms had the greatest total carbohydrate content, 1.68%. The amount of carbohydrate rapidly decreases with an increase in worm size to about 0.9%. The polysaccharide content (0.33% to 0.76%) closely parallels total carbohydrates except in the smaller sized Syngamus where it contributes less markedly to the total carbohydrate content. Therefore, the content of simple sugars in the smaller gapeworms greatly exceeds the relative constant amount which exists in the larger gapeworms. The difference between total carbohydrates and polysaccharides during their parallel decline ranged between 0.4% and 0.65%.

The nature of the red pseudocoelomic fluid of Syngamus was investigated by studies of its absorption spectra under various conditions. Samples of pseudocoelomic fluid were

TABLE 4

RELATIONSHIP OF DRY WEIGHT TO WET WEIGHT OF PAIRED *SYNGAMUS TRACHEA*

Mg Dry Weight *	Mg Wet Weight *	Dry/Wet Weight Ratio
0.188	0.72	0.261
0.237	1.045	0.227
0.239	1.14	0.210
0.253	1.07	0.236
0.273	1.27	0.215
0.437	1.56	0.280
0.446	1.51	0.295
0.775	3.15	0.246
0.778	3.03	0.256
0.827	3.50	0.236
0.947	3.69	0.256
1.190	4.68	0.254
1.470	4.415	0.332
1.520	4.625	0.329
1.730	5.93	0.292
1.770	6.506	0.272
1.830	7.56	0.242
1.830	6.85	0.267
1.980	8.90	0.223
2.040	7.78	0.258
2.070	8.18	0.246
2.350	9.27	0.254
2.370	8.77	0.270
2.400	8.10	0.296
2.420	10.08	0.240
3.290	11.09	0.297
8.540	29.65	0.288

* Weights were averages of a large number of paired *Syngamus* used in other studies.

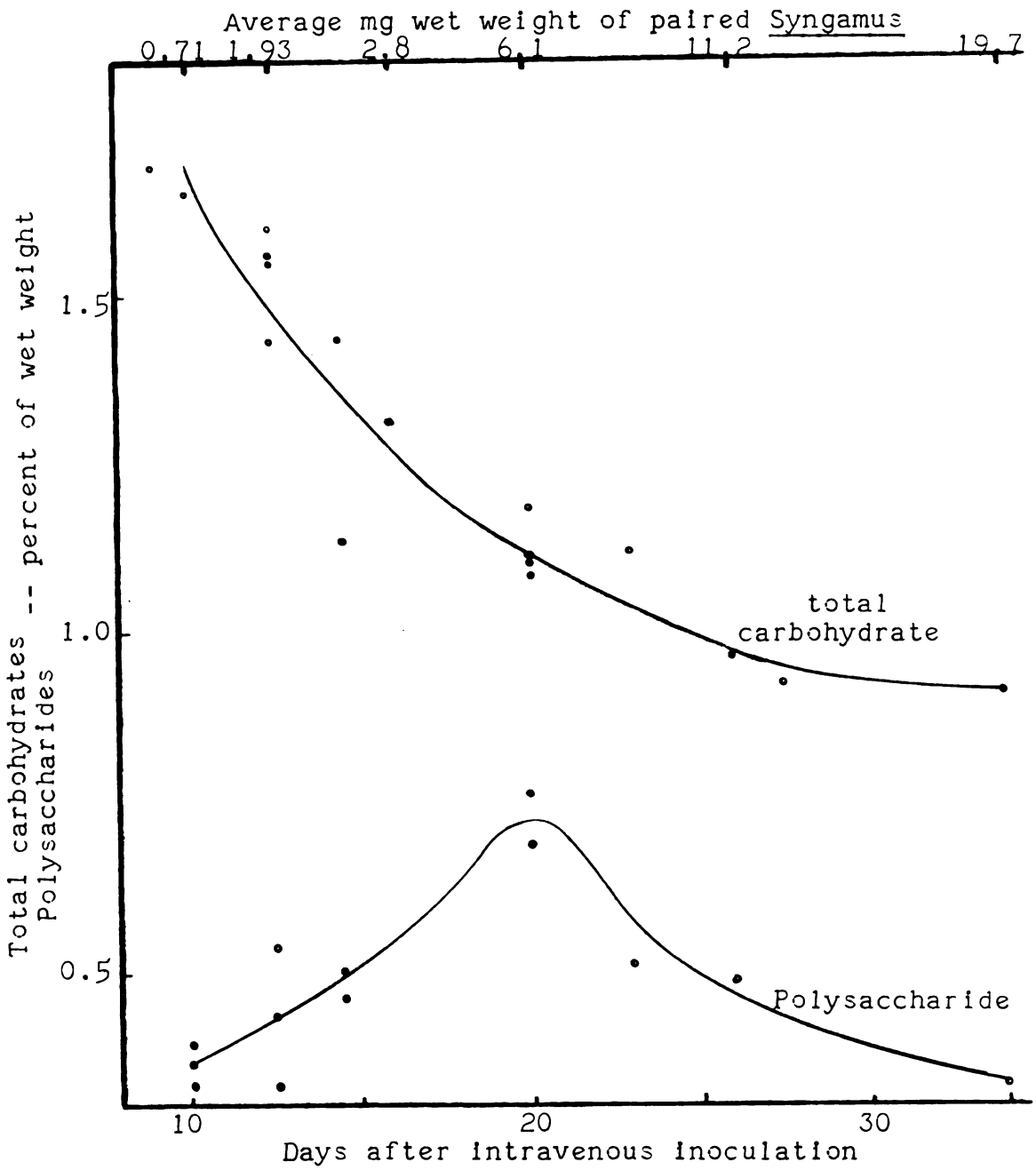


Figure 4. Comparison of percent total carbohydrate and polysaccharide content of paired Syngamus trachea to age and average wet weight

compared with samples of turkey blood used as reference standards.

The absorption curve of Syngamus pseudocoelomic fluid was quite similar to that of oxyhemoglobin of turkey blood as is shown in Figure 5. The α absorption maxima of turkey blood was 575 mμ while for worm pseudocoelomic fluid it was 570 mμ. The β absorption maxima for both samples was 540 mμ.

Similarly treated samples of turkey blood and the pigmented body fluid of Syngamus were deoxygenated by physical and chemical methods. Reduced hemoglobin absorption curves were obtained for each sample. When the samples were deoxygenated by heat, evacuation, and maintained in a vacuum following heating there was a difference of five mμ between Syngamus and the turkey hemoglobin maxima (Figure 6A). Syngamus pseudocoelomic fluid, when deoxygenated by gassing with nitrogen and heated in a vacuum resulted in a broad absorption maximum with a peak at 535 mμ (Figure 7A). When samples were deoxygenated by sodium hydrosulfite and heat in a vacuum similar flat absorption patterns as presented in Figure 8A resulted. In each case when the deoxygenated sample was cooled and aerated, a typical oxyhemoglobin absorption curve with α and β maxima developed (Figures 6B, 7B, and 8B).

Samples of oxyhemoglobin from turkeys treated with sodium hydrosulfite and heated, through not maintained in a vacuum during light absorption studies, resulted in absorption curves of oxyhemoglobin at pH 7 and 8 rather than a

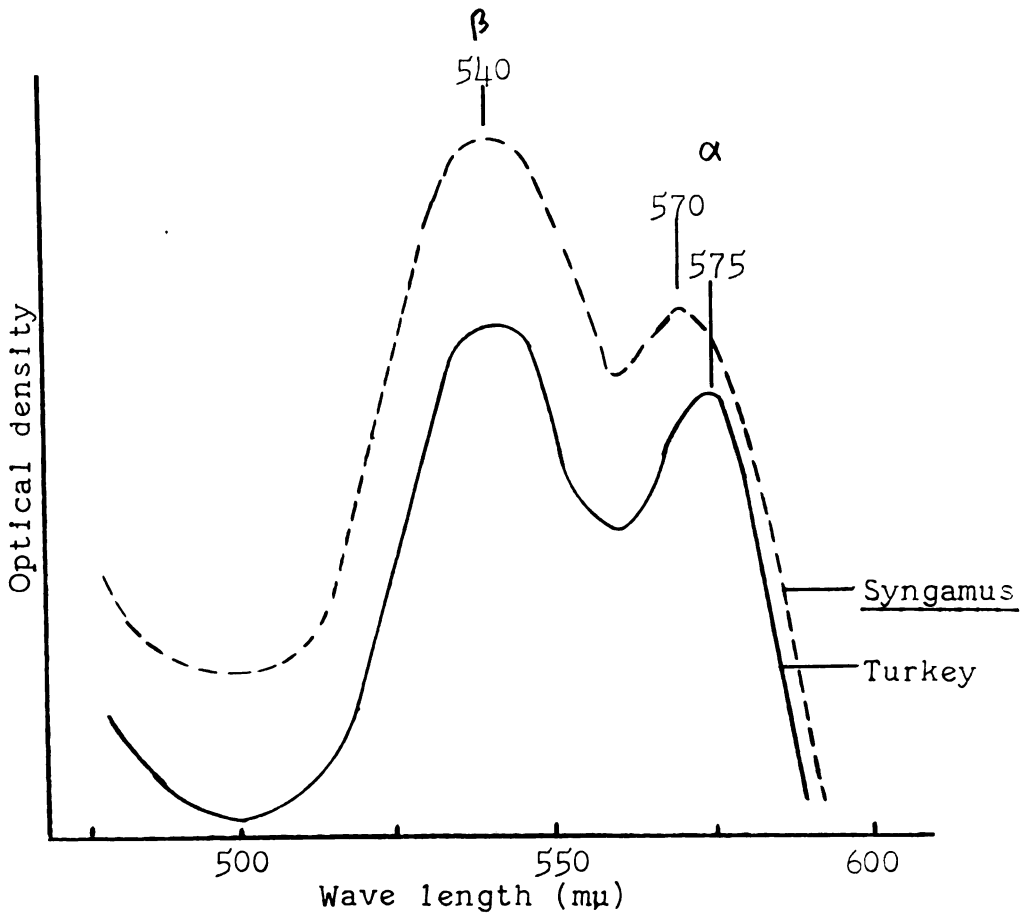


Figure 5. Oxyhemoglobin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid in dilute ammonium hydroxide, pH 8.

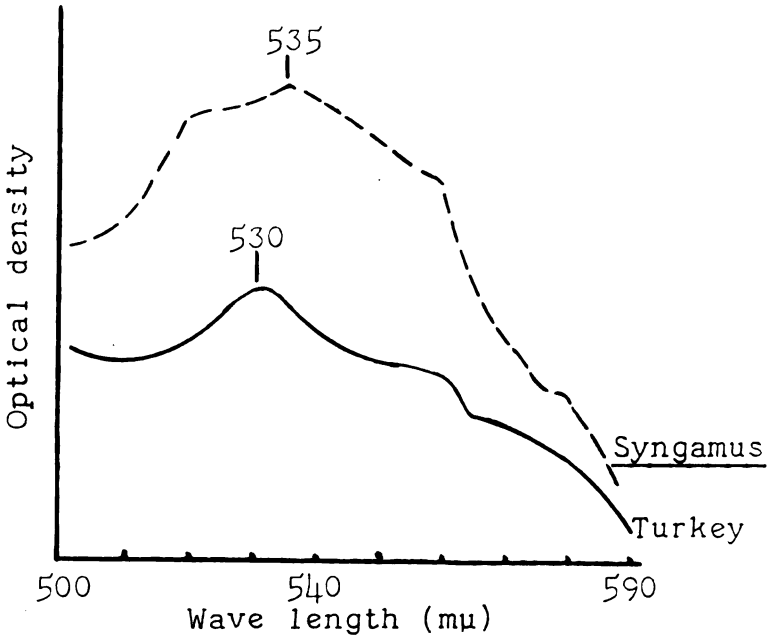


Figure 6A. Hemoglobin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid in dilute ammonium hydroxide and reduced by heat under vacuum.

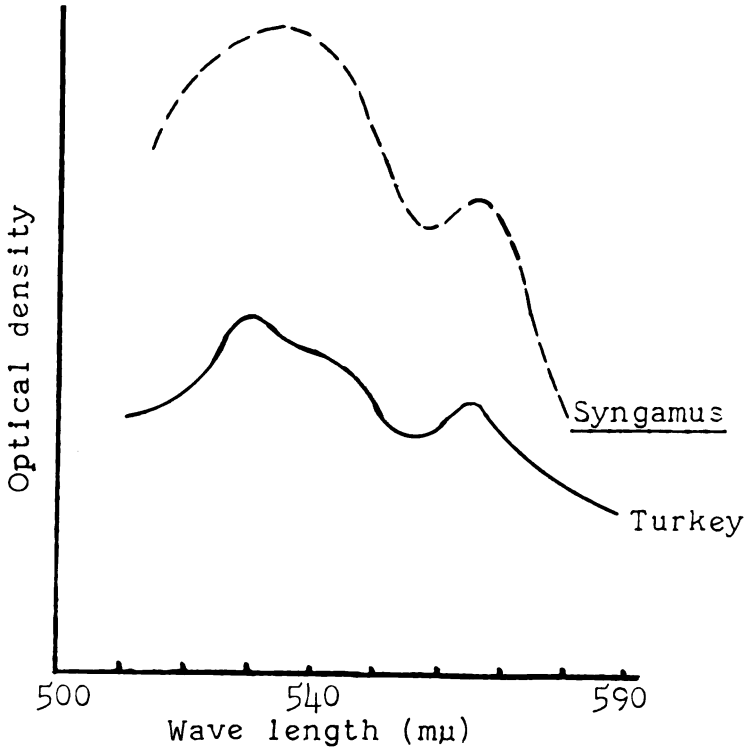


Figure 6B. Absorption curves of above samples following cooling and aeration.

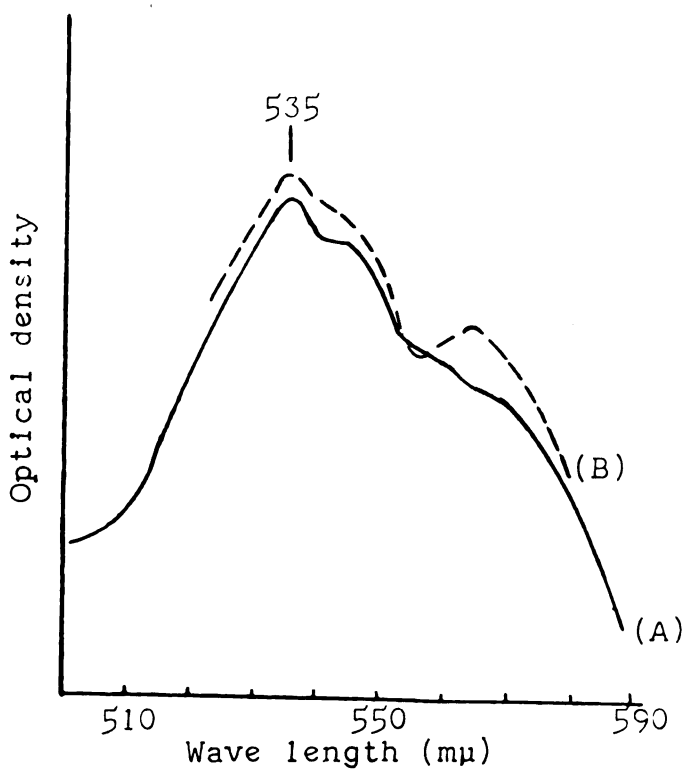


Figure 7. Hemoglobin absorption curve of Syngamus trachea pseudocoelomic fluid in dilute ammonium hydroxide, pH 8.

(A) Reduced by gassing with N_2 and heating under vacuum.

(B) Absorption curve of (A) following cooling and aeration.

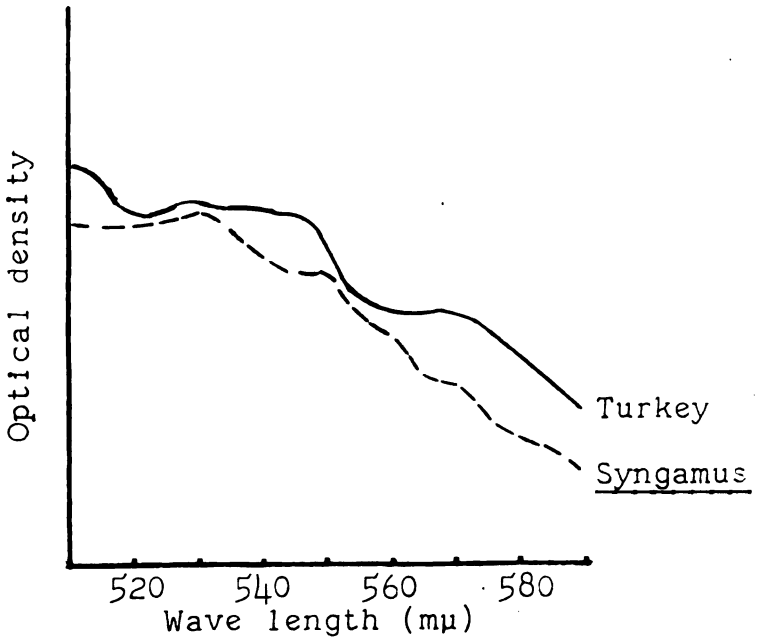


Figure 8A. Hemoglobin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid in dilute ammonium hydroxide, pH 8, reduced by $Na_2S_2O_4$ and heat under vacuum.

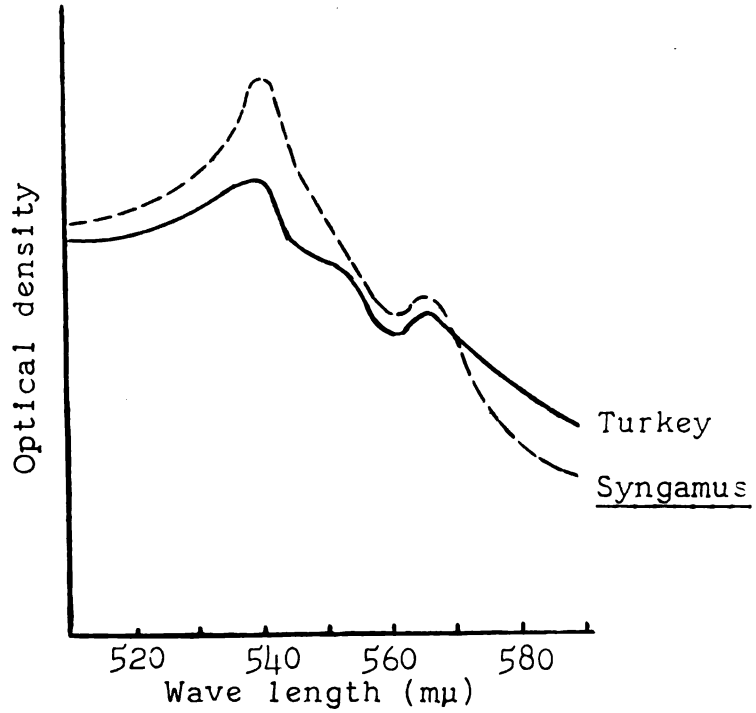


Figure 8B. Absorption curves of above samples following cooling and aeration.

reduced hemoglobin. Similar treatment of Syngamus pseudocoelomic fluid resulted in an absorption pattern of deoxygenated hemoglobin (Figure 9). This absorption pattern at pH 8 was identical to that of hemoglobin which was similarly reduced but maintained in a vacuum, however, at pH 7 there was a slight absorption maximum at 535 mμ.

Absorption curves quite like that of carboxyhemoglobin of turkey blood were obtained with the pseudocoelomic fluid from Syngamus. These absorption curves are presented in Figure 10. Similar absorption maxima were evident at 570 mμ and 540 mμ.

Treatment of turkey hemoglobin with potassium cyanide resulted in oxidation of the ferrous form of hemoglobin to the ferric form, methemoglobin. The absorption curves of turkey methemoglobin were similar at pH 6, 7, and 8. The absorption patterns of Syngamus pseudocoelomic fluid which was treated with KCN were dissimilar at each pH and also different from those of turkey methemoglobin. These differences in absorption patterns can be noted in Figure 11.

The absorption curves of acid hematin of turkey blood and the acid preparation of the body fluid of Syngamus were essentially the same. This can be seen in Figure 12.

Treatment of hemoglobin with concentrated hydrochloric acid results in a hemoglobin artifact, hematoporphyrin. The absorption patterns of the hematoporphyrin of turkey blood and a sample of the pigmented body fluid of Syngamus treated in the same manner are moderately different. Such a difference is apparent in Figure 13 in the wave length range between

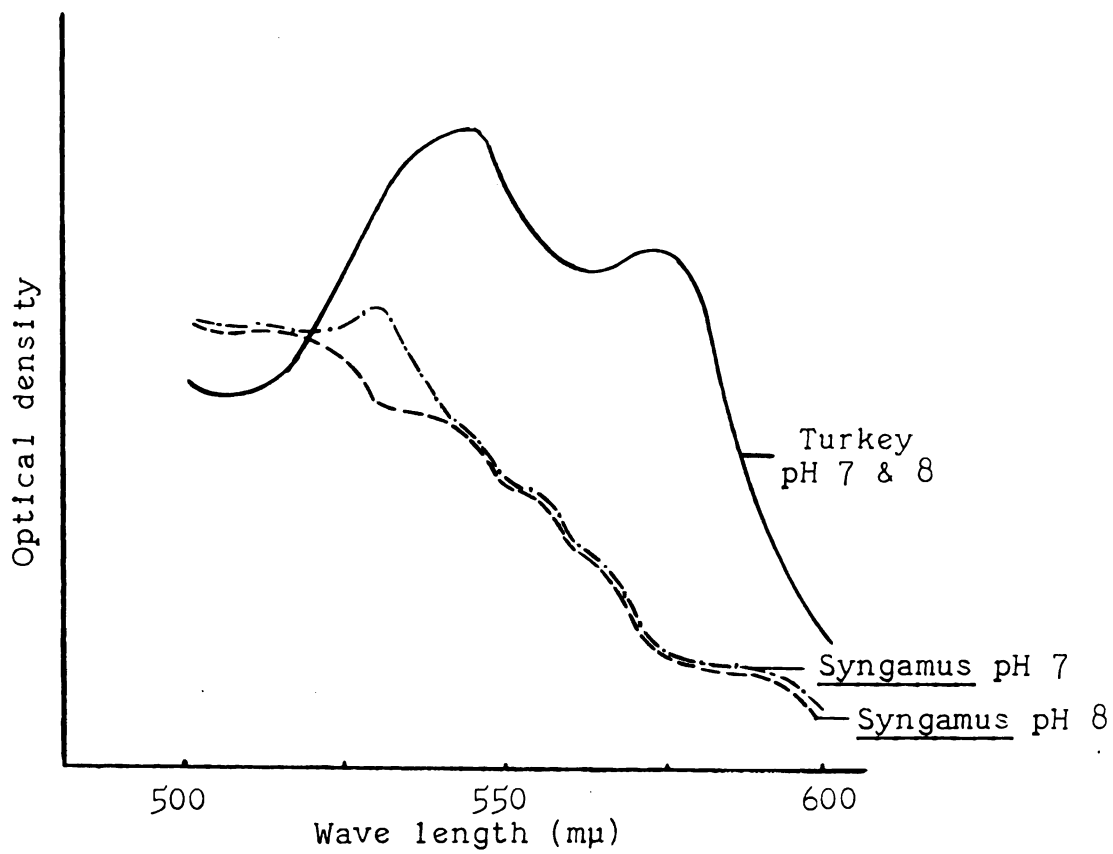
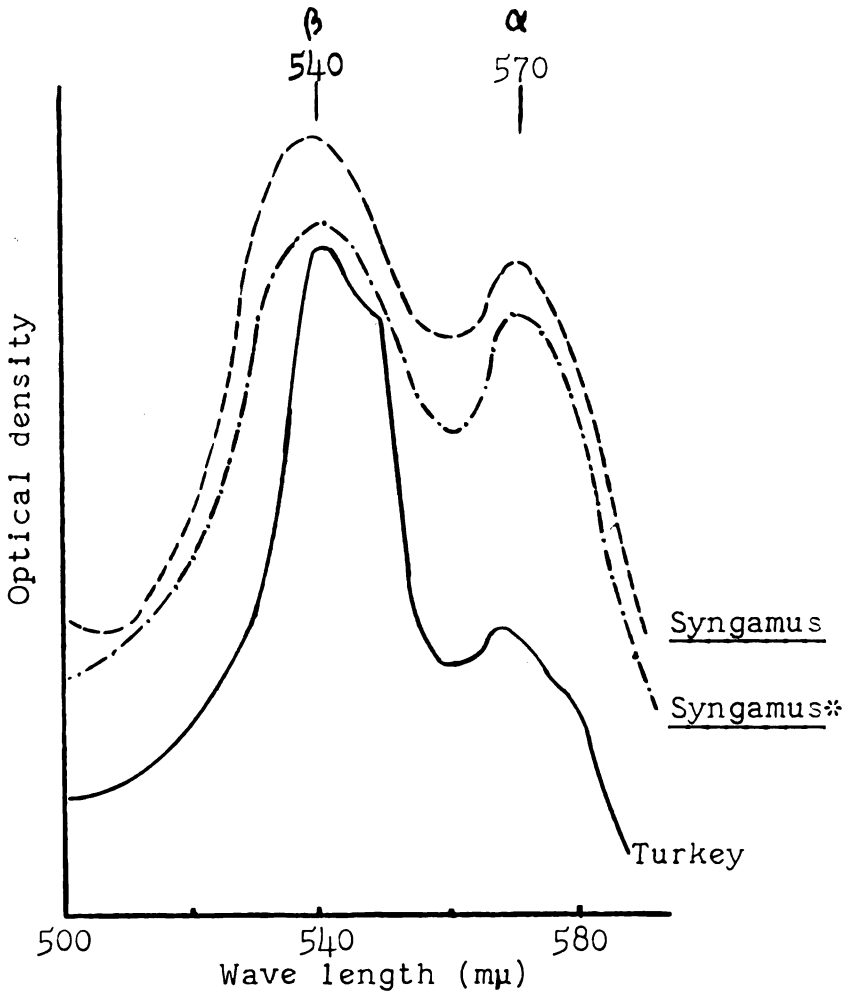
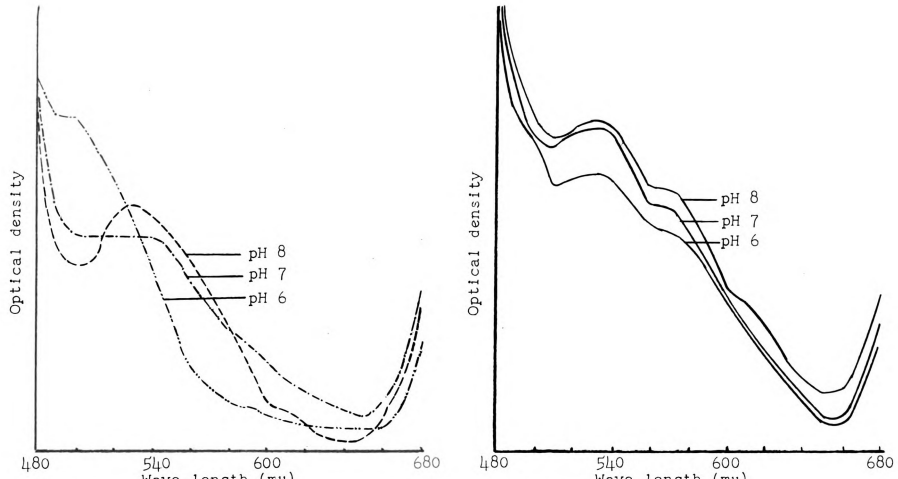


Figure 9. Hemoglobin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid in dilute ammonium hydroxide, pH 7 and 8, reduced by $Na_2S_2O_4$ and heat.



*pseudocoelomic fluid taken from Syngamus after exposure of the intact gapeworm to CO.

Figure 10. Carboxyhemoglobin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid in dilute ammonium hydroxide, pH 8, after exposure to carbon monoxide.



A. Syngamus trachea pseudocoelomic fluid.

B. Turkey blood.

Figure 11. Methemoglobin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid in dilute ammonium hydroxide at pH 6, 7, and 8.

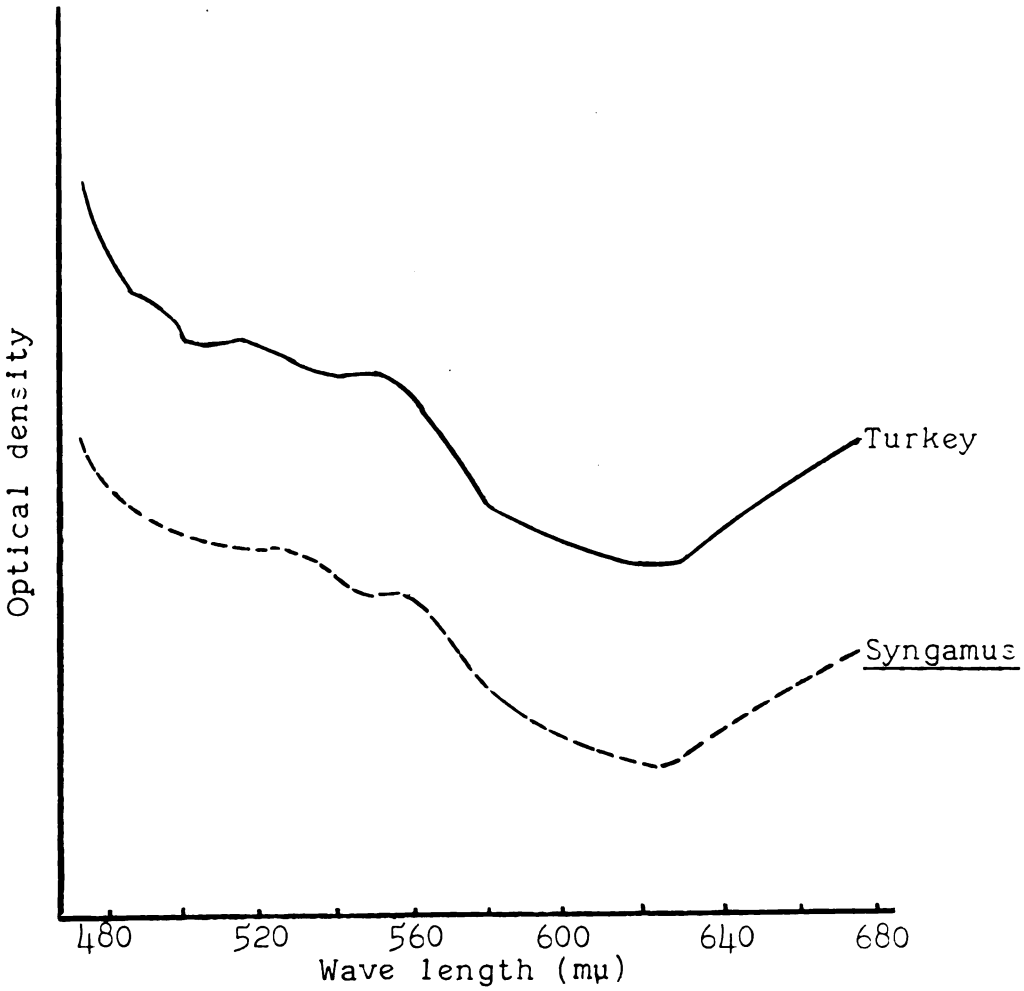


Figure 12. Acid hematin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid.

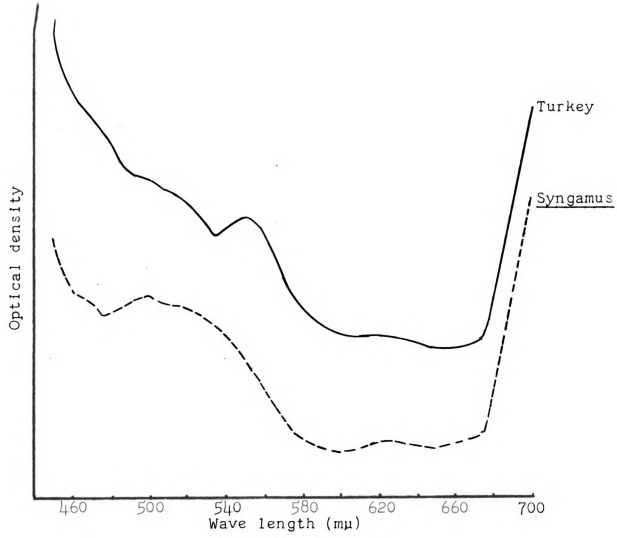


Figure 13. Acid hematoporphyrin absorption curves of turkey blood and Syngamus trachea pseudocoelomic fluid.

470 mμ and 560 mμ.

The endogenous carbohydrate utilization of Syngamus was determined for a 24 hour period. The total carbohydrates and polysaccharides were determined on gapeworms maintained in a phosphate buffered substrate without glucose and on gapeworms kept in the same substrate with 0.005 M glucose. The difference between these carbohydrate values after 24 hours and carbohydrate determinations at zero hours represent the carbohydrate utilization which appears in Table 5. The zero hour carbohydrate values represent the controls. Total carbohydrate was found to be 0.009 mg per mg wet weight of Syngamus, or 0.9% of the wet weight. The polysaccharides contributed 0.51% of the total carbohydrates. The endogenous total carbohydrate utilization of gapeworms maintained in a phosphate buffered substrate was 0.29% of wet body weight while gapeworms from a 0.005 M glucose substrate utilized 0.15%. The endogenous utilization of polysaccharides by Syngamus in a glucose free substrate was 0.35% of wet body weight. In a substrate containing glucose the utilization was 0.38% of wet body weight. The differences between the total carbohydrates and polysaccharides per mg wet weight Syngamus was 0.0039 mg for the controls, 0.0062 mg for Group I I, and 0.0045 mg for Group III.

Exogenous carbohydrate utilization by Syngamus trachea was obtained by determining the amount of glucose removed from a phosphate buffered substrate which contained 0.005 M glucose during aerobic respiration studies. When the

TABLE 5

TWENTY-FOUR HOUR ENDOGENOUS CARBOHYDRATE UTILIZATION BY *SYNGAMUS TRACHEA*[1] IN PHOSPHATE BUFFERED SUBSTRATE AT 37° C

	TOTAL CARBOHYDRATES		POLYSACCHARIDES	
	Mg/Mg Wet Weight *Syngamus*	Utilization Gm/100 Gm Wet Weight[2]	Mg/Mg Wet Weight *Syngamus*	Utilization Gm/100 Gm Wet Weight[2]
Group I Controls	0.0090	----	0.0051	----
Group II 0.005 M Glucose in Phosphate Buffer	0.0075	0.15	0.0013	0.38
Group III Phosphate Buffer No Glucose	0.0061	0.29	0.0016	0.35

[1] average wet weight per paired *Syngamus* was 9.23 mg.

[2] may also be expressed as percent of wet weight.

gapeworms were young and concurrently smallest in size the glucose utilization was greatest. With increased body size there was a decline in exogenous glucose utilization. This can be seen in Table 6.

Exogenous glucose utilization was also determined on gapeworms maintained *in vitro* for ten days at 37° C in a phosphate buffered substrate containing 0.005 M glucose. The daily rate of glucose utilization in terms of mg glucose utilized per gram of wet weight of *Syngamus* per hour for each of the ten days is shown in Figure 14. The rate of exogenous glucose utilization was lowest on the first day of the ten day period — 0.56 mg glucose utilized per gm wet weight of *Syngamus* per hour. A gradual increase in glucose utilization occurred until on the tenth day the rate was 1.36 mg glucose utilized per gm wet weight *Syngamus* per hour. Over the period of ten days the gapeworms decreased in average wet weight from 5.03 mg to 2.61 mg per pair of *Syngamus*.

The aerobic respiration of paired *Syngamus* was investigated by standard manometric procedures based on the Warburg method. The oxygen consumption, carbon dioxide liberation and respiratory quotient were obtained on a size range of *Syngamus* in order to determine the rate of the aerobic respiration with increased age and size. All values of gas exchange were calculated in microliters per mg dried weight per hour.

The general pattern of aerobic respiration can be seen in Figure 15. No attempt was made to correct for absorption

TABLE 6

EXOGENOUS GLUCOSE UTILIZATION BY SYNGAMUS TRACHEA DURING AEROBIC RESPIRATION STUDIES WITH A WARBURG APPARATUS IN A PHOSPHATE BUFFERED SUBSTRATE CONTAINING 0.005 M GLUCOSE

Average mg Wet Weight Per Pair	No. of Pairs Used	Mg Glucose Uptake Per Gram Wet Weight Per Hour	Hours Shaking
0.72	48	3.89	6.5
1.56	72	1.80	9.0
4.42	48	1.36	5.0
5.92	70	0.80	7.0
8.80	72	0.23	4 + 20*

* Twenty hours without shaking.

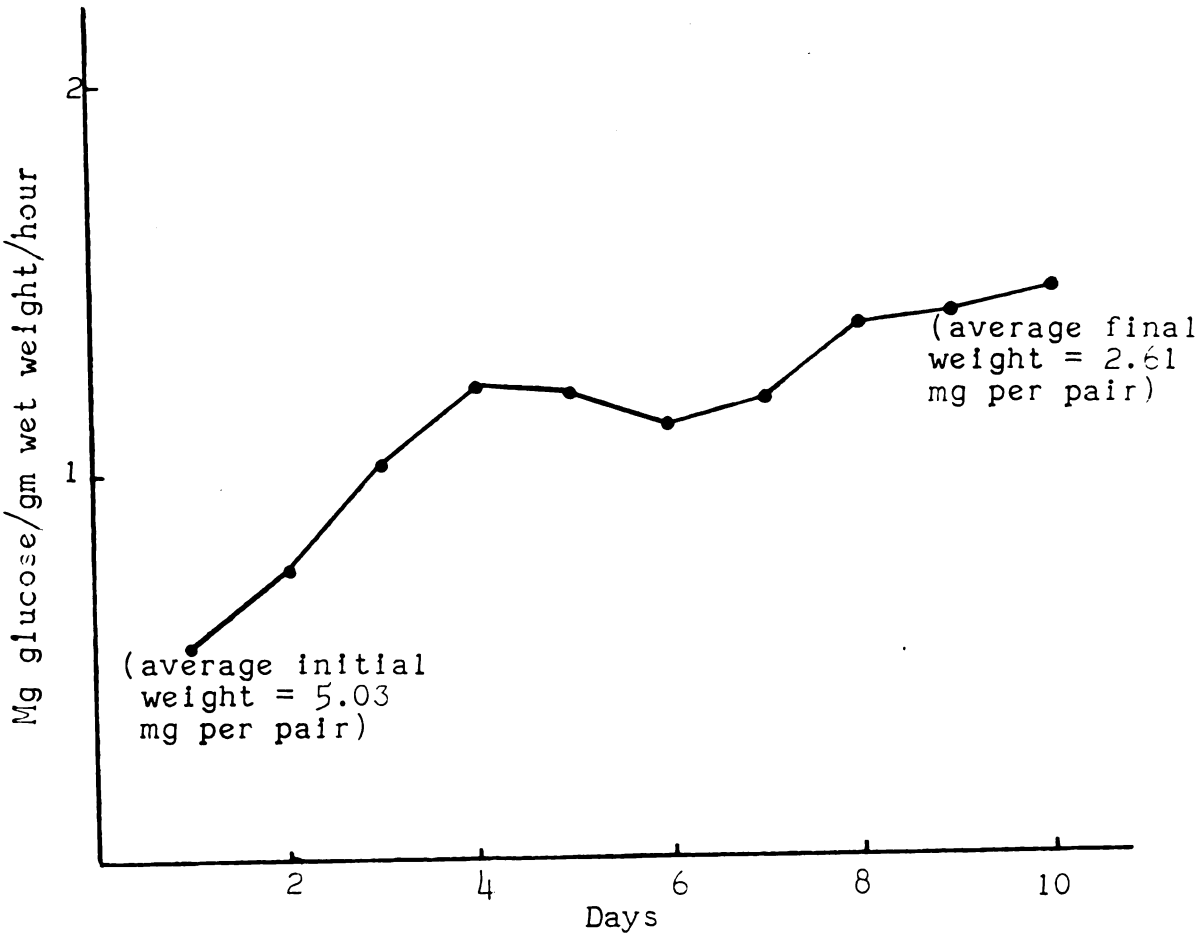


Figure 14. Exogenous glucose utilization* by Syngamus trachea maintained in vitro at 37° C for ten days in phosphate buffered substrate containing 0.005 M glucose.

*utilization based on average initial weight of 5.03 mg per paired Syngamus.

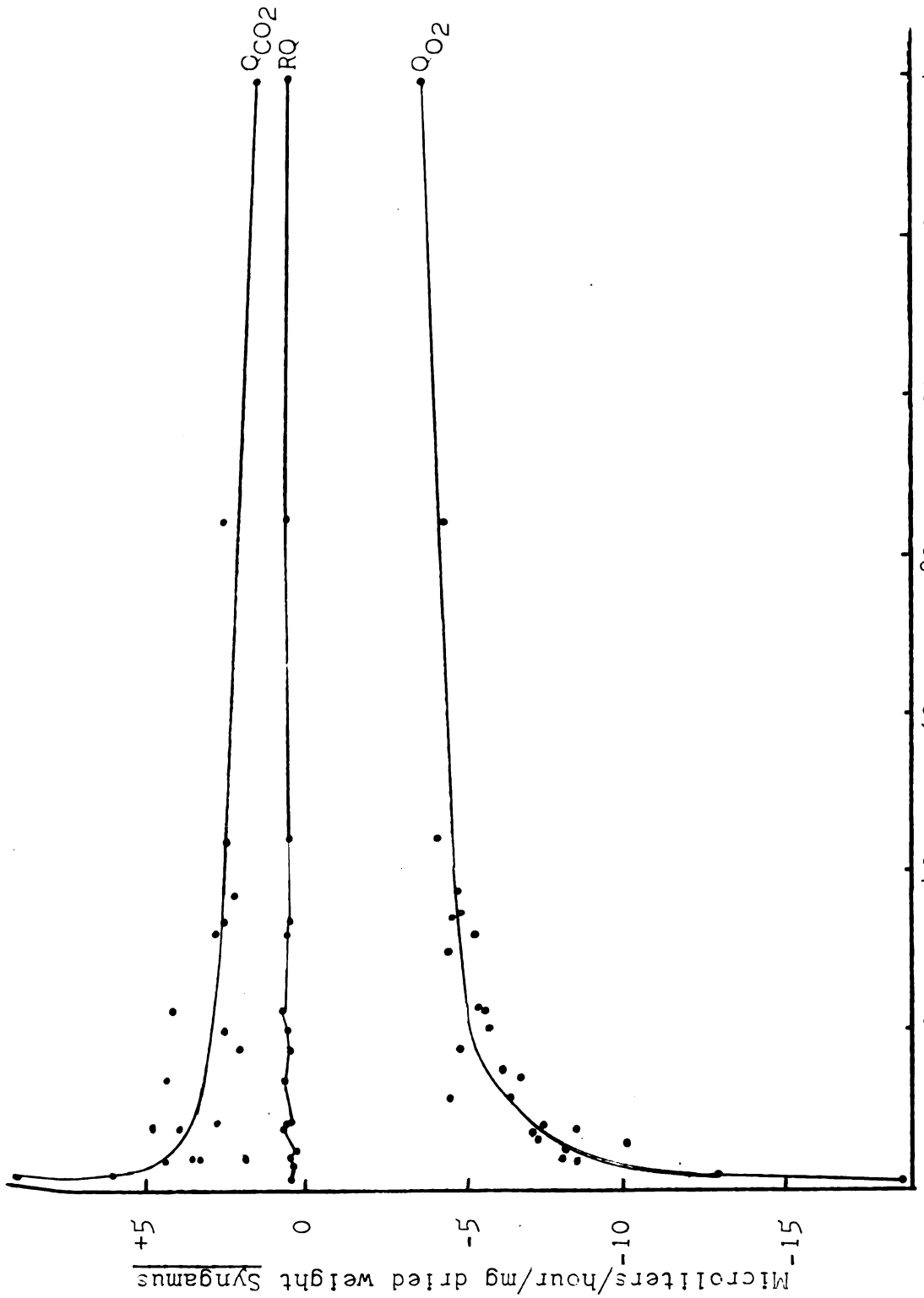


Figure 15. Aerobic respiratory activity of paired Syngamus in a phosphate buffered substrate with 0.005 M glucose as associated with body size.

of some of the CO_2 in the phosphate buffer, consequently, the values for the carbon dioxide liberation and the respiratory quotients are slightly lower. Nevertheless, these respiratory values were useful in determining the comparative relationship of body size to rate of respiration of Syngamus. The rate of respiration may be described as the quantitative oxygen utilization and carbon dioxide liberation per mg dried weight of Syngamus per hour. The highest rate of respiration was obtained in the smallest and youngest gapeworms and a gradual decrease in rate with increased body size was noted. It appears that the gradual decrease in respiratory rate becomes negligible after the paired Syngamus obtain a dried weight size of about 2 mg.

The hourly values for oxygen utilization and carbon dioxide liberation for some of the longer aerobic respiration studies are presented in Figure 16. Examination of the hourly rates of aerobic respiration suggest that as length of time of the study increased the respiration rate of gapeworms in the substrate with glucose increased. This increase in respiration rate appeared to be more pronounced in gapeworms of lighter average weight. The pattern of respiration is rather constant over the hourly period for gapeworms of heavier weight and for gapeworms in a glucose free substrate.

Only the pattern of aerobic respiration for the various weights of Syngamus was represented in Figure 15. The aerobic respiratory activity of Syngamus as measured by the

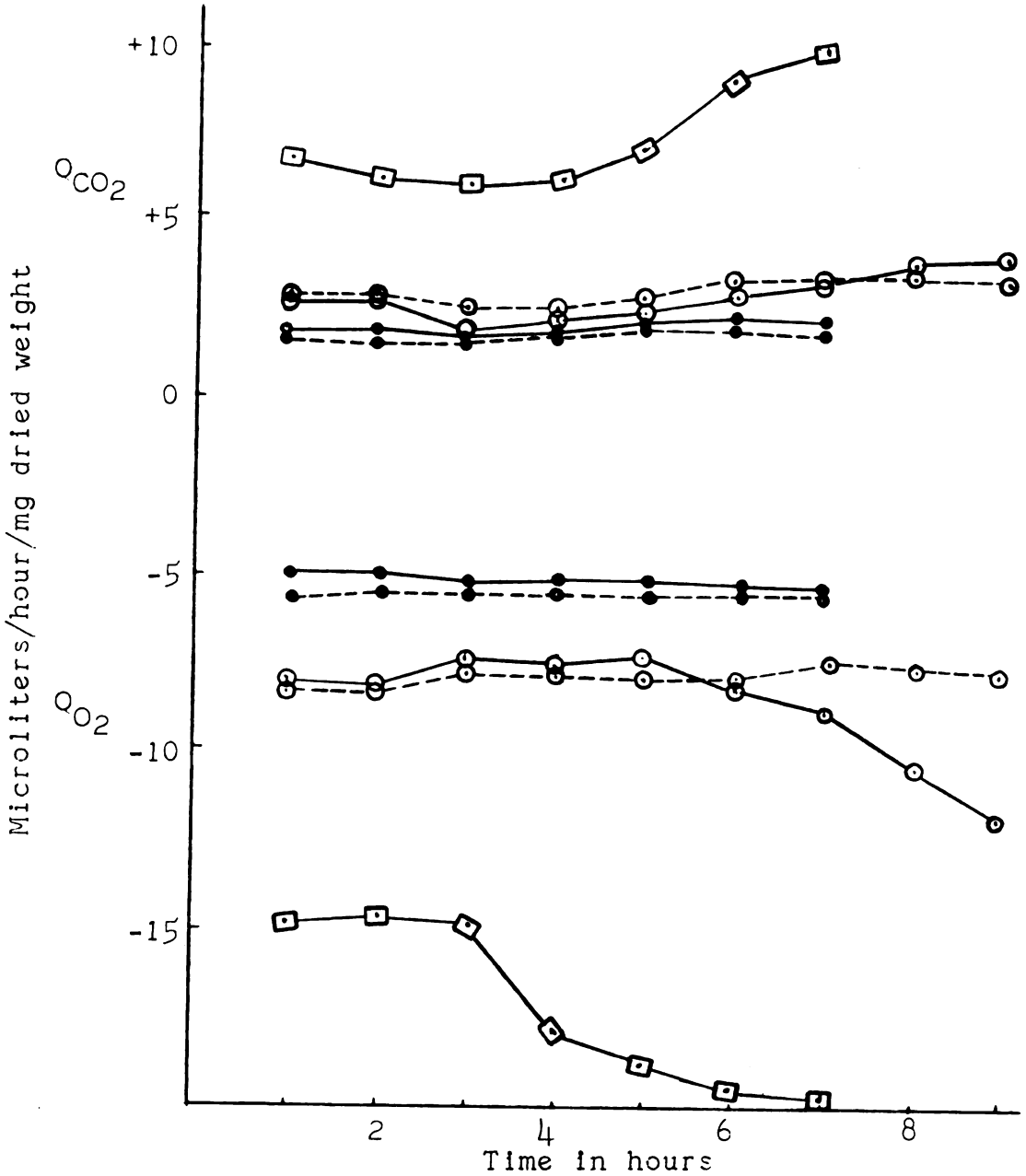


Figure 16. Hourly rate of aerobic respiration of paired Syngamus trachea of different average weights in phosphate buffered substrate, pH 7.54, glucose free or containing 0.005 M glucose.

respiratory quotient (RQ) appears in Table 7. Regardless of the size of the gapeworms used for the determinations there was a rather consistent respiratory quotient (average = 0.866) when the substrate contained 0.005 M glucose. Even when the substrate was free of glucose or butyric acid the respiratory quotient of Syngamus approximated that obtained when gapeworms were present in a substrate containing 0.005 M glucose (average = 0.887). However, when 0.065 M butyric acid was present in the substrate a respiratory quotient of 0.708 was obtained.

The pronounced effect of butyric acid in the substrate on the respiratory quotient of Syngamus was not reflected by a change in oxygen utilization. The rate of oxygen utilization in the presence of 0.065 M butyric acid was the same as when 0.005 M glucose was present in the substrate (Table 8). The effect of butyric acid was due to decreased carbon dioxide liberation, thus causing a lower respiratory quotient.

The rate of aerobic respiration of a brei of Syngamus was greater than an equal weight of intact gapeworms (Table 8). The respiratory quotient of the brei was 0.900 as compared to 0.434 for the control group.

An acetone powder prepared from Syngamus, when in the phosphate buffered substrate containing glucose, produced complete oxidation of the glucose molecule. This is shown by a respiratory quotient of nearly 1.000 (Table 9). The microliters of oxygen uptake and carbon dioxide liberation

TABLE 7

RESPIRATORY QUOTIENTS[1] OF PAIRED SYNGAMUS TRACHEA

Mg Dry Weight Per Pair	Microliters/mg/hr Q_{O_2}	Microliters/mg/hr Q_{CO_2}*	RQ[1]	Phosphate Buffered Substrate, pH 7.54 With:
0.77	-7.05	6.34	0.863	.005 M Glucose
1.07	-5.99	3.95	0.708	.065 M Butyric Acid
1.155	-5.52	4.97	0.900	----------
1.165	-4.68	4.11	0.878	.005 M Glucose
1.58	-6.65	5.82	0.873	----------
1.58	-6.12	5.39	0.882	.005 M Glucose
3.80	-4.10	3.86	0.820	.005 M Glucose
4.48	-4.08	3.61	0.885	.005 M Glucose

[1]Respiratory quotient (RQ) is the ratio of CO_2 liberated to O_2 utilized.

*Corrected for CO_2 retention in the substrate.

TABLE 8

AEROBIC RESPIRATORY ACTIVITY OF PAIRED SYNGAMUS TRACHEA IN PHOSPHATE BUFFERED SUBSTRATE UNDER SPECIAL CONDITIONS

Condition	Mg Wet Weight Per Pair	Microliters/mg/hr Q_{O_2}	Microliters/mg/hr Q_{CO_2}	RQ
.005 M Glucose	1.60	-12.63	----	----
.065 M Butyric Acid	1.78	-12.65	----	----
.005 M Glucose	4.68	- 6.39	----	----
.005 M Glucose	4.73*	-10.67	----	----
.005 M Glucose	7.78	- 5.90	2.62	0.434
.005 M Glucose	7.98*	- 7.75	7.00	0.900

*Brei of Syngamus trachea.

Q_{O_2} - Microliters of oxygen utilized per mg dried weight of Syngamus per hour.

Q_{CO_2}- Microliters of carbon dioxide liberated per mg dried weight of Syngamus per hour. No correction has been made for retention of CO_2 in the substrate.

RQ - Ratio of CO_2 liberated to O_2 utilized.

TABLE 9

RESPIRATORY ACTIVITY OF AN ACETONE POWDER PREPARED FROM SYNGAMUS TRACHEA IN PHOSPHATE BUFFERED SUBSTRATE[1]

Ml Acetone Powder Used[2]	Phosphate Buffered Substrate, pH 7.54 With:	Microliters Per Two Hours		RQ[3]
		X_{O_2}	X_{CO_2}[a]	
1.3	0.005 M Glucose	-20.07	+21.67	1.078
1.3	----------	0	0	-----
0.5	0.005 M Glucose	-20.75	+22.25	1.072
0.3	0.005 M Glucose	-20.35	+20.42	1.005
0.3	0.005 M Glucose and 10^{-2} M Azide	0	---	-----
0.3	0.005 M Glucose and 10^{-2} M Iodoacetate	- 3.89	---	-----
0.3[b]	0.005 M Glucose	-11.45	---	-----
0.3	----------	0	0	-----
0.1	0.005 M Glucose	- 3.62	+ 3.60	0.994

[1] Calcium free substrate.

[2] Acetone powder was dissolved in phosphate buffered substrate. Final volume used for manometric study was 2.8 ml.

[3] Respiratory quotient (RQ) is the ratio of carbon dioxide liberated to oxygen utilized.

[a] corrected for carbon dioxide retention in substrate.

[b] Acetone powder emptied from sidearm into reaction vessel one hour after trial began.

were nearly the same for all volumes of the acetone powder in a glucose substrate, except for the smallest volume (0.1 ml). No gaseous exchange occurred when the acetone powder was in a glucose free substrate. The addition of potential inhibitors of respiration and glycolysis, azide and iodoacetate, produced a marked decrease in oxygen consumption. When acetone powder was emptied from the sidearm into the reaction vessel after the manometric trial had begun, the one hour volume of oxygen consumption was approximately one-half of the standard two hour oxygen consumption of a paired sample.

The anaerobic respiratory activity of Syngamus trachea, as reflected by carbon dioxide liberation ($Q^{N}_{CO_2}$) in the presence of various substrates, appears in Table 10. The rate

TABLE 10

ANAEROBIC* RESPIRATORY ACTIVITY OF PAIRED SYNGAMUS TRACHEA IN PHOSPHATE BUFFERED SUBSTRATE

Substrate with:	Mg Dry Weight Per Paired Syngamus	$Q^{N}_{CO_2}$ Microliters
0.005 M Glucose	0.201	+2.84
	1.190	+2.00
	4.460	+1.76
0.065 M Butyric Acid	0.893	+3.26
	1.400	+2.22
No Glucose	0.825	+3.59
	1.320	+2.48

* Nitrogen atmosphere (95% N_2 - 5% CO_2).

of anaerobic activity may be considered to be the quantitative rate of carbon dioxide liberated in manometric investigations. Regardless of the substrate used in the anaerobic investigations the rate of anaerobic respiration as expressed by carbon dioxide liberation was decidedly less than the Q_{CO_2} of aerobic respiration (Table 7). It appeared that the anaerobic respiration of Syngamus in a phosphate buffered substrate and in one with 0.065 M butyric acid was about the same while it was slightly less in the buffered substrate containing 0.005 M glucose. As was previously noted for aerobic respiration the rate of anaerobic respiration decreased with increased weight of the gapeworms.

The effect of various recognized inhibitors of metabolism on the aerobic respiration of Syngamus is summarized in Table 11. A decrease in oxygen utilization from the normal was used as a criterion of metabolic inhibition. The rate of oxygen utilization was markedly inhibited by azide, cyanide, and 2,4-dinitrophenol. In the presence of fluoride, iodoacetate, and malonate there was decreased oxygen uptake; however, this inhibition was less pronounced. Fluoride and malonate were inhibitory only in a calcium free phosphate buffered substrate.

When Syngamus was exposed to carbon monoxide (95% CO - 5% O_2) the pseudocoelomic fluid changed from a dark to a bright red color and a decrease in oxygen utilization occurred. The difference between oxygen uptake in the presence and absence of light appears in Table 12. In the presence

TABLE 11

EFFECT OF VARIOUS INHIBITORS ON THE OXYGEN UTILIZATION OF PAIRED SYNGAMUS TRACHEA IN PHOSPHATE BUFFERED SUBSTRATE OF pH 7.54 CONTAINING 0.005 M GLUCOSE

Inhibitor	Concentration (Molarity)	Mg Dry Wt. Per Pair	Ratio[1]
AZIDE	10^{-2}	0.733	0.264
	10^{-4}	6.80	0.612
	10^{-6}	0.73	0.895
CYANIDE	10^{-2}	0.273	death
	10^{-4}	0.239	0.088
		1.83	0.077
	10^{-6}	0.237	0.716
		2.40	0.491
2,4-DINITRO-PHENOL	10^{-2}	0.708	0.387
	10^{-4}	0.80	0.68 *
		6.50	0.852*
	10^{-6}	0.60	0.79 *
		0.84	0.812
		1.38	0.89 *
	10^{-8}	0.76	0.89 *
FLUORIDE	10^{-2}	0.618	1.00
		0.75	0.83 *
		1.38	0.86 *
		brei	1.00
IODOACETATE	10^{-2}	0.69	0.633
		7.10	0.76
MALONATE	10^{-1}	brei	0.63 *
	10^{-2}	0.67	0.97
		0.80	0.79 *
		1.38	0.94 *
		brei	0.88 *

[1]Ratio is the oxygen utilization in the presence of the inhibitor to the oxygen utilization in the absence of the inhibitor.

*Calcium free substrate.

TABLE 12

EFFECT OF CARBON MONOXIDE ON OXYGEN UTILIZATION OF PAIRED *SYNGAMUS TRACHEA* UNDER CONDITIONS OF LIGHT OR DARKNESS IN 0.005 M GLUCOSE PHOSPHATE BUFFERED SUBSTRATE

Atmosphere[1]	Q_{O_2}[a] in Light	Q_{O_2} in Light	Q_{O_2} in Darkness
AIR	-2.83	-4.08	-6.57
95% CO - 5% O_2[b]	-1.58 (0.56)[c]	-2.01 (0.495)	-1.83 (0.278)
AIR	-1.54 (0.54)	---	-1.78 (0.270)

[1] Oxygen utilization determined in the following air-carbon monoxide-air atmosphere succession on the same group of gapeworms.

[a] Q_{O_2} - microliters of oxygen utilization per mg dried weight of *Syngamus* per hour.

[b] Forty to sixty minute gassing period.

[c] Figures in parentheses represent the ratio of O_2 utilization in atmosphere cited to initial oxygen utilization of the group.

of light the rate of oxygen utilization by Syngamus decreased to about one-half, and in darkness it decreased to about one-quarter the original rate of oxygen utilization. The inhibition which resulted from gassing with carbon monoxide continued even after an equilibration period in air.

DISCUSSION

As was previously mentioned in the historical review, infections of Syngamus trachea can be obtained in birds through the use of a transport host, such as the earthworm, and by oral administration of suspensions of infective larvae. The degree of infection obtained by these methods was shown to be highly variable and uncontrollable. Intravenous inoculation of chickens and turkeys with suspensions of infective larvae had never been used as a means of establishing Syngamus infections. It is apparent from the results of this investigation that the intensity of infections can be well controlled when turkeys are inoculated intravenously with infective larvae. The technique for controlling the intensity of infection now opens new avenues for such investigations as determination of factors of stress and resistance in birds infected with gapeworms, studying the genetics of Syngamus spp., use of Syngamus as a tool for chemotherapeutic studies, etc. The application of the technique of intravenous inoculation of infective larvae might be applied to other parasitic nematodes. When applied to Syngamus the intravenous route of inoculation might help explain the route of migration of the larvae. A final benefit of the method of intravenous inoculation is the savings in time and energy in infecting birds as compared with the efforts required to obtain infective earthworms and sufficient larvae for per os

inoculation.

This investigation resulted in the substantiation of existing information and the addition of new information on the life cycle of Syngamus trachea. Intravenous inoculation of birds made it possible to know the exact hour of tissue invasion by infective larvae. This served as a "zero" time on which to base subsequent observations on the parasite. Paired gapeworms were recovered as early as eight days in turkeys inoculated intravenously. When earthworms were used as inoculum recovery of gapeworms was on the ninth day. It is generally agreed (Morgan and Clapham, 1934; Wehr, 1937; and Guilford and Herrick, 1954) that the paired Syngamus reach the trachea on the ninth day. Consistent recovery of the parasite on the eighth day would mean the elimination of an initial period of 24 hours during which the larvae were associated with the intestinal tract of the turkey. The same could also apply to the prepatent period. When turkeys were infected intravenously the prepatent period was $14\frac{1}{2}$ days but with oral infection patency occurred on the 15th day. As previously mentioned, a wide range of 14 to 25 days has been given for prepatency. In this investigation eggs from gapeworms recovered on the $14\frac{1}{2}$ day, after embryonation, developed into larvae which were infective for turkeys. Ortlepp (1923) believed that the sexes reached maturity in ten to 14 days after infection but first produced "normal" eggs in 17 to 20 days.

Even in the presence of a great deal of information on

the life cycle of Syngamus there still remains doubt as to the route of migration of larvae from the intestinal tract to the lungs. The discovery that larvae may be given intravenously with resulting infections strongly suggests that the route of migration is by way of the blood. The lack of infectivity of larvae given subcutaneously and intraperitoneally can be considered as evidence that infective larvae migrate from the intestinal lumen to blood vessels in close association with the intestinal lumen, otherwise inoculation by intraperitoneal or subcutaneous routes would have resulted in infection. Likewise, the sensitivity of infective larvae to incubation at 37° C for 48 hours would suggest that if larvae migrated through the body tissues of the bird, the body temperature would probably kill them. It is probable that the decrease in the time required for the appearance of gapeworms in the trachea and the prepatency following intravenous inoculation is due to the by-passing of the intestinal phase of the regular life cycle. Ortlepp (1923), Wehr (1937), Clapham (1939), and Guilford and Herrick (1954) found the third stage infective larvae in the lungs, liver, air sac, and postcaval vein approximately 18 to 24 hours after infection by way of the intestine. After consideration of the above circumstancial evidence and the consistent and intense infection obtained by intravenous inoculation it would appear that there is little doubt that the route of migration in nature is from the intestine through the blood vascular system to the lungs.

Attempts to infect chickens over three weeks of age

generally resulted in failure. Even after ten to twelve days of age it appeared that the intensity of infection obtained in chickens decreased. This is in agreement with the findings of Wehr (1939) and Olivier (1944). They found chickens refractory to infection at an early age. Even intravenous inoculation of chickens older than 30 days resulted in no infection. It would appear that the refractiveness of chickens is associated with some phase of the life cycle after the larvae leave the intestinal lumen. In agreement with Olivier (1944) the infection of older turkeys presented no problem.

After infection of turkeys and chickens with Syngamus the parasite numbers remained rather constant for about three weeks. After 24 days a decline in the number of worms in turkeys was noted. By the 37th day of infection the number of Syngamus recovered was negligible. This decrease in intensity of infection was also observed by Morgan and Clapham (1934), Wehr (1939), and Olivier (1944). Guilford and Herrick (1954) noted that the loss of gapeworms in pheasants began at about 27 days and at the end of 48 days almost every bird was free of infection.

Previous investigators have determined the size of Syngamus by linear measurements of the female worm. Frequently an estimate is not made on the male gapeworm. The manipulations required for linear measurement are time consuming and tedious and often result in injury to the gapeworm. In this investigation the comparative size of paired gapeworms on various days following inoculation was rapidly and accurately

obtained by dry and wet weight determinations. Similar information on the ratio of dry and wet weight of other parasites might prove to be more useful to parasitologists than linear measurements.

The relative water content of biological material can be obtained by determining the relationship of dry weight to wet weight, and is expressed as percent dried weight. A comparison of the range of dried weight values (15% to 25%) of other parasites obtained by investigators mentioned in the review of literature, reveals that the paired Syngamus possess the highest percent dried weight (26.2% ± 2.9%). Only the 25% dried weight of the larvae of Eustrongylides ignotus reported by von Brand (1938) approaches the dried weight of gapeworms. The determinations were not designed to determine why the percent dry weight of Syngamus is higher than values found for other parasites. Dry weight determinations on gapeworms were made for a comparison of the relative water content with other nematodes and as a basis for manometric studies on the respiration of Syngamus.

There were no records of the uniformity of the percent dry weight during the life span of other nematodes with which comparisons can be made. No direct connection between the uniformity of percent dried weight to other information gained in this investigation can be made. The percent dried weight would not be accurate enough to reflect changes in carbohydrate contents because such differences were less than one percent. It is interesting to note, however, the

uniformity of percent dried weight in light of such a remarkable increase in weight.

The total carbohydrate (0.9% to 1.68%) and polysaccharide (0.3% to 0.76%) contents of Syngamus are almost identical to that of the filarial worm, Litomosoides carinii (0.99% to 1.73% and 0.73%) reported by Bueding (1949). Of several nematodes discussed by von Brand (1950) only Dipetalonema gracilis contained less polysaccharides, 0.2%, than Syngamus. The polysaccharide values (1.6% to 8.7%) for three ascarides and two strongyles of the intestinal tract, as summarized by von Brand (1952), were all greater than that of Syngamus. Based on a possible two to ten times greater polysaccharide content of these nematodes of the intestinal tract, as opposed to that of Dipetalonema of the abdominal cavity, Litomosoides of the pleural cavity, and Syngamus of the trachea, it would appear that the greater polysaccharide content might be associated with environment or feeding habits.

Ten day old gapeworms, as well as 35 day old gapeworms, contained about the same percent of polysaccharide (0.33%). It appears that this is the basic minimum polysaccharide content of Syngamus. Baldwin and King (1942) found that the stored polysaccharide of nematodes was glycogen. With this in mind, it is probable that the stored polysaccharide in Syngamus is also glycogen. The difference between the least amount (0.33%) or basic amount of polysaccharides, which is probably glycogen, and the increased amounts of polysaccharides found in gapeworms between ten and 35 days might be attributed

to the development of eggs as well as stored polysaccharide. von Brand (1952) stated that the egg shells of most nematodes are composed of the polysaccharide, chitin. It appears that some of the rise in polysaccharide content might be attributed to the chitin in the eggs. There seems to be a correlated rise in polysaccharides during the period of greatest egg production and the gradual decline parallels decreased egg numbers in old worms.

The difference (0.4% to 0.65%) between the total carbohydrates and polysaccharides content of Syngamus represents primarily mono- and disaccharides. This difference compares favorably with the difference (0.39%, 0.45%, and 0.62%) which existed between the gapeworms used in the endogenous carbohydrate study. One might consider this as the basic amount of low molecular weight carbohydrates of Syngamus.

In spite of the similarities of low carbohydrate content in Litomosoides and Dracunculus with Syngamus, the endogenous and exogenous carbohydrate utilization of Syngamus is decidedly less. Carbohydrate utilization is Syngamus was more like that of Eustrongylides larvae or even the intestinal nematodes. There appeared to be only a slight difference in the rate of endogenous carbohydrate utilization between gapeworms maintained in solutions containing glucose and solutions without glucose. The utilization of glucose from the substrate was evidenced by a lessened rate of endogenous carbohydrate utilization.

The rate of exogenous carbohydrate utilization of

Syngamus decreased with increased body size. This follows the general pattern for worms as stated by von Brand (1952) that smaller organisms metabolize at a higher rate than larger ones. The rate of exogenous glucose utilization increased greatly when gapeworms were maintained *in vitro* for ten days. During this same time there was a continual decrease in body weight. In the presence of only a carbohydrate during this period it would appear that the decrease in weight might be attributed to utilization of body substances. The increased rate of exogenous glucose utilization appears to reflect a more predominant carbohydrate utilization only after endogenous substances were depleted. It is recognized that the rates of carbohydrate utilization in the unphysiological conditions of *in vitro* maintenance might not reflect the true *in vivo* rate. Nevertheless, the results obtained concerning carbohydrate utilization demonstrates the importance of carbohydrate metabolism and are useful for a comparison of the rate of utilization between similarly studied nematodes.

The procedure most frequently used by investigators to study hemoglobin in nematodes is spectroscopic analysis. Usually absorption curves or bands for oxyhemoglobin and deoxygenated hemoglobin are determined in order to support their views (von Brand, 1937; Stannard *et al.*, 1938; Wharton, 1941; Davenport, 1949; Rogers, 1949a; and Goldberg, 1956). Oxyhemoglobin is usually converted to hemoglobin by the addition of sodium hydrosulfite, but occasionally evacuation

or gassing with nitrogen is used to deoxygenate the oxyhemoglobin.

The red pseudocoelomic fluid of Syngamus trachea appears to be an iron porphyrin compound quite similar to the hemoglobin of turkeys but distinct in a number of ways. Some of the differences between turkey hemoglobin and the pigmented body fluid of Syngamus were: turkey oxyhemoglobin has an α absorption maximum at 575 mμ, while it is at 570 mμ for Syngamus pseudocoelomic fluid; the span between the α absorption maxima of oxyhemoglobin and carboxyhemoglobin is about 5 mμ for turkey hemoglobin while little or no span exists between the maxima of the same absorption curves of Syngamus; treatment with potassium cyanide results in different absorption patterns for the two samples; and slightly different absorption curves for acid hematoporphyrins were obtained. Variations in the absorption curves for acid hematoporphyrins would suggest different porphyrins. The deoxygenation by evacuation of turkey and Syngamus samples required about the same effort and subsequent oxygenation of both pigments took place equally well.

The absorption maxima of oxygenated pseudocoelomic fluid of Syngamus were at 570 mμ and 540 mμ. Only the α maximum of Syngamus differed from the absorption maxima of oxyhemoglobin of other nematodes which have been studied. These were five to ten mμ higher than the α maximum of Syngamus.

Physical deoxygenation of the pigmented fluid of Syngamus resulted in an absorption maxima at 535 mμ. This

is ten to 20 mμ lower than the hemoglobin maxima reported by Wharton (1941) for Camallanus trispinosus and by Rogers (1949b) for Nematodirus spp. and Haemonchus contortus.

Chemical deoxygenation of turkey blood without maintenance in a vacuum during the absorption study resulted in an absorption curve of oxyhemoglobin rather than that of reduced hemoglobin. Samples of pseudocoelomic fluid were reduced and remained deoxygenated. It would appear that chemically deoxygenated Syngamus pseudocoelomic fluid is less sensitive to oxygenation than turkey hemoglobin. However, when the pH of the deoxygenated pseudocoelomic fluid was changed from pH 8 to pH 7 a slight absorption maxima was noted at 535 mμ. This maximum appears to represent a greater sensitivity to oxygen and is suggestive of the "Bohr effect" in which the sensitivity of hemoglobin to oxygen increases with decreased pH.

Rogers (1949b) obtained absorption curves of carboxyhemoglobin after gassing with carbon monoxide. He found a span of about six mμ between the α absorption maxima of oxyhemoglobin and carboxyhemoglobin of Haemonchus and Nematodirus. As has been seen no span of this nature for Syngamus was obtained.

It has been shown that the pseudocoelomic fluid of gapeworms is similar, except in the instances noted, to turkey hemoglobin and to the identified hemoglobins of other nematodes. On the basis of these comparisons and observations it would appear that the pigmented pseudocoelomic fluid

of Syngamus trachea contains a hemoglobin.

Although the functional nature of hemoglobin of nematodes has not been settled it is generally thought that it serves a useful function. Trichinella larvae were shown to have hemoglobin which probably aids in their respiration (Stannard et al., 1938). Davey (1938) and Wharton (1941) suggest that in view of the physical properties of hemoglobin it may act as an oxygen carrier. Rogers (1949a, 1949b) called attention to the high affinity of hemoglobin for oxygen and its low affinity for carbon dioxide. He thought that the hemoglobin may carry oxygen to the cytochromes as well as transporting oxygen to the tissues of the parasites when the partial pressure of oxygen in the medium is below five mm mercury. Davenport (1949) and others state that there is no evidence that hemoglobin carries on a respiratory function. Observations made during this investigation suggest that Syngamus hemoglobin might be functional in nature. The aerobic oxygen utilization was inhibited by carbon monoxide in the presence of light. The effect of carbon monoxide on the utilization of oxygen in the absence of light suggests that Syngamus possesses a cytochrome system similar to that found in mammalian tissue. Carbon monoxide is known to form complexes with heavy metal enzymes and such iron complexes are photolabile. Whether the inhibition of oxygen utilization by carbon monoxide in the presence of light resulted from a combination with a functional hemoglobin, or if it was incomplete dissociation of

cytochrome carbon monoxide complex by certain wave lengths of light was not learned. The well known inhibiting action of azide, cyanide, fluoride, and malonate on cytochromes and other heavy metal enzymes lends support to the observation of the inhibition of carbon monoxide in the absence of light on oxygen utilization and suggests the presence of a functional cytochrome system in gapeworms.

The smallest gapeworms appeared to have the highest rate of oxygen utilization and carbon dioxide liberation. This rate decreased rapidly with increased body weight until about the 28th day, when the decrease became more gradual. This general observation holds true for other parasites (von Brand, 1952), invertebrates and vertebrates (Prosser et al., 1950). It is a well established fact that the decline in respiration with increased weight is largely eliminated if the surface area is taken into account. von Brand (1942), Rogers (1948), and Lazarus (1950) found that with nematodes calculations for surface area do not completely eliminate or explain the size-rate relationship. As with the carbohydrate content, endogenous and exogenous carbohydrate utilization the rate of oxygen consumption appears to be associated with egg production.

Comparison of a brei of the gapeworms with an equal wet weight of intact gapeworms did not resolve the problem of decreased rate of respiration with increased body weight. Oxygen utilization was greater with a brei, probably indicating the elimination of some of the physio-chemical

problems involving permeability and diffusion of gases and nutrients.

The oxygen utilization of Syngamus might be compared with that found by Lazarus (1950) on such blood sucking nematodes as Haemonchus contortus, Ostertagia circumcincta, Strongylus equinus, and S. vulgaris. It would appear that, in general, the oxygen consumption rate of Syngamus is slightly greater than these blood sucking nematodes, and far greater than that of any of the intestinal nematodes which have been studied.

The respiratory quotient of gapeworms (0.887) compares favorably with an average respiratory quotient of animal tissues in which proteins, lipids, and carbohydrates are metabolized. The lack of a higher respiratory quotient in the presence of 0.005 M glucose does not preclude an inability to metabolize carbohydrates. Exogenous and endogenous utilization of carbohydrates by Syngamus has been demonstrated in this investigation. Suitable constituents for carbohydrate metabolism were demonstrated by the use of an acetone powder prepared from gapeworms. Likewise, inhibition of the oxygen uptake by 2,4-dinitrophenol, fluoride, iodoacetate, and malonate demonstrated that some of the well known pathways of carbohydrate metabolism are present in Syngamus.

If manometric studies on gapeworms are conducted over a period of three hours, the absence or presence of glucose in the substrate does not affect the rate of aerobic respiration. A slight increase in the rate of gaseous exchange does take

place after three to four hours in longer determinations only in a substrate containing glucose. This was most pronounced in the smaller gapeworms. It would appear that endogenous nutrients were becoming depleted and a greater amount of exogenous glucose was being utilized to serve the energy requirements of the parasite. This, coupled with the observation of increased exogenous carbohydrate utilization with time, appears to offer some explanation of the role of carbohydrates in Syngamus. That carbohydrates are not the only nutrient utilized by Syngamus might be noted by the respiratory quotient (0.708) which resulted when butyric acid was present in the substrate. The decreased respiratory quotient resulted from decreased carbon dioxide liberation, not from any change in oxygen utilization. This would suggest that butyric acid is not inhibitory but rather is utilized by gapeworms. Utilization of fatty acids, then, as well as carbohydrates, is part of the metabolic complex of Syngamus trachea.

The respiratory quotient of Litomosoides carinii reported by Bueding (1949) and of Ostertagia circumcincta reported by Lazarus (1950) compare favorably with that of Syngamus. Most nematodes of the intestinal tract have low respiratory quotients. Some few have respiratory quotients greater than one, indicating incomplete oxidation of nutrients. In general it would appear that respiratory quotients of parasites parallel the availability of oxygen and type of nutrition in their environments.

The anaerobic respiratory activity of Syngamus was determined by measurement of carbon dioxide liberated by the worms in a nitrogen atmosphere. Under anaerobic conditions the rate of carbon dioxide liberated decreased to about one-half that of the aerobic rate. If the rate of carbon dioxide liberation is considered as a reflection of metabolism one might conclude that the presence of oxygen is of primary importance to the normal metabolism of Syngamus. If anaerobiosis causes a decrease in metabolism, as shown by a decreased carbon dioxide liberation, then the nature of respiration of Syngamus is probably aerobic. This implies that the normal energy and nutritional requirements of the parasite are in excess of those supplied under anaerobic conditions. Even if it appeared that the basic requirements of Syngamus were met by anaerobic metabolism, aerobic metabolism would be a more logical means of satisfying energy and nutritional requirements if only on the basis of the oxygen rich environment in which gapeworms live. The rate of carbon dioxide liberation of Syngamus, when in an anaerobic condition, decreased with increased body size. If the sizes of other nematodes are considered, the differences in rate of carbon dioxide liberation decreases to a point where one might state that there appears to be no difference between the $Q^{N}_{CO_2}$ of Syngamus and the $Q^{N}_{CO_2}$ of tissue inhabiting nematodes, blood sucking nematodes, or intestinal nematodes.

By the use of some of the well known inhibitors of

respiration and metabolism it was demonstrated that Syngamus quite possibly possesses enzyme systems similar to those present in higher animal tissues. Syngamus appears to possess some of the enzymes and components of the Meyerhof-Embden scheme of glycolysis, tricarboxylic acid cycle, and heavy metal enzymes which are probably part of a cytochrome system.

SUMMARY

The infection of chickens and turkeys with Syngamus trachea by various routes of inoculation was studied. Birds were infected by feeding of earthworms containing encysted larvae and by orally or intravenously administered suspensions of larvae. The degree and intensity of gapeworm infections established by means of earthworms or orally introduced suspensions of larvae was highly variable. Quantitative gapeworm infections were obtained by intravenous inoculation of infective larvae. This previously untried route of infection proved to be superior to established methods for obtaining infection. The advantages of this method to investigations of Syngamus and other nematodes was discussed.

The pathway of migration of infective larvae from the intestinal tract to the lungs is proposed and discussed on the basis of observations made concerning route of infection.

Syngamus was recovered from the trachea as early as eight days following intravenous infection and a prepatent period of 14½ days was observed. Oral inoculations resulted in paired gapeworms in the trachea on the ninth day and a prepatent period of 15 days.

After approximately two weeks of age chickens became more difficult to infect and after three to four weeks of age became refractory to infection. Turkeys from one to 186 days of age were easily infected. The intensity of

gapeworm infections in turkeys began to decline after 24 days and by the 37th day infections were negligible, but gapeworms were recovered in a few instances as late as 82 days after inoculation.

The red pseudocoelomic fluid of Syngamus was studied spectrophotometrically and was compared with turkey hemoglobin. The pseudocoelomic fluid appears to contain an iron porphyrin compound which is probably a hemoglobin. This hemoglobin is not identical with that of the host's hemoglobin. These hemoglobins are compared and similarities and differences are noted.

Wet weight and dry weight values covering the life span of Syngamus in the trachea of turkeys are given. The average percent dry weight of the paired Syngamus was 26.2% ± 2.9%.

Total carbohydrate content of paired Syngamus ranged from 1.68% of wet weight in the youngest gapeworms to 0.9% in the oldest. The range of polysaccharides was 0.76% to 0.33%. The increases in polysaccharides which might be contributed in the form of chitin and glycogen are discussed.

Endogenous and exogenous carbohydrate utilization was determined for paired Syngamus. The endogenous total carbohydrate utilization was less when gapeworms were maintained in a glucose substrate than when in a glucose free substrate. Polysaccharide utilization was about the same under both conditions. The rate of exogenous glucose utilization decreased during the life span of the parasite from 3.89 mg/gm in the young worms to 0.23 mg/gm wet weight/hour in the old worms.

There was a pronounced increase in rate of exogenous glucose utilization when gapeworms were maintained *in vitro* for ten days.

The rate of aerobic respiration was most pronounced in the youngest gapeworms and gradually decreased in older worms. There appeared to be a relationship of rate of respiration to the egg producing period and carbohydrate content of *Syngamus*. The rate of oxygen utilization decreased from 18.54 to 4.08 microliters per mg dried weight of the paired *Syngamus* per hour as the worms aged and increased in weight. Oxygen utilization rate of a brei of *Syngamus* was greater than that obtained with the whole paired gapeworms.

The respiratory quotient for *Syngamus* in a phosphate buffered substrate, with or without glucose, was approximately 0.87. In a substrate with 0.065 M butyric acid the respiratory quotient was 0.708; there was no increase in oxygen utilization but there was a decrease in carbon dioxide liberation.

The respiratory quotient of an acetone powder preparation of *Syngamus* in a substrate with glucose was 1.0. Azide and iodoacetate inhibited the rate of oxygen utilization of this powder.

The rate of oxygen consumption of gapeworms was decreased by such inhibitors of respiration and carbohydrate metabolism as azide, cyanide, 2,4-dinitrophenol, fluoride, iodoacetate, and malonate. Oxygen utilization also decreased following gassing with carbon monoxide. Carbon

monoxide inhibition was greater when gapeworms were maintained in darkness for the determination than when present in light.

Carbon dioxide liberation by gapeworms in a nitrogen atmosphere appeared about one-half that which occurred in an aerobic atmosphere. It appeared to be slightly less in a substrate containing 0.005 M glucose than one without glucose or one with 0.065 M butyric acid.

The relationship of aerobic and anaerobic respiration of Syngamus with carbohydrate and butyric acid metabolism is discussed. Parallels between Syngamus and other parasitic nematodes are pointed out.

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