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THE UNIVERSITY OF OKLAHOMA

GRADUATE COLLEGE

A PHYSIOLOGIC AND ANATOMIC STUDY OF CANINE DIROFILARIASIS

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A PHYSIOLOGIC AND ANATOMIC STUDY OF CANINE DIROFILARIASIS

APPROVED BY

DISSERTATION COMMITTEE

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A PHYSIOLOGIC AND ANATOMIC STUDY OF CANINE DIROFILARIASIS

CHAPTER I

INTRODUCTION

The dog heart worm, Dirofilaria immitis Leidy 1856, is a common parasite of members of the canine family in many areas of the world. The disease produced by this nematode varies greatly in intensity. While many lightly infected dogs apparently remain normal, in others right heart failure and death ensue. Between these extremes exists a group of animals which appears normal at rest but develops signs of impaired cardiac and pulmonary function upon exercise. While descriptions of the lesions associated with this parasite have been the subject of numerous reports, there is a paucity of information concerning the pathophysiologic changes occurring in infected animals. Further understanding of these changes is important from several points of view.

D. immitis infection presents a major problem to the dog owner and veterinarian in endemic areas. In these localities there is a high incidence of infection in the dog population and many animals are killed or disabled annually. Present therapy is directed toward killing off the adult and larval forms of the parasite and is unsatisfactory in that further

encroachment on cardiac reserve is not prevented. The long term effect of surgical removal of the adult parasites has not been extensively investigated. Inasmuch as rational therapy depends on a knowledge of the etiology, pathogenesis, and pathophysiology, further studies of the nature of the anatomic and physiologic changes should help to define a therapeutic course as well as to delineate the nature of the functional deficit.

The dog is one of the most frequently used experimental animals and, with the expanding quantity of medical research, larger numbers are required for this purpose. Although some animals are specifically raised for scientific usage, most dogs are acquired from pounds and similar agencies. In the Southern United States up to one-third of the dogs so obtained are infected with D. immitis. Since only outwardly normal animals are utilized, a heavy infection of D. immitis will not be encountered in research, but the problem of the dog with a light infection must be elucidated.

D. immitis infection is unique in that it offers a naturally occurring pulmonary hypertension in a laboratory animal of convenient size. Thus dogs naturally or experimentally infected with this parasite might be used to study various factors occurring in cor pulmonale as they apply to the human beings.

For these reasons further study of D. immitis infection, specifically relating to correlation of the functional and anatomical alterations occurring in the host, was deemed desirable.

The Parasite

The adult forms of D. immitis inhabit the right ventricle and pulmonary arteries (LaPage, 1962). These nematodes are slender white parasites which vary in length from 16 to 30 cm. The posterior end of the male is coiled spirally and the tail has small lateral alae. The ovoviviparous females produce larvae, known as microfilariae, which measure 218 to 329 microns in length and circulate with the blood. This species of nematode is known to occur in Southern Europe, India, China, Japan, Australia, and North and South America.

The existence of worms in the heart of the dog has long been known, the first published reports probably having been made by Panthot (1679) and la Peyronie (1793). It is impossible, however, to determine the exact location of the parasites referred to in these works. Peysson (1806) observed long, slender parasites in the lumen of a dog's heart. The clinical signs exhibited by this animal included convulsions, a cough, and "lack of activity." The presence of the parasite in the United States was reported by Osborne (1847), Leidy (1850, 1856, 1880), French (1899), and Howard (1904). Leidy used the designation Filaria canis cordis in his cases, a name which was later changed to Filaria immitis. The genus Dirofilaria was established by Raillet and Henry in 1911.

In the first half of the Twentieth Century D. immitis infection was diagnosed in many areas of the North American Continent. Incidences of infection as high as 75 per cent were reported in the Southeastern states

(Brown, 1939). The parasite was observed in New Jersey (Ryan, 1919), Virginia (Underwood, 1933), Hawaii (Moss, 1940), California (Roberts, 1946), Mississippi (Ward and Franklin, 1953), Tennessee (Eyles et al., 1954), Canada (Neilson, 1954), and Michigan (Stone, 1957). Some have considered that dirofilariasis kills more dogs than canine distemper (Vet. Med., 1933).

Since most studies on the prevalence of dirofilariasis were based on the occurrence of microfilaria in the peripheral blood, a re-evaluation of the problem was necessitated by the discovery of Dipetalonema reconditum, a second filaroid parasite of dogs previously unrecognized in the United States (Newton and Wright, 1956, 1957). Recent studies show much variation in the prevalence of the two filariids in different parts of the country (Wallenstein and Tibola, 1960; Rothstein et al., 1961; Lindsey, 1961; Thrasher et al., 1963). Rates of infection by D. immitis were observed to be as high as 44 per cent and those of D. reconditum as high as 50 per cent in different localities.

Until the end of the 19th century the life cycle of D. immitis was unknown and many theories were debated. Grassi and Noe' (1900) and Bancroft (1901, 1903, 1904) showed that certain species of mosquitoes were the intermediate hosts of this nematode. Mosquitoes acquire the parasite and later transmit it during the process of feeding. The developmental period in the intermediate host is necessary for maturation of the nematode. Hu (1931) reported transmission of D. immitis by 17 species

of mosquitoes including members of Aedes, Culex, and Anopheles genera. While Brown and Sheldon (1940), Summers (1940), and Steuben (1954) considered that fleas were also intermediate hosts of D. immitis, Otto and Baumm (1959) questioned that these arthropods were capable of transmitting this parasite. Newton and Wright (1957) considered that mosquitoes act as intermediate hosts for D. immitis and that fleas serve as the intermediate host for D. reconditum.

Kume and Itagaki (1955) studied the development of D. immitis in dogs artificially infected with massive doses of larvae. Immature worms were found in large numbers in the submuscular membrane, subcutaneous tissue, adipose tissue, subserosa, and muscles. Migration to the right ventricle occurred via veins 80 to 120 days after infection. Orihel (1961) found that microfilariae appeared in the blood of artificially infected dogs 191 to 197 days after infection.

In addition to the dog (Canis familiaris), D. immitis infection has been diagnosed in several other species of mammals, most commonly in animals relating to the family Canidae. Table 1 gives a partial list of such species in which naturally occurring infections have been observed. Mann and Pratts (1953) produced experimental infections in domestic cats by transplanting adult forms of D. immitis from dogs.

Orihel (1961) discussed seven human cases of filarial infection reported in the United States and indicated that either microfilariae or adult males are necessary for positive species identification. Faust

TABLE 1

SPECIES OF ANIMALS REPORTED TO HARBOR D. IMMITIS

Species	Common Name	Reference
Canis dingo	Dingo	Faust et al., 1941
Canis sp.	Wolf (Japan)	Janson, 1892a
C. rufus floridans	Red Wolf	Faust et al., 1941
C. nubilus	Gray Wolf	Klemper and Moschowitz, 1938 Hartley, 1938
C. occidentalis	Timber Wolf	Coffin, 1944
C. brachyurus		Faust et al., 1941
Felis catus domestica	Domestic Cat	Griffiths et al., 1962 Tornes and Sambol, 1959
F. tigris	Tiger	Faust et al., 1941
F. tigris sondiaca		Faust et al., 1941
F. onca	Jaguar	Faust et al., 1941
	Fox (captive)	Goss, 1942
Vulpes fulva	Fox (wild)	Erickson, 1944
Ondatra zibethica macrodon	Muskrat	LeCompte, 1933 Smith, 1934
O. zibethica zibethica	Muskrat	Goble, 1942
Nasua narica	Mexican Coati	Cabellero, 1944

et al. (1941) described a mature male worm obtained from the inferior vena cava of a resident of New Orleans. Since this nematode differed morphologically from all known species, it was designated Dirofilaria louisianensis. Faust et al. (1952), Sams and Beck (1959), and Jung and Harris (1960) observed subcutaneous nodules in human patients which contained filarial worms. Immature or infertile female nematodes were dissected from some of these lesions. Filarial parasites occurring in

subcutaneous nodules in man have usually been called Dirofilaria conjunctivae, but mature worms have not been collected for morphological study. These workers discussed the possibility that D. conjunctivae is actually D. immitis in an abnormal host.

The adult forms of D. immitis usually inhabit the right ventricle and pulmonary arteries, but are not uncommonly found in other anatomical locations. Otto (1949) considered that the right ventricle is the normal location for these parasites and that they occur elsewhere only when their number is too great for accommodation by this structure. Leidy (1856), Janson (1892a), Hinman (1935), and Jackson et al. (1962) observed cases in which these parasites were present in the right atria and venae cavae. Janson (1892a), Crocker (1919), Hartley (1938), Adams (1956), and von Lichtenberg et al. (1962) observed adult forms of D. immitis in the hepatic veins. The occurrence of this nematode in the renal, jugular, and brachial veins was detected by von Lichtenberg et al. (1962). Janson (1892b) reported the presence of these parasites in the left atria and pulmonary veins of a dog which had a patent foramen ovale. Van Meter (1892) described a dog in which 15 to 20 adult parasites extended from the right atrium through the interatrial septum and left atrium into the left ventricle. Janson (1892b) also referred to the infrequent finding of adult parasites in the cavity of the left ventricle, aorta, or femoral artery.

Schnelle and Jones (1945) reported two cases in which adult forms of D. immitis were found in aberrant locations. In one dog an immature

male occurred in the eye and was observed to move back and forth between the anterior and posterior chambers. An immature female was found in an interdigital cyst of another dog in which adult parasites were also present in the heart. Dibbel (1951), during a surgical operation on a dog, observed a female D. immitis in the abdominal cavity. Leidy (1880) reported the presence of these parasites in the trachea of a dog and Hartley (1938) in the bronchus of a wolf. Turk et al. (1956) detected D. immitis in the bronchioles of one dog and also observed another animal which coughed up the parasites shortly before its death. Tajuchi et al. (1969) reported the presence of D. immitis in the lateral ventricles of the brain of a dog which had symptoms of rabies.

Clinical Manifestations

While D. immitis infections have often been observed at autopsy in dogs which had no clinical signs of disease and died of accidental causes, in other cases this nematode may produce death of the host. The usual signs attributed to infection by this parasite are lack of stamina, dyspnea, and a cough (LaPage, 1962; Smith and Jones, 1961). Edema, venous congestion, hepatomegaly, ascites, and hemoptysis are also reported (Hobbs, 1940; Schnelle, 1945; Dibbel et al., 1950; Patterson, 1962). Various other clinical manifestations, including eczema, vomiting, generalized pain, enlarged lymph nodes, and petechiae of the mucous membranes, have been described (Bader, 1938; Hutyra et al., 1949; Soltys, 1956; Bailey and Hoerlein, 1962). Pollack (1948) observed

eosinophilia in a case of dirofilariasis. In addition to the symptomatology listed above, LaPage (1956) included edema of the hind legs and scrotum, paraplegia, bronchopneumonia, and symptoms resembling those of rabies. Porter (1951) considered the clinical syndrome resulting from D. immitis infection to be that of right heart failure—edema, ascites, hepatomegaly, and easily induced dyspnea.

The clinical manifestations produced by the parasite in aberrant locations vary with the anatomical structures involved. Some cases have been asymptomatic; in others too few cases have been reported to generalize on the clinical disease. Jackson et al. (1962) studied 18 dogs with D. immitis infection of the posterior vena cava. The clinical syndrome exhibited by these dogs included weakness, collapse, hemoglobinuria, bilirubinuria, and rapid progression to death from hepatic and renal failure.

Pathology

The anatomical changes associated with D. immitis infections have been the subject of numerous reports. Probably the first comprehensive study of the pathology of this disease was made by Janson (1892b) in Japan. Hypertrophy and dilation of the right ventricle were common findings, as were aneurysms and thrombosis of the pulmonary arteries. Emboli resulted in pulmonary infarction and abcess formation. There were atelectasis and edema of the lungs, interstitial hepatitis, and parenchymatous and interstitial changes in the kidney.

Hopkins (1906) studied six cases of dirofilariasis and observed the pulmonary arteries of some dogs to be studded with fine granules. Histological examination showed these granules to be subendothelial fibrous nodules.

In a wolf infected with D. immitis, Hartley (1938) observed marked right ventricular hypertrophy and the pulmonary ring was dilated. A thrombus, with entrapped parasites, filled the right ventricle and extended into the pulmonary artery. Aneurysmal dilatations and thrombosis were present in some branches of this artery. The lining of the main pulmonary artery was roughened with shallow pit-like depressions and networks of fibrous bands and strings extended across the lumina of smaller arteries. Many infarcts and areas of pneumonitis were present in the lungs. The liver was enlarged, and both acute and chronic passive congestion were evident. Adult forms of D. immitis were present in a thrombus which filled the right main branch of the hepatic vein.

Histological examination revealed degenerative changes in the myocardium. Fibrous intimal proliferation thickened the wall of the pulmonary artery and organizing thrombi were present in the lumina of some branches. In the lung the alveolar septa were thickened and contained inflammatory cells. Fibrosis of the lung suggested organized infarcts. Centrolobular congestion with atrophy of hepatic cells was evident in the liver and in these areas cavernous sinusoids had replaced the hepatic tissue.

Moss (1940) observed verrucous growths in the heart and pulmonary arteries of Hawaiian dogs infected with D. immitis. Thrombi were present which contained dead parasites. Hepatitis, cirrhosis, and nephritis were also reported.

Porter (1951) studied six D. immitis infected dogs and observed lesions only in the heart, lungs, and liver. There was marked right ventricular hypertrophy. In some areas of the pulmonary artery there was subintimal fibrosis and in other areas nodules of subintimal connective tissue projected into the lumen. Endothelial proliferation narrowed the lumina of smaller pulmonary arteries and medial hypertrophy occurred in pulmonary arterioles. There was marked hepatomegaly.

Nissen (1953) and Weatherford (1954) described cases (probably the same dog) in which adult forms of D. immitis were present in the right side of the heart, posterior vena cava, and [sic] portal circulation. In the liver there was marked congestion of central veins and loss of hepatic cells in the central areas of hepatic lobules. The latter was often so complete that these areas seemed to consist only of dilated blood spaces and connective tissue. Localized inflammatory foci were present in the alveolar walls of the lungs and various degrees of thrombosis were observed in the intrapulmonary arteries.

Adams (1956) described a dog in which the lungs, pulmonary arteries, right heart, vena cava, and hepatic veins contained adult forms of D. immitis. Histological examination of the lung revealed broncho-

pneumonia and the liver displayed an increase in interlobular connective tissue which encroached upon the hepatic lobules.

The group of dogs studied by Hennigar and Ferguson (1957) contained both symptomatic and asymptomatic cases of dirofilariasis. In these dogs dilatation and hypertrophy of the right heart was common and occasionally there was hypertrophy of the left heart. Atherosclerosis and endothelial cushions were observed in the pulmonary arteries. Medial hypertrophy and endothelial thickening were present in smaller arteries, and medial hypertrophy occurred in arterioles.

Jaques and Hyman (1957) observed villous intimal thickening in the large pulmonary arteries of 17 dogs infected with D. immitis.

Balch et al. (1957) studied dogs which had been treated with an arsenical compound to destroy the adult forms of D. immitis. Fibrosis and calcification of the tricuspid valve were present in one dog. Microscopic study revealed fragments of necrotic worms surrounded by partially calcified fibrous connective tissue. Granulomatous reactions occurred in the walls of arteries which contained dead worms. The thrombi which formed around parasites in the arterial lumina were eventually disintegrated.

Bailey (1958a) observed right ventricular hypertrophy and fibrous thickening of the right ventricular endocardium in untreated cases of dirofilariasis. There was sclerosis of the pulmonary artery and the intima had a "pebbled" appearance. Masses of worms were entrapped in

gelatinous thrombi. Pulmonary infarcts were observed following arsenical treatment to kill the adult nematodes and organized thrombi were associated with degenerating parasites (Bailey, 1958b). Purulent inflammatory reactions and recanalization were apparent in arteries which had undergone thrombosis. A subacute inflammation of the portal triads was reported to be present in the liver.

Winter (1959), working with dirofilariasis in Australian dogs, observed the lungs to be firm in consistency, rust-brown in color, and to contain irregular nodules of consolidation. The livers were enlarged and congested, and there was slight splenomegaly. In advanced cases the kidneys had a brown discoloration. Microscopic examination of the lungs revealed inflammatory infiltration, hemosiderosis, and fibrosis. Congestion, erythrophagocytosis, hemosiderosis, and anthracosis were observed in the lymph nodes. The spleens were congested, contained increased amounts of hemosiderin, and passive congestion was apparent in the livers. Hemoserosis occurred in the livers and was present in the kidneys of one dog.

Wilcox (1960) reported pulmonary endarteritis and intimal proliferation in 15 of 20 dogs from which adult forms of D. immitis were removed surgically.

While Adcock (1961) observed congestion, edema, hemorrhage, hemosiderosis, infarction, and interstitial pneumonitis in the lungs of D. immitis infected dogs, he considered these lesions to be less

significant than those of the pulmonary arteries. In these vessels inflammatory and proliferative fibrosis of the intima and media were associated with living parasites and a granulomatous response was present in arteries containing dead parasites. Rugose or villous structures, consisting of plump, loosely arranged fibroblasts with an endothelial covering extended into the lumina of large elastic arteries. Fibrosis of intima and media, which often contained infiltrations of inflammatory cells, was also observed. Proliferation of the intima, and less often the media, were observed in small muscular arteries and arterioles. Thrombosis occasionally resulted in pulmonary infarction.

The lesions occurring in 15 dogs infected with D. immitis were described by von Lichtenberg et al. (1962). Twelve of these animals had the clinical syndrome associated with the presence of adult parasites in the posterior vena cava. Microscopically, the most constant lesion was found to be rugose or villous endarteritis. Thrombosis was a common finding, occurring both in the main branches and in the distal intrapulmonary branches of the pulmonary artery. Thrombosis or embolization of arterioles occurred sporadically. Intimal and muscular proliferation thickened the walls of small arteries. Granulomatous lesions were associated with both living and dead worms. Congestion, interstitial fibrosis, focal inflammation, and thickening of interalveolar capillaries occurred in the lungs of most animals.

The livers exhibited bile stasis, centrolobular necrosis, and fibrosis. Increased numbers of leucocytes and histiocytes were present in

sinusoidal vessels, sometimes forming small nodules. A "cavernomatosis change of the hepatic veins" was noted in the livers of ten of the dogs which had adult parasites in the posterior vena cava. Here the centrolobular venule had been replaced by many dilated vessels. These thin-walled, dilated vessels were said to resemble cavernous capillary hemangiomata. Phlebosclerosis of the larger collecting veins, and rarely thrombosis, were associated with the carernomatous change.

Passive congestion and microfilariae were present in other organs. Small interstitial foci of lymphocytes and plasma cells occurred in the cardiac muscle. Hemosiderosis was commonly observed in the kidneys.

Jaques (1963) studied 22 dogs infected with D. immitis and observed lesions in the lungs, heart, liver, spleen, and kidneys. Infarcts were present in the lungs, and the intima of the pulmonary arteries had a rugose appearance. Eccentric thickening of the intima, often in filariform configurations, was accompanied by endothelial proliferation. At times lymphocytes and plasma cells had infiltrated these fibroblastic thickenings. Microfilariae were observed in association with thrombosis of small veins and arterioles, and granulomatous inflammation, without giant cells, occurred in the older lesions. Inflammatory reactions, apparently produced by dying microfilariae, were present in the hearts of four dogs. These lesions, characterized by perivascular infiltrations of polymorphonuclear leucocytes and chronic inflammatory cells, often extended into the adjacent myocardium. In the livers there were

hyperplasia of Kupffer cells and occasionally focal areas of necrosis. The etiology of renal lesions was confused by the concomitant presence of pyelonephritis.

Pathophysiologic Manifestations

In reviewing the rather voluminous body of literature regarding canine dirofilariasis, the small number of studies dealing with the functional changes occurring in infected animals was striking. Blackberg and Ashman (1930) observed no electrocardiographic differences between controls and infected dogs in the resting state. After exercise, consisting of a ten minute run on a treadmill, marked changes were noted in the infected animals. The QRS interval was lengthened and there was decreased amplitude of the R wave. In one dog, the T wave was inverted. These alterations were considered to indicate depression of intraventricular conduction and inadequate blood and oxygen supply of the cardiac muscle. Patterson et al. (1961) did not observe diagnostic electrocardiographic changes in cases of dirofilariasis.

Jackson (1962) reported an increased circulation time in dogs infected with D. immitis. In normal dogs, fluorescein injected intravenously in the foreleg was detected in the nictitating membrane or oral mucosa in less than 12 seconds. This interval was found to be greater than 12 seconds in animals infected with D. immitis.

Wallace (1959, 1962) and Wallace and Hamilton (1962) reported elevated pulmonary arterial and right ventricular pressures in dogs

infected with D. immitis. Systolic pressures as high as 158 mm. Hg. were observed in severely afflicted animals. Beasley and Jaques (1963) observed mean pulmonary arterial pressures ranging from 6 to 73 mm. Hg. in a group of infected dogs having no clinical indications of infection.

Wallace (1959) and Wallace and Hamilton (1962), in studies of canine congestive heart failure and including dogs infected with D. immitis, reported increased blood volumes and marked salt and water retention. Severe pulmonary hypertension and right ventricular hypertrophy were observed.

CHAPTER II

EXPERIMENTAL PROCEDURES

Twenty-three dogs with D. immitis infection were used during the course of this study. Eight of the animals were acquired at the Houston City Dog Pound, Houston, Texas, and 15 through the Department of Vivarium, Baylor School of Medicine. In the selection of these dogs, no consideration was given to age, sex, breed, or size; the sole criterion being the presence of microfilariae in smears of peripheral blood. No attempt was made to determine the species of microfilariae. None of the dogs had symptoms of congestive heart failure or other signs of dirofilariasis. Two of the animals had a generalized dermatitis which responded to repeated topical applications of chlordane (Engo[1]), and three were in a rather poor nutritional state. The latter was promptly corrected by isolation and proper diet.

Nine uninfected control dogs were obtained from dog pounds in the area of central Oklahoma. Neither the presence of microfilariae in blood smears nor evidence of cardiac or pulmonary disease were observed in these animals. In selecting the control dogs an attempt was made to

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[1]Fort Dodge Laboratories

include animals comparable in age and size to the dogs in the infected group; however, an adequate number of aged dogs was not available.

All dogs were separately caged in screened quarters in the Animal House at the University of Oklahoma Medical Center, and were fed a commercial ration.

Physiologic and Anatomic Alterations of D. Immitis Infection

Eighteen infected dogs and nine controls were utilized in this phase of the project. Multiple pulmonary artery, right ventricular, and femoral artery pressures and electrocardiograms were taken of each animal at intervals of two to four weeks. These procedures were carried out under sodium pentobarbital (Nembutal[1]) anesthesia. Although the usual methods of sterile surgery were practiced, each dog received antibiotic therapy (Strep-Combiotic[2]) for three days following catheterization.

Pressures in the lesser circulation were measured by right-sided cardiac catheterization through No. 5 or 6 Cournand catheters and recorded with a Sanborn Model 267BX pressure transducer and Sanborn 150 Polyviso carrier preamplifier. After the jugular vein had been isolated surgically and ligated, the catheter was inserted through a small incision proximal to the ligature and passed through the right heart into the pulmonary artery. The presence of the catheter in the artery was

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[1]Abbott Laboratories

[2]Pfizer

verified fluoroscopically in half of the dogs and in others by the pressure readings and curve characteristics occurring during its withdrawal. Heparinized saline (one unit per ml.) was perfused through the catheter in ten of the experiments and in the remainder only isotonic saline was used.

Femoral artery pressures were measured through a Henry needle inserted directly into the artery and recorded with the Sanborn preamplifier. In a few dogs it was necessary to expose the artery surgically before insertion of the Henry needle was possible.

Electrocardiographic studies were made on each dog following the pressure recordings while the animal was still under the effect of the anesthetic. These studies were made with a Model 15-1600 Sanborn ECG preamplifier. The areas of attachment for the electrodes were shaved to insure adequate contact and standard leads 1, 2, 3, and V1, V2, V3, and V4 were recorded.

Following these studies euthanasia was performed with an overdose of sodium pentobarbital. At necropsy, adult parasites were removed from the right ventricles and pulmonary arteries and the gross lesions observed. In an effort to detect early right ventricular hypertrophy, the hearts were dissected in the manner to be described below and the weights and thicknesses of the various parts were recorded. Tissue sections were prepared from the lungs, pulmonary arteries, heart, aorta, stomach, liver, intestine, pancreas, gall bladder, adrenal glands, thyroid and parathyroid glands,

brain, spinal cord, eyes, and genital tract of each dog, and from other tissues when indicated. Multiple blocks of tissue were taken from standard locations in each lobe of the lungs, although other areas were sectioned when lesions were suspected. The site from which the routine blocks were taken was an area approximately one cm. from the hilus, thus including the major bronchi and large elastic arteries.

All blocks of tissue were fixed in 10 per cent neutral formalin, embedded in paraffin, and sectioned at a thickness of five microns. The sections were stained by the Harris hematoxylin and eosin method. Weigert's elastic tissue stain counterstained with Van Gieson's picric acid-fuchsin mixture was employed on selected sections of lung and pulmonary artery. Masson's trichrome, Prussian blue, reticulum, Sudan IV, Gram, Giemsa, acid fast, and periodic acid-Schiff stains were also used.

Effect of Treatment to Destroy Adult Worms

Five dogs with D. immitis infection were used to determine the effect of dead parasites on the pulmonary circulation. Pulmonary artery, right ventricular, and femoral artery pressures were measured as before and electrocardiographic studies were made. Each dog then received three intravenous doses of sodium p-bis (carboxymethylmercapto) arsinobenzamide (Filcide[1]) in the amount of 0.2 ml. (arsenic 0.36 mg.) per pound of body weight on three consecutive days. At three and five weeks

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[1]Pitman-Moore Co.

after treatment pressures were again measured and the electrocardiograms repeated. Euthanasia was performed on four of the dogs immediately after the last catheterization and necropsies were conducted. Tissues were subjected to the procedures described previously. The fifth dog was held for 82 days after treatment. Euthanasia was then performed and, in addition to the usual procedures, blocks of tissue from the lung were obtained for electron microscope study.

Determination of Cardiac Hypertrophy

In order to detect early right ventricular hypertrophy it was necessary to know the normal range in weight and thickness of the various parts of the canine heart, their relationship to each other, and the relationship of heart weight to body weight. This was accomplished by dissecting and weighing the hearts from 50 normal dogs. All of these dogs were apparently in good health and no gross lesions were observed in the heart, lungs, liver, or kidneys. After breed, age, sex, and weight of each dog was determined, the heart and lungs were removed and the heart was severed from the major vessels. Dissection of the hearts was carried out in a modification of the manner described by Stewart (1911).

Using the left longitudinal groove as a guide, the right ventricle and interventricular septum were separated from the level of the orifice of the pulmonary artery to the apex of the heart. The process was repeated separating the left ventricle and septum, beginning at the

aortic orifice and extending to the apex, still using the left longitudinal groove as a guide. The heart was then opened, washed, and the chordae tendineae severed. After reflecting the valves, the auricles were separated from the ventricles by cutting along the line of valvular attachment. Separation of the ventricle and interventricular septum was completed by extending the incisions from the apex of the heart back to the respective orifices, using the right longitudinal groove as a guide. The portions of the heart were washed and dried, and the fat was trimmed away from the muscle. After weighing, the thickness of the right ventricle was determined at the center of the pulmonary outflow tract. The thickness of the left ventricle was measured at a point midway between the papillary muscles, and that of the interventricular septum at its center. Weights and thicknesses were recorded for statistical analysis. The hearts of the dogs with D. immitis infection and those of the nine controls were subjected to these same procedures so that comparison would be possible.

Determination of length and interval of myocardial nuclei, utilizing the method of Linzbach (1956), was employed to obtain further evidence of cardiac hypertrophy. The right ventricles of the nine control dogs and those of the infected dogs, which exceeded the expected weight, were sectioned at a thickness of 12 microns and stained with hematoxylin and eosin. The lengths of 100 nuclei and 100 internuclear distances, when three consecutive nuclei were present in a single fiber, were measured in each dog. An eyepiece micrometer, which had previously

been calibrated with a stage micrometer, was used in this procedure. All measurements were made with the high dry objective at a magnification of 450X. From this data the mean nuclear length, mean internuclear distance, and mean length of the myocardial muscle fiber segment (mean nuclear length plus mean internuclear distance) were calculated for each dog. Statistical methods were utilized to determine any real difference between these values in the control group and those of the infected dogs.

Preparation of Tissue for the Electron Microscope

Tissue from the lungs of dog No. 4, which had been treated to destroy the adult parasites, was prepared for study with the electron microscope. Small blocks of tissue were removed from both apical lobes and fixed in 1.0 per cent buffered Palade Solution at 4° C. Tissues were then embedded in Maraglas Mixture C (Freeman and Spurlock, 1962) and sectioned at a thickness of 500 to 600 A° using glass knives in a Porter-Blum microtome. The sections were placed on 200-mesh copper grids coated with Formvor film, stained with lead hydroxide, and examined with a Model 3FD RCA electron microscope.

In conjunction with this work microfilariae were subjected to selected histochemical procedures. Following fixation in alcohol or acetone smears of blood containing larvae were stained by the Feulgen method, periodic acid-Schiff, oil red 0, alcian blue, Toluidine blue, methyl green-pyronine, and Astrablau at pH 0.25. Some slides were subjected to malt diastase digestion prior to staining with periodic

acid-Schiff; others were stained without periodic acid oxidation. Acridine orange stains were made following fixation in acetone.

CHAPTER III

RESULTS

Measurements of the Normal Canine Heart

The group of dogs from which hearts were obtained for measurement was made up of eight Hounds, six Cocker Spaniels, two Boxers, two Collies, two Pointers, one German Shepherd, one Weimaraner, and 28 mongrels of undetermined breeding. Twenty-eight of the dogs were males and 22 were females. Six of the animals were considered to be under the age of one year, 32 to be more than one but less than four years, and 12 to be greater than four years old. Table 2 gives the sex, approximate age, body weight, and weight and measurements of the hearts of these 50 normal dogs.

Weight of the Heart and Component Parts

The wide range in body weight, 6.4 to 29.2 kg., presented by this group of dogs is paralleled by a large range of values in the weight of the heart and its component parts. In order to reduce these large variations to more workable values, and in accordance with previous work, ratios of heart weight to body weight and percentages of the component

TABLE 2

BODY WEIGHT AND HEART MEASUREMENTS OF 50 NORMAL DOGS

Age (years)	Body Wt. (kg.)	Ventricular Mass						Atria (gm.)	Total Heart (gm.)
		R.V.		L.V.		I.V.S.			
		(gm.)	(mm.)	(gm.)	(mm.)	(gm.)	(mm.)		
Females									
2	6.4	12	4	25	15	11	12	6	51
3	7.3	14	4	27	12	12	13	5	58
2	7.7	11	3	21	10	11	11	5	48
4	8.1	12	3	23	9	11	10	6	52
1	8.3	13	5	23	10	11	12	6	53
3	8.6	15	5	28	10	14	10	7	64
0.5	9.7	17	4	33	10	16	12	8	75
2	10.1	15	3	32	15	16	11	9	72
3	11.4	17	4	32	9	12	10	10	71
3	11.6	17	3	34	12	15	13	8	74
3	12.0	22	4	43	12	19	13	8	92
0.7	12.2	14	4	28	10	12	12	8	62
2	12.9	20	5	40	12	18	12	9	87
2	13.6	19	4	39	18	18	14	11	87
2	14.3	25	5	42	13	26	13	11	104
3	15.5	22	5	42	15	18	15	11	93
2	16.6	23	4	46	17	22	12	12	103
5	16.6	29	5	57	13	31	13	15	132
4	17.2	28	5	60	13	25	15	14	127
2	17.6	33	5	68	17	33	12	12	146
2	19.1	29	5	54	13	29	15	12	124
Old	24.9	29	6	69	17	27	19	18	143

TABLE 2 - Continued

Age (years)	Body Wt. (kg.)	Ventricular Mass						Atria (gm.)	Total Heart (gm.)
		R.V.		L.V.		I.V.S.			
		(gm.)	(mm.)	(gm.)	(mm.)	(gm.)	(mm.)		
				Males					
2	7.3	15	4	25	9	13	10	7	60
3	8.3	17	4	30	10	15	11	9	71
2	8.6	15	5	32	16	18	12	10	75
1	9.3	13	4	26	9	14	10	7	60
3	10.9	18	4	37	15	17	13	11	83
5	12.0	21	5	44	12	17	10	10	92
2	12.3	17	3	40	13	16	16	9	82
0.5	12.4	14	3	37	10	15	10	5	71
3	13.4	21	5	40	13	22	10	10	93
3	13.5	20	4	46	12	24	15	11	101
3	13.8	22	5	42	12	17	13	10	91
5	14.1	28	4	52	14	23	12	14	117
2	15.0	16	4	40	10	16	11	10	82
2	15.5	21	5	42	17	21	10	12	96
3	17.7	26	5	48	13	25	12	13	112
3	18.0	25	5	55	13	23	14	11	114
4	18.1	35	6	71	15	33	16	17	156
0.5	18.1	22	4	44	12	23	13	10	99
Old	18.5	29	6	69	11	26	14	13	137
3	18.6	24	4	48	12	27	11	13	112
3	18.8	31	4	61	16	29	15	13	134
3	20.2	27	5	52	12	28	18	12	119
3	23.1	42	7	90	12	39	12	17	188
Old	24.5	28	5	62	16	26	15	12	128
4	25.6	38	5	85	14	34	19	15	172

TABLE 2 - Continued

Age (years)	Body Wt. (kg.)	Ventricular Mass R.V. (gm.)	R.V. (mm.)	L.V. (gm.)	L.V. (mm.)	I.V.S. (gm.)	I.V.S. (mm.)	Atria (gm.)	Total Heart (gm.)
				Males					
Old	28.3	42	7	101	16	41	16	21	205
Old	28.6	48	5	102	15	50	14	33	233
3	29.2	40	7	87	20	44	15	27	198
Range	6.4-29.2	11.0-48.0	3-7	21.0-102.0	9-20	11.0-50.0	10-19	5.0-33.0	48.0-233.0
Mean	15.11	23.02	4.58	47.42	13.04	22.04	12.92	11.46	104.16
S.D.	5.83	8.72	0.98	20.04	2.64	9.02	2.31	5.12	41.98

parts of the heart were calculated. Heart weight/body weight ratios were expressed as grams per kilogram. Weights of the ventricles and the interventricular septum were expressed as percentages of the combined weight of the ventricular mass (right ventricle + left ventricle + interventricular septum). Table 3 gives the range and mean of these values calculated from data obtained from the 50 dogs.

TABLE 3

RANGE AND MEAN OF HEART WEIGHT/BODY WEIGHT RATIOS AND CARDIAC COMPONENT PERCENTAGES OBSERVED IN 50 NORMAL DOGS

	Range	Mean
Heart Weight/Body Weight (gm/kg)	5.08 - 8.72	6.94
R. Ventricle/Ventricular Mass * %	20.21 - 28.30	25.12
L. Ventricle/Ventricular Mass * %	45.16 - 56.06	50.98
I.V. Septum/Ventricular Mass * %	19.67 - 27.95	23.87

* RV + LV + IVS

Since weight of the muscular mass is the point of interest in cardiac hypertrophy, these ratios and percentages were determined using weights of the hearts after the removal of the fat. The weight of fat removed from the hearts had a range of 2.0 to 17.0 gm., a mean of 6.54 gm., and standard deviation of 3.45 gm.

The weight of the left ventricle made up approximately one half of the weight of the ventricular mass. The weight of the right ventricle was essentially half that of the left and was approximately equal to the weight of the interventricular septum. However, the wide range in these ratios indicated the great variation occurring among individual dogs.

In order to determine the possible influence of body weight on the HW/BW ratio and on the percentages of the various components of the heart, the dogs were divided into four groups according to body weight. The HW/BW ratio and the cardiac component percentages were then calculated for the various weight groups and the results are shown in Table 4.

TABLE 4

MEAN HEART WEIGHT/BODY WEIGHT RATIOS AND CARDIAC COMPONENT PERCENTAGES OF 50 DOGS GROUPED ACCORDING TO BODY WEIGHT

Body Weight	No. Dogs	HW/BW*	RV/VM%	LV/VM%	IVS/VM%
6.0 - 11.9 kg	15	7.29	26.01	49.78	24.20
12.0 - 17.9 kg	20	6.82	25.06	51.26	23.66
18.0 - 23.9 kg	9	6.83	24.78	50.87	24.33
24.0 - 29.9 kg	6	6.65	23.62	53.26	23.11

* gm/kg

Dogs in the lightest weight group had a higher HW/BW ratio and RV/VM percentage and lower LV/VM percentage than those in the

heavier weight groups. Thus it appeared that the relative weights of the component parts change as the heart increases in size. In order to properly evaluate these factors linear regression studies were made on the data obtained from the 50 dogs.

Using the equation $Y = a + bX$, where b is the regression coefficient, regression lines were fitted to heart weight (Y) on body weight (X), ventricular weight (Y) on weight of the ventricular mass (X), and weight of the interventricular septum (Y) on weight of the ventricular mass (X). The 95 per cent confidence belts for each line were then calculated. Table 5 gives the regression coefficient (b), standard deviation from regression (Sy.x), standard deviation of regression coefficient (S_b), and limits of the 95 per cent confidence belt for each of the calculations.

TABLE 5

REGRESSION DATA OF HEART WEIGHT ON BODY WEIGHT AND OF VENTRICULAR AND I.V. SEPTUM WEIGHTS ON WEIGHT OF THE VENTRICULAR MASS OBTAINED FROM 50 DOGS

	b	Sy.x	S_b	Limits of Confidence Belt for Y	
HW/BW	6.684	15.44	0.373	$\pm \frac{(x=7.3\ kg.)}{37.02\ gm.}$	$\pm \frac{(x=30.0\ kg.)}{38.53\ gm.}$
RV/VM	0.231	1.135	0.004	$\pm \frac{(x=40\ gm.)}{2.35\ gm.}$	$\pm \frac{(x=210\ gm.)}{2.52\ gm.}$
LV/VM	0.532	2.232	0.008	$\pm$ 4.61	$\pm$ 4.94 gm.
IVS/VM	0.263	1.857	0.007	$\pm$ 3.84 gm.	$\pm$ 4.11 gm.

Regression of heart weight on body weight and the 95 per cent confidence belt are shown in Figure 1. Figure 2 shows the regression of

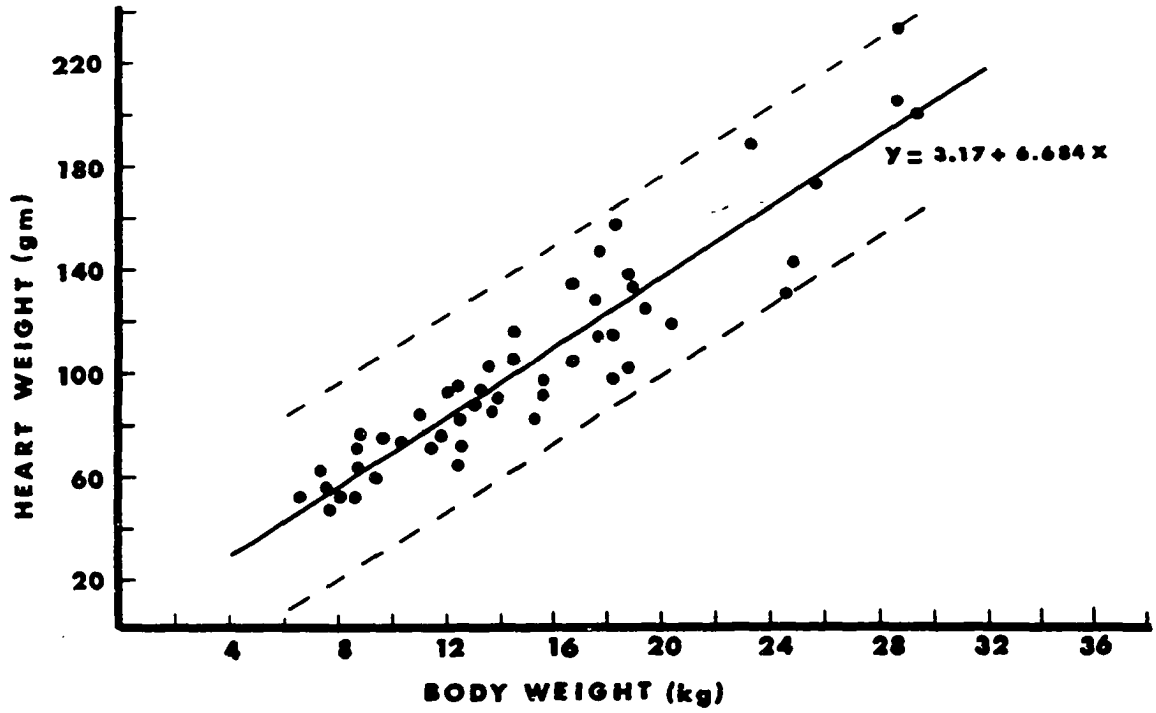


Figure 1. Regression of heart weight on body weight (50 normal dogs). The broken lines indicate the 95 per cent confidence belt.

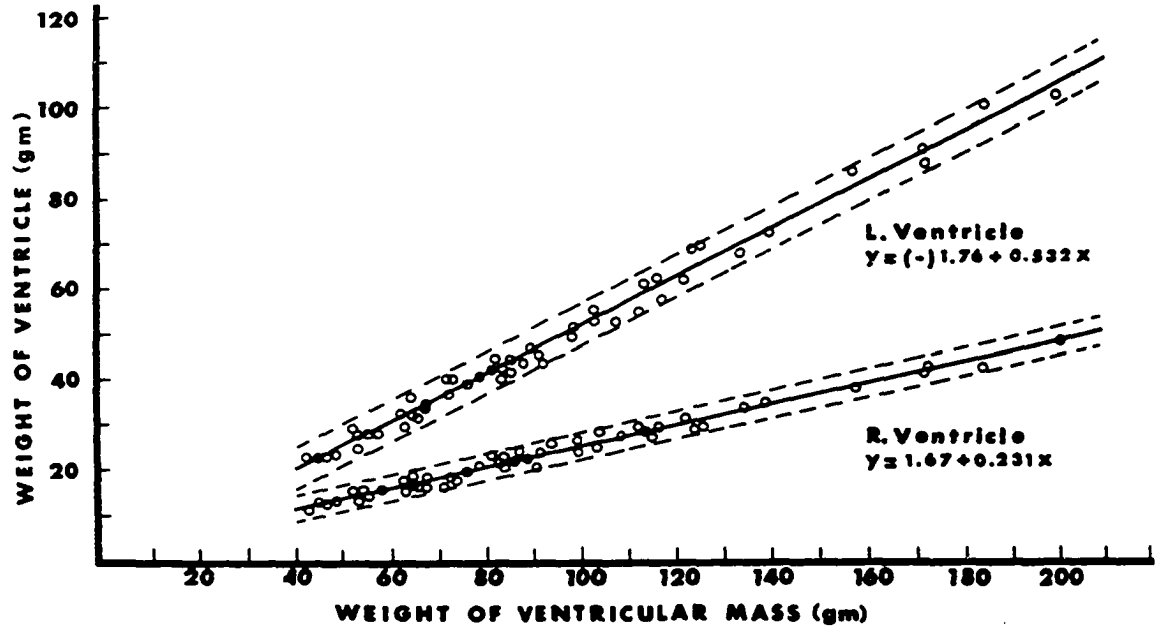


Figure 2. Regression of ventricular weights on weight of the ventricular mass (50 normal dogs). The broken lines indicate the 95 per cent confidence belts.

weight of right and left ventricles on the weight of the ventricular mass and the respective 95 per cent confidence belts. The formula for regression of weight of interventricular septum on weight of ventricular mass is

$$Y = 2.27 + 0.0263X .$$

The possibility of a difference in the relationship of heart weight to body weight by sex was investigated by comparing the slope of the regression lines obtained from data on the male dogs with that of the females. Figure 3 shows the regression of heart weight on body weight. The regression coefficient (b) for the males was 6.833 and that of the females was 6.243. Figure 4 shows the regression of weight of the right ventricle on the weight of the ventricular mass. The regression coefficient (b) for the males was 0.232 and that of the females was 0.235. Little difference is noted between the sexes.

In order to further determine the possibility of sex difference in cardiac weight, the HW/BW ratios and the RV/VM percentages of the 28 male dogs were compared with those of the 22 females. The mean HW/BW ratio of 7.00 and standard deviation of 1.02 in the males contrasted with a mean of 6.83 and standard deviation of 0.83 in the females. Analysis of these data by Student's "t" gave a t-value of 1.512 with 48 degrees of freedom, for which $0.7 < P < 0.8$; thus indicating no significant difference between the sexes. The mean RV/VM

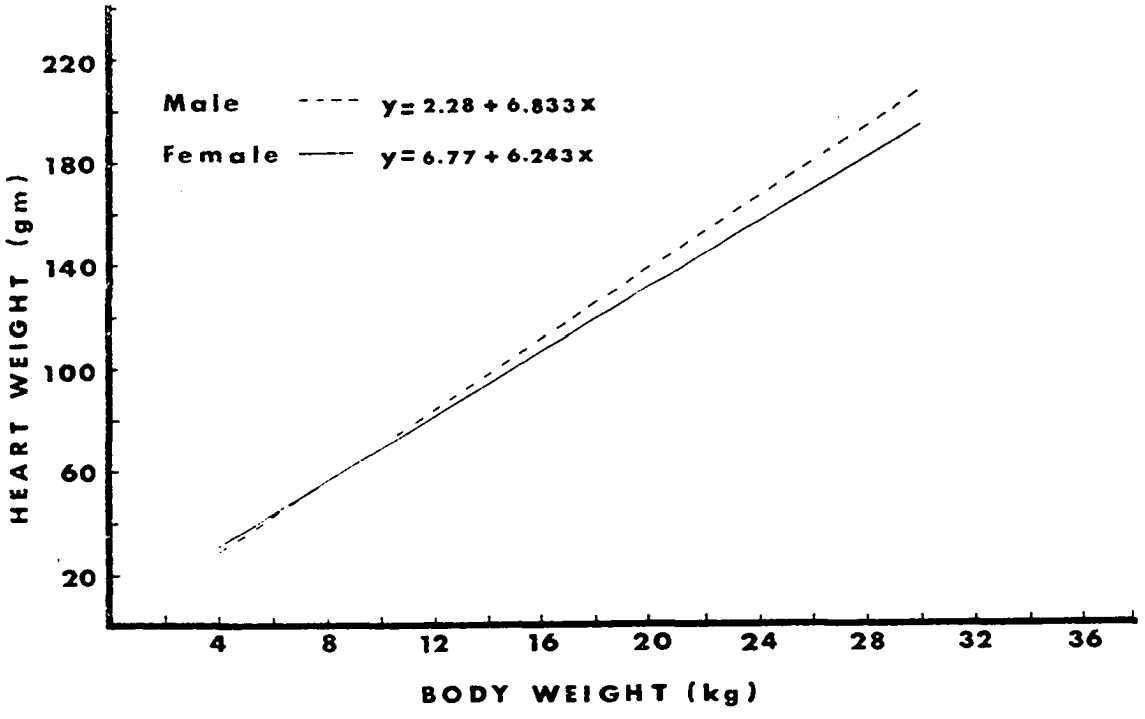


Figure 3. Comparison of regression of heart weight on body weight in male and female dogs (50 normal animals: 28 males, 22 females).

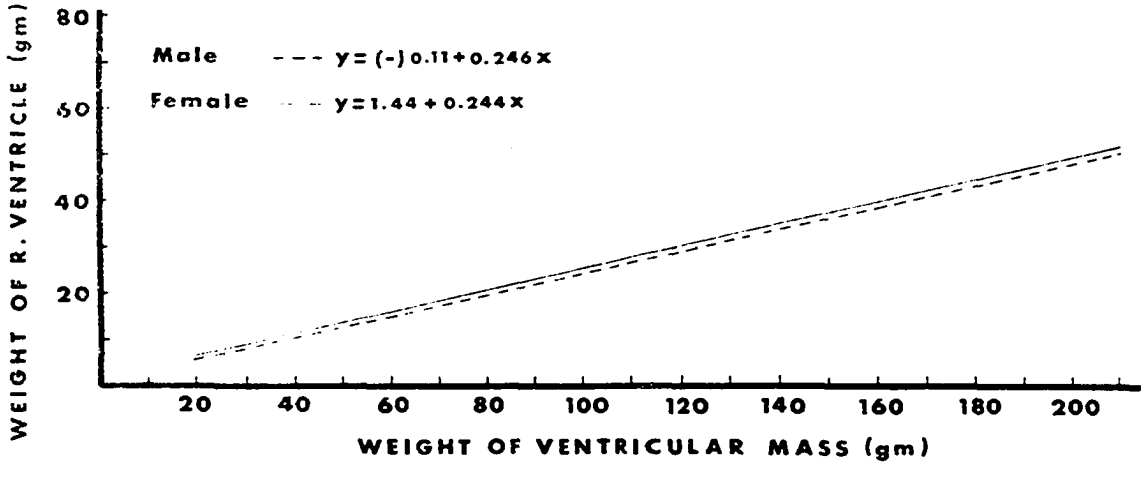


Figure 4. Comparison of regression of right ventricular weight on weight of the ventricular mass in male and female dogs (50 normal animals: 28 males, 22 females).

percentage for the males was 24.57 with a standard deviation of 1.63 and that of the females was 25.62 with a standard deviation of 1.05. Analysis of these data by Student's "t" gave a t-value of 2.527 (df = 48) for which $0.02 < P < 0.01$. P-values at this level are of borderline significance.

Thickness of the Ventricles of the Canine Heart

The ranges in thickness of the various parts of the heart were as great as those of the weights. Values of the greatest magnitude occurred in heavier hearts from the larger dogs. Table 2 gives the range, mean, and standard deviation in thickness of the ventricles and interventricular septa observed in the 50 dogs. The thickness of the left ventricle and that of the interventricular septum were essentially equal and were approximately three times as thick as the right ventricle.

In order to better define the normal limits, linear regression studies relating thickness of the ventricles (Y) to weight of the ventricular mass (X) were made on the data from the 50 dogs. The 95 per cent confidence belts for "Y" were then calculated. These data are shown in Table 6.

Regression of thickness of the right ventricle on weight of the ventricular mass and the 95 per cent confidence belt is shown in Figure 5. Figure 6 shows the regression of thickness of the left ventricle on weight of the ventricular mass and the 95 per cent confidence belt.

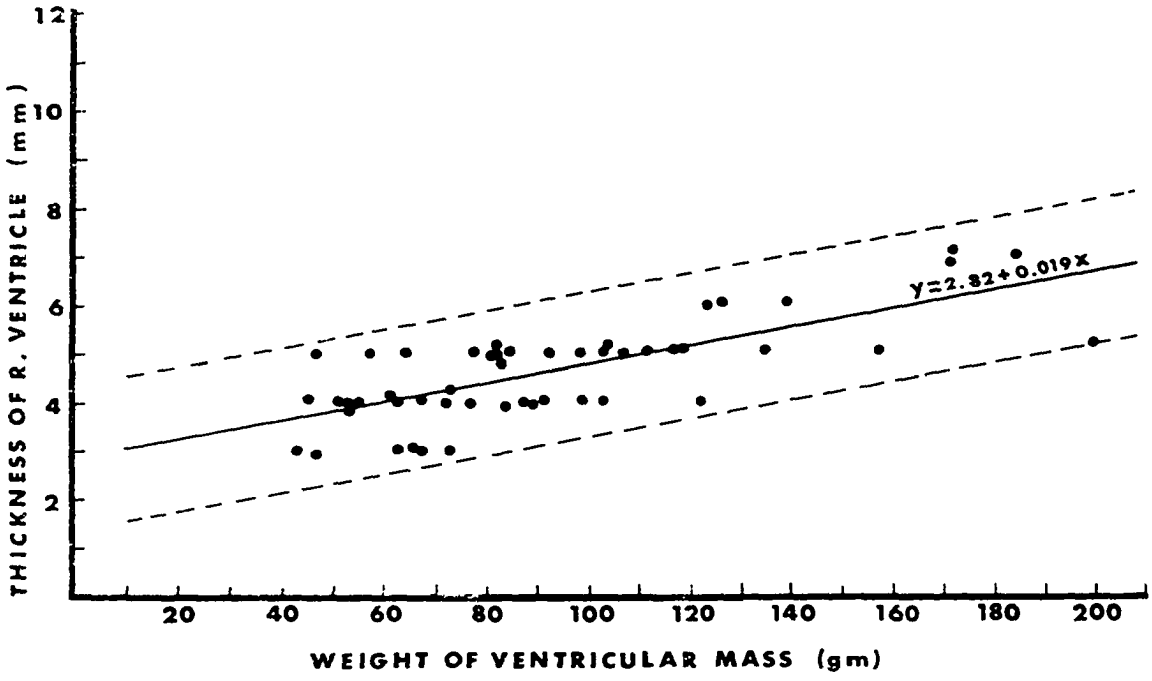


Figure 5. Regression of right ventricular thickness on weight of the ventricular mass (50 normal dogs). The broken lines indicate the 95 per cent confidence belt.

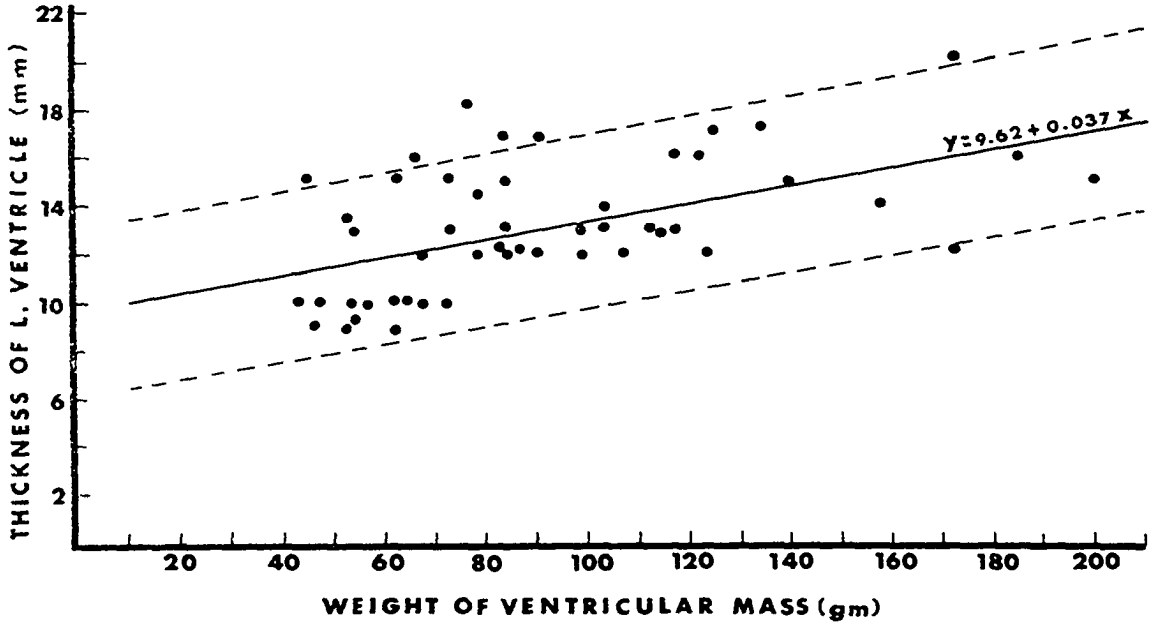


Figure 6. Regression of left ventricular thickness on weight of the ventricular mass (50 normal dogs). The broken lines indicate the 95 per cent confidence belt.

TABLE 6

REGRESSION DATA OF THICKNESS OF THE VENTRICLES ON WEIGHT OF THE VENTRICULAR MASS OBTAINED FROM DATA FROM 50 NORMAL DOGS

	b	Sy.x	S_b	Limits of Confidence Belt for Y	
				X=40 gm.	X=210 gm.
RV/VM	0.019	0.692	0.0026	± 1.43 mm.	± 1.54 mm.
LV/VM	0.037	1.676	0.006	± 3.47 mm.	± 3.72 mm.

Physiologic and Anatomic Alterations

Control Dogs

The nine control dogs included four mongrels, two Cocker Spaniels, one Chow, one Beagle, and one German Shepherd. Five of the dogs were females and four were males. In age, four of the dogs were approximately one year old, four were more than one but less than four years, and one dog was considered to be more than four years old.

Hemodynamic study. A great deal of variation was observed in both the systemic and pulmonary pressures in the dogs of this group and in the two measurements made on individual animals. Table 7 gives these pressures for each of the control dogs. The mean systolic pulmonary arterial pressure for the group (24 mm. Hg.) is slightly lower than expected; however, the means of the diastolic (9 mm. Hg.) and mean

TABLE 7

PULMONARY ARTERIAL, RIGHT VENTRICULAR, AND FEMORAL ARTERIAL PRESSURES OF THE CONTROL DOGS
(values mm. Hg.)

Dog	Pulmonary Artery			Right Ventricle				Femoral Artery		
	S	D	M	S	D	M	End.	S	D	M
V	19	6	11	24		6	3	135	110	125
	15	3	9	19	3	8	7	115	85	95
C	24	12	17	37		12	5			
	19	4	10	25		8		190	140	160
E	30	6	13	32		12	7	125	100	115
	19	5	12	24		10	7	130	100	115
T	23	10	14	25		8	5	130	100	115
B	25	11	16	27		10	4	125	50	80
W	28	9	18	37		4		145	105	115
				35		14	3	125	90	100
D	21	15	18	21		14	7	155	115	125
A	35	12	22	36		13	5	100	57	75
U				42		14		125	85	100
	33	17	25	33	5	19	10	115	80	90
Mean	24	9	15	30		11	6	132	94	108

S - systolic

D - diastolic

M - mean

End. - end-diastolic

(15 mm. Hg.) pressures approximate those reported to occur in normal dogs. While the mean pressures of dogs "A" and "U" are slightly elevated, their systolic and diastolic pressures fall within the normal range. Disregarding these animals, the pulmonary arterial pulse pressures for the group had a range of 6-24 mm. Hg. and a mean of 13.7 mm. Hg.

Right ventricular pressures for the group (systolic 30 mm. Hg. and mean 11 mm. Hg.) are compatible with those reported in normal dogs. The mean right ventricular pressures of dogs "U", "A", "D", and "W" are slightly elevated; however, the systolic pressures of these animals are within the normal range. Measurable right ventricular diastolic pressures were observed in dogs "V" and "U". The systolic pressures of the right ventricle and pulmonary artery approximated each other except in dogs "C" and "W", in which a difference of 13 and 9 mm. Hg. were observed. In each measurement the mean pulmonary arterial pressure exceeded the mean right ventricular pressure.

The systemic pressures of this group of dogs fell within the normal range and no apparent relationship existed between sex and blood pressure in either the greater or lesser circulatory systems. The highest systemic pressure occurred in a male ("C") and the highest pulmonary arterial pressure in a female ("U"). The oldest dog ("C") had the highest systemic pressure. While in individual animals higher systemic pressures were associated with higher pressures of the lesser circulation, dog "C" had the highest systemic pressure and one of the lower pulmonary arterial pressures.

Electrocardiographic study. No abnormalities were observed in electrocardiographic study of any of the control dogs.

Anatomic study. Necropsy of the control dogs confirmed that these animals were not infected by D. immitis, nematodes not being observed in any animal of the group. Likewise, no gross lesions were present in the respiratory or cardio-vascular systems. Dog "B" had an exudative dermatitis and dog "V" was heavily infected with Dipylidium caninum. The uterine horns of dog "V" contained nodular enlargements, which measured 1.5 cm. in diameter and resembled the changes of early pregnancy.

Microscopic examination of the lungs of dog "A" revealed an interstitial pneumonitis, characterized by thickened alveolar septa containing lymphocytes and mononuclear macrophages. Similar lesions of milder intensity and focal distribution were present in the lungs of the other control dogs. Neither fibrinous nor purulent pneumonia was observed. No lesions were apparent in the heart or pulmonary arteries of any of the control dogs and microfilariae were not identified in their tissues.

Histopathologic examination revealed the presence of lesions in other organs of some of the control dogs. The kidneys of dogs "C" and "D" contained scattered and small infiltrations of lymphocytes and other mononuclear cells which were widely dispersed in the interstitial tissue. These inflammatory foci occurred in both the cortex and medulla and occasionally were present beneath the epithelium of the renal pelvis.

Cystic glandular hyperplasia produced the nodular enlargements observed grossly in the uterus of dog "V". Mild encephalitis, characterized by perivascular lymphocytic infiltrations and small glial foci, occurred in the brain of this animal. Demyelinating lesions were not present and no inclusion bodies were identified. Scattered foci of lymphocytes admixed with a few polymorphonuclear leucocytes were present in the liver of dog "E". These foci had no definite relationship to lobular architecture and special stains failed to reveal organisms.

D. Immitis Infected Dogs

Only 16 of the 18 animals could be utilized in the study. One dog died before hemodynamic studies could be made and no parasites were observed in the heart or pulmonary arteries of the other animal. This group of dogs was made up of four Boxers, two Collies, one Hound, one Greyhound, one Dalmation, one Cocker Spaniel, one Pointer, one Boston Terrier, and four mongrels. In sex the group was equally divided, eight being males and eight females. Four of the dogs were estimated to be less than two years old, two to be more than two but less than four, and 10 of the dogs were considered to be more than four years old.

Hemodynamic study. Table 8 gives the results of the hemodynamic study made on the infected dogs. While there was much variation in the measurements, some evidence of elevated pressure was observed in the lesser circulatory system in each of the 16 animals. Pulmonary arterial pressures were not obtained in dog "Y". Mean pulmonary arterial

TABLE 8

PULMONARY ARTERY, RIGHT VENTRICLE, AND FEMORAL ARTERY PRESSURES OF D. IMMITIS INFECTED DOGS
(values mm. Hg.)

Dog	Pulmonary Artery			Right Ventricle				Femoral Artery		
	S	D	M	S	D	M	End.	S	D	M
Y				25	10	18	9	145	90	115
X				40	5	21	10	95	75	80
	9	3	6	38	4	15	10	100	75	85
G	16	8	12	41	5	18	6	90	50	65
L	24	13	19	30		13	5	90	60	75
	30	15	23	30		15	6	115	55	80
Q	31	19	25	36		14	6			
	32	18	26	40	4	20	8	165	135	145
F	31	23	27	31	5	18	7	100	75	85
N	36	16	28	56	8	29	16	185	145	155
	29	17	24	32	4	17	15	170	120	135
I	33	27	30	33	19	25	21	155	120	130
	30	19	25	31	5	17	8	100	65	75
H	39	18	30	42	3	21	18	115	80	95
	40	17	29	48	2	20	15	115	80	95
S	40	27	33	44	12	25	14	120	75	98
K	24	15	19	54	18	37	33	105	70	80
	40	27	33	40	7	19	10	165	110	130
J	31	22	26	39	11	22	8	100	50	70
	39	30	37	40	10	20	12	128	115	120
M	45	20	40	50	20	42	34	135	100	115
	40	24	37	40	5	23	20	157	127	137
O	50	33	42	55	16	30	22	175	130	150
	50	30	41	59	14	35	20	85	70	75
R	51	32	44	51	10	27	13	165	130	140
	60	38	48	60	2	29	16	165	120	137
P	29	6	20	45	7	23	18	150	120	130
	82	71	73	82	52	73	64	185	145	160

S - systolic
D - diastolic
M - mean
End. - end-diastolic

pressures greater than 22 mm. Hg. were observed in 13 of the dogs; however, in only three cases ("O", "R", and "P") were the systolic pressures above the normal range of 45 mm. Hg. These three animals also had elevated diastolic pressures. Six other dogs ("M", "J", "K", "S", "I", and "F") had pulmonary arterial diastolic pressures which exceeded 20 mm. Hg. Marked differences in pressure were observed in the two determinations made on dogs "K" and "P". While the pulse pressure of dog "M" (first measurement) was slightly widened, in all other dogs these pressures fell within the range observed in the control group.

Elevated right ventricular mean pressures were observed in all 16 infected dogs; however, in only seven animals ("P", "R", "Q", "M", "K", "H", and "N") were the systolic pressures greater than 45 mm. Hg. Measurable right ventricular diastolic pressures occurred in 15 dogs and in nine animals these pressures were at least 10 mm. Hg. End-diastolic pressures as great as 10 mm. Hg. occurred in 11 dogs. On at least one determination the mean right ventricular pressure of six animals exceeded the mean pulmonary arterial pressure and a substantial difference in these pressures occurred in three dogs ("X", "G", and "K"). The right ventricular systolic pressures of dogs "K", "G", "N", and "X" also exceeded the pulmonary arterial systolic pressures by at least 20 mm. Hg. Marked differences in pressure were observed in the two measurements made on dogs "K" and "P".

Much variation in systemic blood pressure was noted in this group of dogs and in the two determinations made on individual animals. While the pressures of all animals fell within the normal range, dog "G" was at the lower limits of normal. When divided into groups according to age, the average pressures of those dogs considered to be over the age of four years (ten animals) had a range of 65-160 mm. Hg. and a mean of 108 mm. Hg.; those under the age of four years (six animals) had a range of 75-150 mm. Hg. and a mean of 100 mm. Hg. In individual animals higher pulmonary arterial pressures were associated with higher systemic pressures.

Electrocardiographic study. No significant abnormalities were observed in electrocardiographic study of any of the 16 infected dogs.

Anatomic study. At necropsy all 16 of the experimental dogs were found to harbor adult forms of D. immitis; However, the intensity of the infection varied greatly. The extremes occurred in dogs "Y" and "K" from which, respectively, 4 and 84 nematodes were recovered. Except for dogs "I", "S", and "Y", in which all of the worms were contained in the right ventricle, parasites were present in the lumina of both the right ventricles and the pulmonary arteries. In most cases the parasites were observed to extend from the lumen of the right ventricle through the pulmonary valves into the pulmonary artery.

Gross lesions of the cardio-pulmonary system were observed in only a few animals. The intima of the main pulmonary artery had a granular appearance in the more heavily infected dogs. This appearance was

produced by small projections extending from the intima of the vessel and was more apparent under the low magnification of a hand lens. A small thrombus adhered to the endocardium at the junction of the vena cava and right atrium in dog "N" and thickening of the endocardium in the pulmonary outflow tract was observed in dogs "N", "O", and "Q". Cardiac dilatation and hypertrophy were not apparent grossly in any of the experimental animals. Pulmonary infarction was observed only in dog "K". In this animal, one hemorrhagic infarct was present in the diaphragmatic lobe of the left lung. Nodules, varying in size up to one cm. in diameter and containing adult parasites, were palpable in the lungs of dogs "F", "H", "J", "K", and "X". The lungs of dogs "P", "R", "S", and "X" contained irregular gray areas, which upon palpation had a rubbery consistency. Small white foci were present in the visceral pleura of dogs "I", "K", and "L".

Several of the dogs possessed lesions which had questionable relationship to dirofilariasis. A submucosal nodule measuring one cm. in diameter was present in the esophagus of dog "J". This structure, containing purulent material and several Spirocerca lupi, occurred in the mid-thoracic region and opened into the lumen of the esophagus through a small sinus. The right third mammary gland of dog "K" contained a mass which measured 5 mm. in diameter. This growth had a rather firm consistency and its cut surface was solid and gray. Several healed infarcts were present in the kidneys of dog "F" and the renal

cortices of dogs "G", "M", "O", "P", and "R" were pitted with small depressions. Small gray streaks and spots were apparent on the cut surfaces of these organs. Muco-purulent endometritis occurred in dogs "I" and "K", both animals having whelped shortly before termination of the experiment. In all dogs there was marked congestion of the spleen.

In contrast to the vague and inconsistent gross lesions, marked and specific microscopic alterations were present in the tissues of the infected dogs. Changes in the ventricles of the hearts included the presence of microfilariae in capillaries between the muscle fibers (Figure 7) and the occurrence of small focal and perivascular infiltrations of lymphocytes and plasma cells (Figure 8). These infiltrations often surrounded degenerating microfilariae and were observed in the right ventricles and interventricular septa of 12 of the dogs and in the left ventricles of 11 animals. A small area of granulomatous inflammation, which had a necrotic center and contained a few polymorphonuclear leucocytes, occurred in the left ventricle of dog "R". Dead microfilariae could not be associated with this lesion. Loosely arranged fibrous connective tissue separated the muscle fibers in the right ventricle of dog "K" and fibrous thickening of the endocardium occurred in the right ventricles of dogs "N", "O", "Q", and "K".

Lesions in the atria resembled those of the ventricles. These changes included the presence of microfilariae in capillaries of the atrial myocardium and the occurrence of focal and perivascular infiltrations of



Figure 7. Microfilaria in a capillary of the myocardium. There is complete absence of inflammatory response. H&E, X960



Figure 8. Degenerating microfilaria in the myocardium with focal inflammatory reaction composed of lymphocytes and mononuclear macrophages. H&E, X960

lymphocytes and plasma cells. The latter were present in the atria of 14 of the animals. Intimal and medial proliferation thickened the walls of scattered arterioles in the atria of ten dogs (Figure 9). Focal areas of mural endocarditis, characterized by infiltrations of lymphocytes and polymorphonuclear leucocytes, occurred in dogs "M" and "X" and there was a similar area of pericarditis in dog "R". A thrombus, containing lymphocytes and polymorphonuclear leucocytes, adhered to the atrial endocardium in the area of the opening of the anterior vena cava in dog "N". No changes were observed in the pulmonary or tricuspid valves of any of the animals.

Microscopic lesions were observed in the major pulmonary arteries and their intrapulmonary branches in 14 of the infected dogs. These changes, usually limited to the intima, consisted of villous processes projecting into the lumen and diffuse irregular intimal thickening (Figures 10 and 11). Both reactions were composed of plump fibroblasts, at times containing a few lymphocytes, and were covered by endothelium. Small inflammatory foci and perivascular infiltrations were observed in the elastic layers of the wall in the major vessels of a few animals. These inflammatory infiltrations were composed of lymphocytes and plasma cells and were not associated with the presence of microfilariae. In addition to the usual lesions, the pulmonary artery of dog "G" contained focal areas of dystrophic calcification (Figure 12). These lesions, occurring in the main artery and its two branches, were subintimal in location and varied



Figure 9. Intimal proliferation narrowing the lumen of an arteriole in the atrium. H&E, X740



Figure 10. Diffuse intimal fibrosis in a major branch of the pulmonary artery. H&E, X100

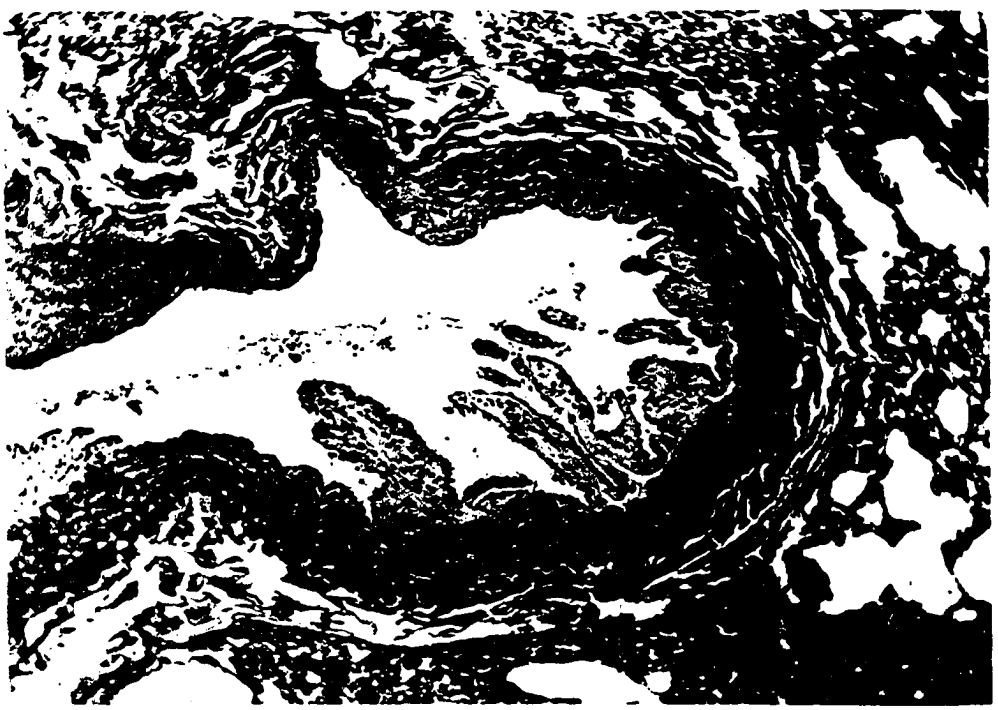


Figure 11. Intimal fibrosis forming villous processes in a large intrapulmonary artery. H&E, X100

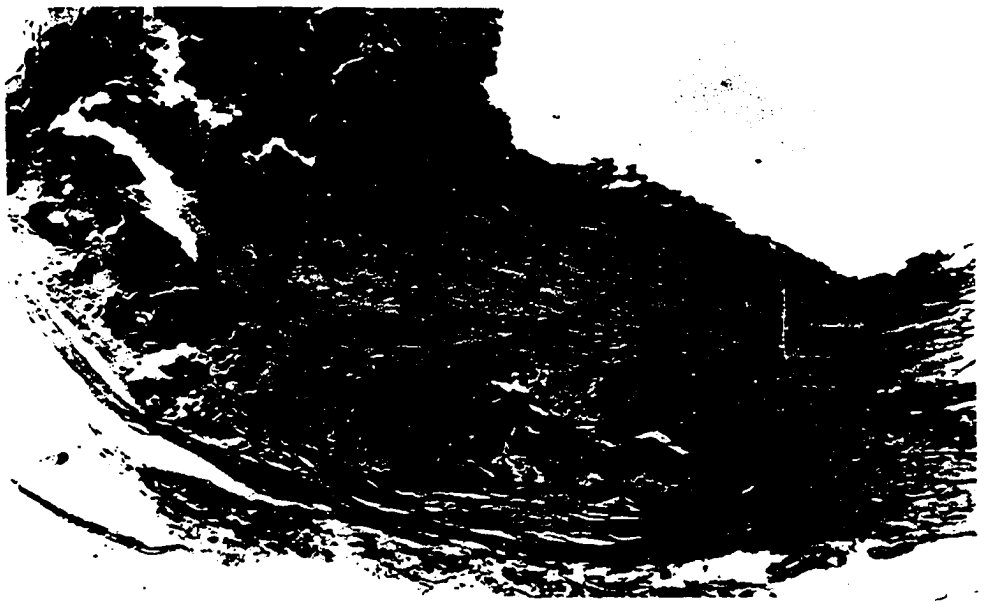


Figure 12. Calcified plaques in the wall of a major branch of the pulmonary artery. H&E, X100

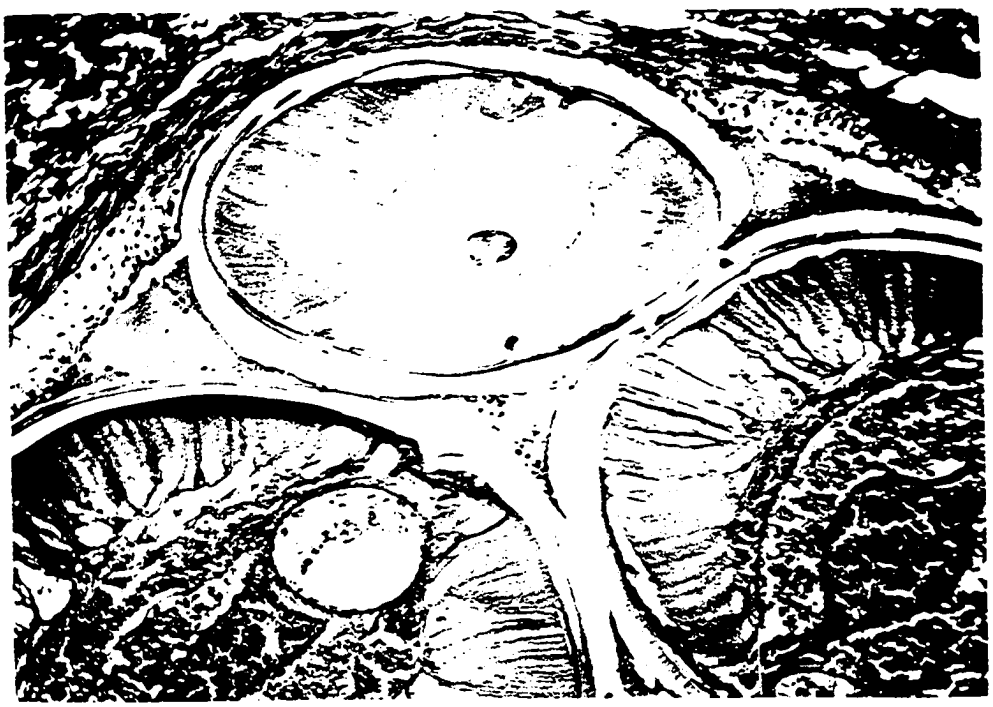


Figure 13. Adult D. immitis in the lumen of an intrapulmonary artery. There is mild intimal fibrosis and little inflammatory response. H&E, X100

greatly in size. Adult forms of the parasite were present in the intrapulmonary arteries of four dogs (Figure 13).

Intimal and endothelial proliferations were observed in the muscular arteries of 14 dogs. Here the intimal reaction was often eccentrically located and varied greatly in thickness (Figure 14). Endothelial proliferation occurred in conjunction with intimal changes in muscular arteries and occasionally was observed in arterioles and capillaries. Neither medial hypertrophy in arteries nor lesions of the venous system were observed in any of the animals.

A severe arteritis was noted in the lungs of four of the infected dogs (Figure 15). While in three animals these changes occurred in arteries containing degenerated parasites, in one of the dogs some of the involved vessels contained well preserved nematodes and in another animal no parasites were present in the intrapulmonary arteries. Elastic arteries were usually affected and the inflammatory process, involving all layers of the vessel wall, consisted of polymorphonuclear leucocytes, lymphocytes, and mononuclear macrophages. Eosinophils were rarely observed. While necrosis was not prominent, in dog "H" the response was granulomatous and contained focal areas of calcification.

Thrombosis of both elastic and muscular arteries was a prominent lesion in the lungs of nine dogs (Figure 16). While masses of fibrin partially surrounded the nematodes in the elastic arteries, the lumina of other vessels were often obliterated by organized fibrin and in many

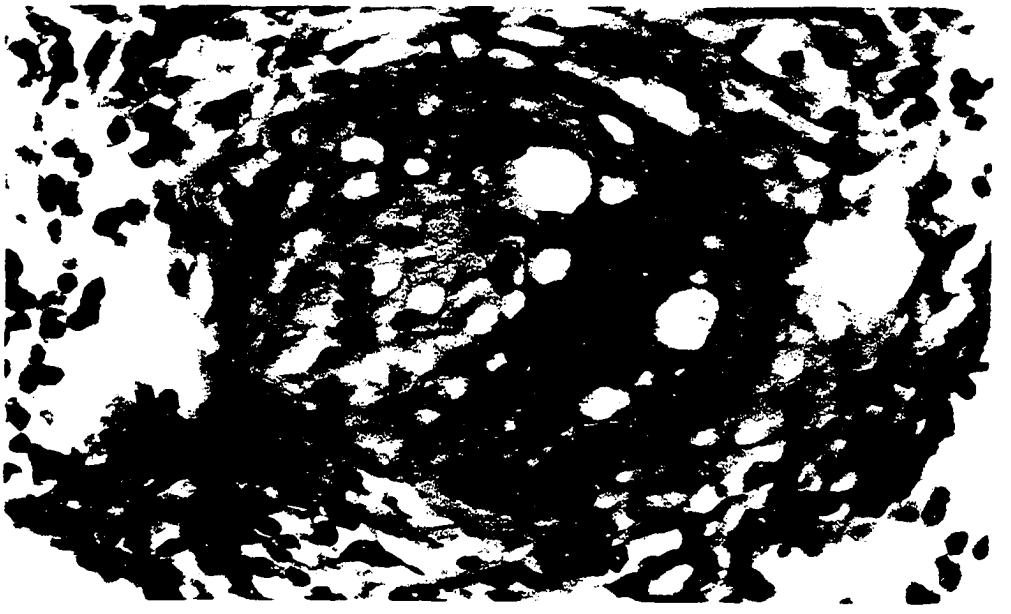


Figure 14. Eccentric intimal thickening in an arteriole of a lung. H&E, X470

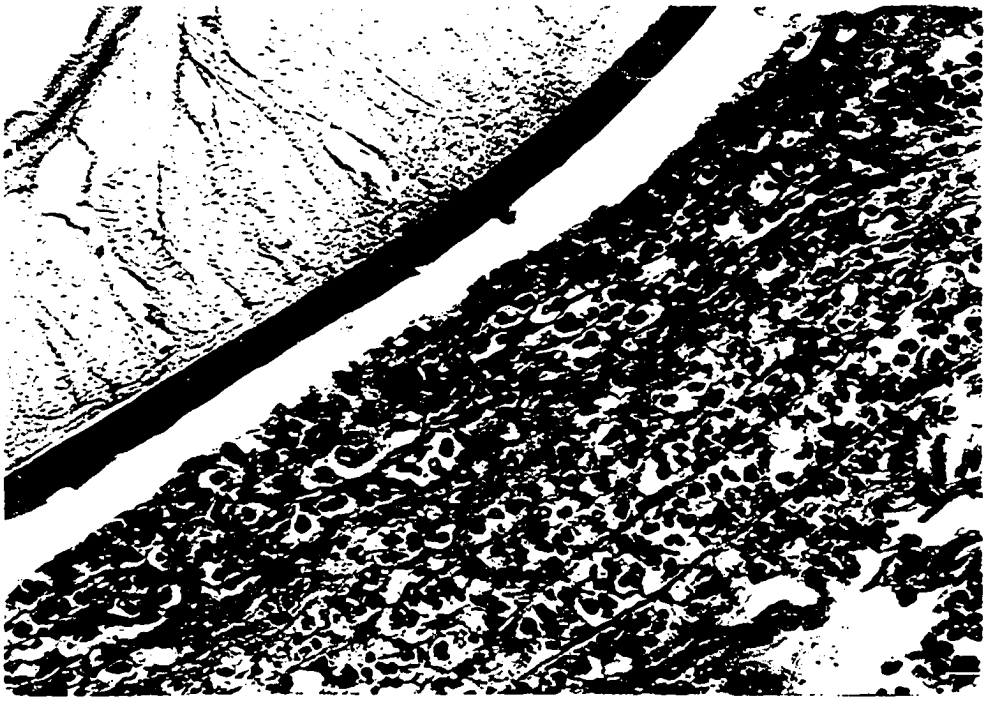


Figure 15. Arteritis in an intrapulmonary artery. Lymphocytes and polymorphonuclear leucocytes infiltrate all layers of the vessell wall and an adult parasite is in the lumen. H&E, X365

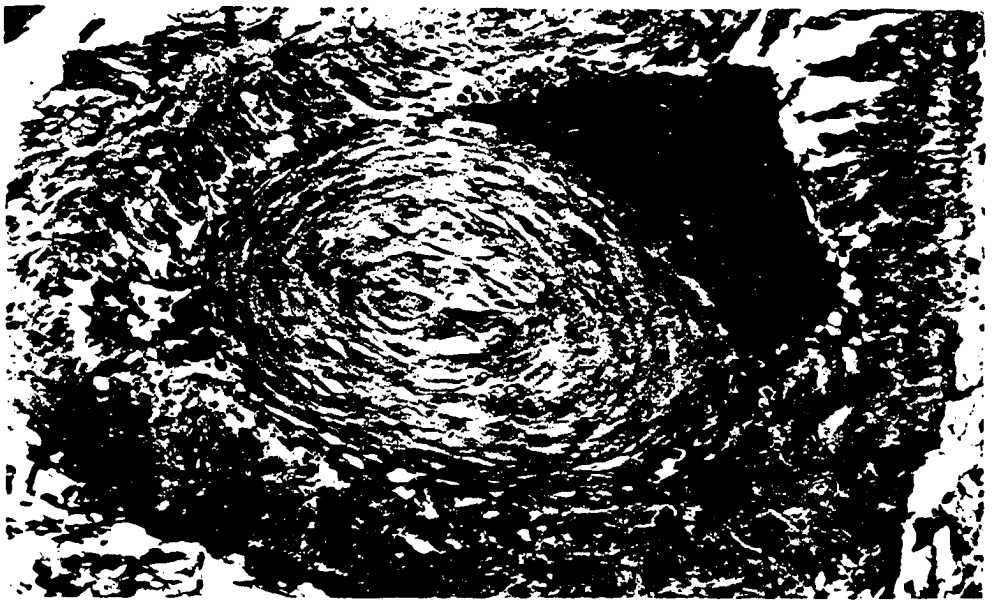


Figure 16. An organized thrombosis occludes the lumen of an intrapulmonary artery. H&E, X380

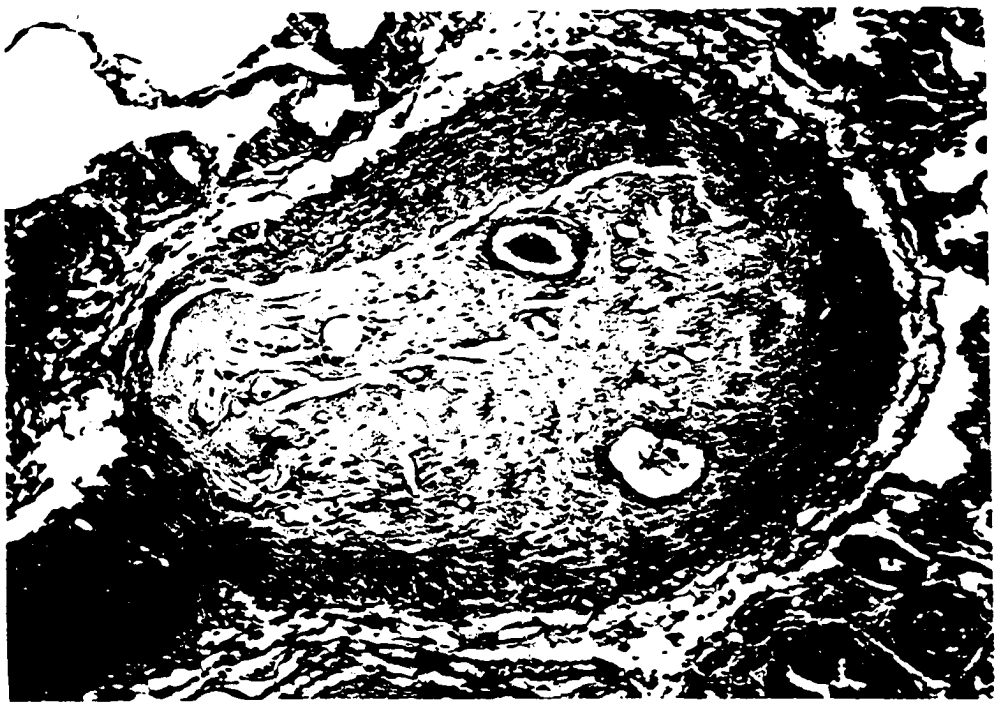


Figure 17. Recanalization of an organized thrombosis in an intrapulmonary artery. H&E, X100

arteries recanalization was apparent. Several large endothelial-lined channels were observed in the organized thrombi filling the lumina of some vessels (Figure 17). In dog "S" minute masses of fibrin occluded the lumina of scattered arterioles and capillaries (Figure 18).

While thrombi filled the lumina of large numbers of arteries in the lungs of nine dogs, infarction occurred only in dog "K". There was necrosis of alveolar septa in this hemorrhagic area and an organizing thrombus occluded the lumen of a large muscular artery at the base of the lesion.

Other alterations occurring in the lungs of the infected dogs included interstitial pneumonitis, fibrosis, and foci of calcification and ossification. Interstitial pneumonitis, characterized by thickened alveolar septa containing infiltrations of lymphocytes and mononuclear macrophages, was present in the lungs of all infected dogs (Figure 19). These lesions were patchy in distribution and varied greatly in severity. Occasionally inflammatory foci, consisting of lymphocytes and macrophages surrounding degenerating microfilariae, occurred in the alveolar walls. Areas of fibrosis were observed in the lungs of 12 dogs. In some animals these areas were small, occurred near major vessels, and consisted of fibrosis of alveolar septa. In other dogs the fibrotic areas were larger and located farther toward the periphery of the lung. Here the normal architecture of the lung was destroyed by the desmoplastic process. Minute foci of calcification and focal areas of ossification were present in the



Figure 18. Fibrin embolus in an arteriole of the lung of a dog heavily infected with D. immitis. H&E, X960

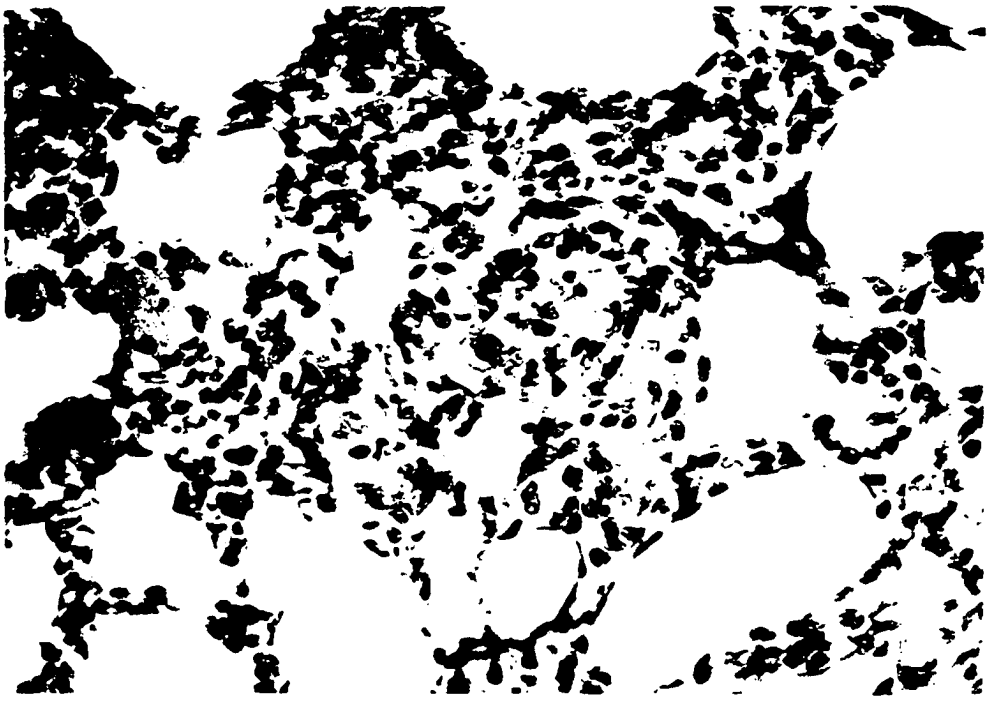


Figure 19. Interstitial pneumonitis characterized by thickened alveolar septa containing lymphocytes, other mononuclear cells, and polymorphonuclear leucocytes. Degenerating microfilariae are surrounded by inflammatory cells. H&E, X400

alveolar septa in the lungs of six dogs. Both of these lesions occurred simultaneously in three of the animals.

Microscopic lesions were present in the livers and kidneys of most of the infected dogs. Periportal infiltrations of lymphocytes were present in the livers of 11 dogs and scattered inflammatory foci occurred in 12 animals. These foci had no definite relationship to lobular architecture and consisted of lymphocytes, mononuclear macrophages, a few polymorphonuclear leucocytes and occasionally multinucleated giant cells. At times these infiltrations were associated with small areas of necrotic hepatic cells and degenerating microfilariae could often be identified in the lesions (Figure 20). While similar inflammatory foci were observed in the kidneys only of dog "F", interstitial infiltrations of lymphocytes were present in the kidneys of 14 of the dogs. These lesions occurred in both the cortex and medulla and were most numerous at the corticomedullary junction. Similar infiltrations also occurred beneath the epithelium of the renal pelvis. Extensive renal lesions were present only in dogs "F", "G", "M", and "R". No evidence of the inflammatory process was observed in the kidneys of dogs "I" and "O" and in the remaining animals the inflammatory foci were small and widely scattered.

Some degree of glomerulitis was apparent in the kidneys of 15 of the infected animals. These lesions varied greatly in both severity and in number of glomeruli involved. There was fibrous thickening of Bowman's membrane and nodular areas of increased cellularity distorted



Figure 20. Degenerating microfilaria in the liver surrounded by lymphocytes, mononuclear macrophages, and a multinucleated giant cell. H&E, X830

glomerular tufts. In these areas both endothelial and epithelial cells were increased in number and synechia often joined the capillary tufts and Bowman's membrane (Figure 21). Microfilariae were present in the capillaries of both normal and altered glomeruli. While in dog "Y" interstitial nephritis occurred in the absence of glomerular lesions only marked glomerulitis was present in the kidneys of dogs "J" and "O".

A mild dermatitis was observed in dogs "F", "G", and "K". This non-specific lesion was characterized by hyperkeratosis and infiltrations of lymphocytes and plasma cells, the infiltrations surrounding adnexa and occasionally forming small foci in the dermis. Microfilariae were observed in association with these lesions in dogs "G" and "K".

Lesions directly related to dirofilariasis were not observed in other organs. Chronic prostatitis occurred in six of the old male dogs and in dogs "I" and "K" there was muco-purulent endometritis, both animals having whelped shortly before termination of the experiment. The mammary tumor in dog "K" was an edenoma with prominent fibrous stroma and the esophageal lesion of dog "H" was typical of S. lupi infection.

Microfilarae were widely distributed in the tissues of the infected dogs and rarely were associated with inflammatory reactions. The larvae were present in arteries, veins, and capillaries and there was much variation in the frequency with which they were encountered. While this variation was marked in dogs parasitized in different degrees, there also was much difference in microfilarial distribution in different tissues of

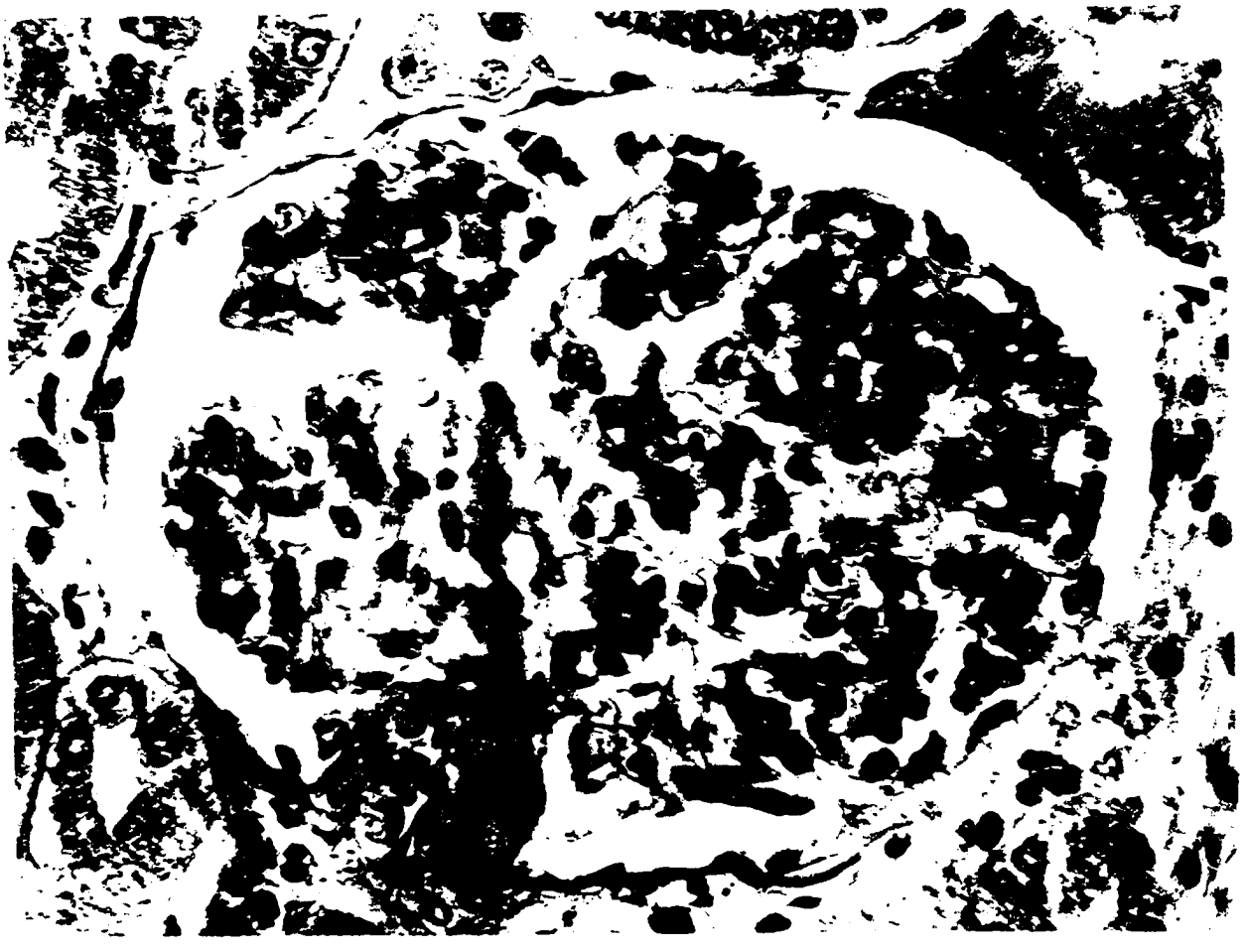


Figure 21. Glomerular lesion consisting of cellular nodules in the capillary tuft and synechia join the tuft and Bowman's membrane. H&E, X650

the same dog. Microfilariae were present in the lungs of all of the infected dogs. In the hearts the larvae occurred in the right ventricles and interventricular septa of 12 dogs, in the left ventricles of 11 dogs, and in the atria of six animals. The occurrence of microfilariae in other organs included the kidneys in 14 dogs and the livers of 12 animals. Larvae were observed in the brains of 11 dogs and in capillaries of the adrenal cortex in seven animals. In six of the dogs microfilariae were present in the intestines where they were most easily identified in capillaries in the tips of villi (Figure 22). Autolysis of the intestinal mucosa aided in recognition of microfilariae in this location. Microfilariae were observed occasionally in most of the tissues of the body, including the eye of one dog and the testicle of another animal. It is noteworthy that the larvae were identified in the spleens of only two dogs.

Relationship of Hemodynamic Changes to Pulmonary Lesions

While pulmonary hypertension, parasites, and pulmonary lesions were observed in most of the infected dogs, the interrelationship of these factors was difficult to establish. Figure 23 shows the pressures in the pulmonary and systemic circulatory systems and the parasite load in each of the infected dogs. The more heavily parasitized animals had greater elevations in pulmonary arterial pressure than did those harboring fewer worms, but direct relationship did not exist between the degree of infection and absolute elevation in pressure. Six dogs had pulmonary arterial pressures equal to or greater than dog "K", the most heavily



Figure 22. Microfilaria in the tip of a villus in the duodenum. There is postmortem autolysis and desquamation of epithelium. H&E, X700

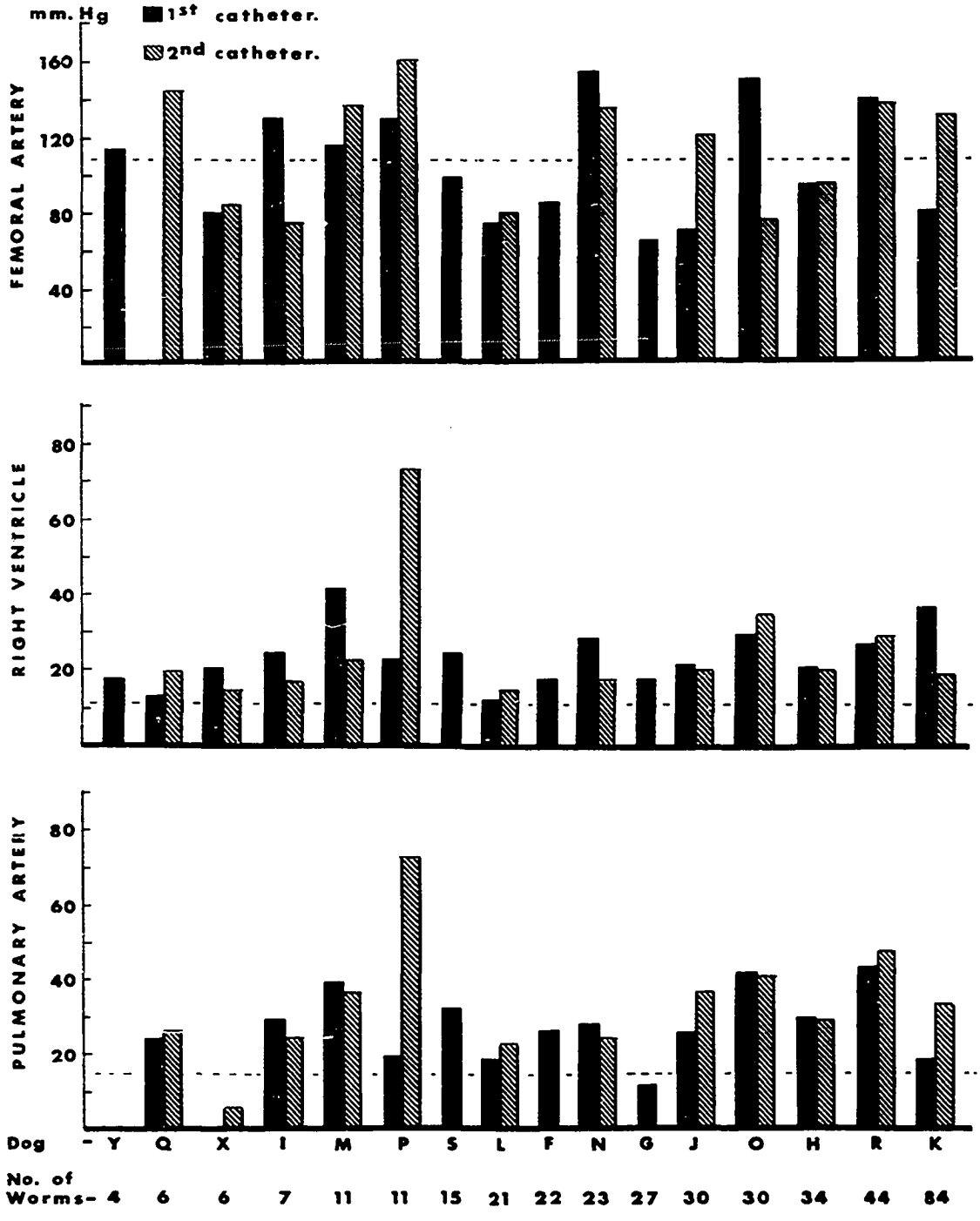


Figure 23. Mean pressures of the systemic and pulmonary circulatory systems compared to the degree of parasitism in 16 D. immitis infected dogs. The broken lines indicate the mean values of the normal controls.

parasitized animal. Dog "P" had the highest pulmonary arterial pressure but only 11 worms. This pressure in dog "Q", with only six parasites, was elevated slightly above the normal range.

The amount of tissue damage occurring in the lungs of the infected dogs was investigated by comparing, in each animal, the number of sections of lung in which specific lesions occurred to the total number of sections of lung examined. This gave no indication as to the severity of lesions but provided evidence of their distribution throughout the lung. Table 9 gives the frequency of lesions, parasite load, and pulmonary arterial pressures in the infected dogs. No one specific lesion predominated in dogs with higher pulmonary arterial pressures. The frequency with which lesions occurred had a closer relationship to parasite load than to degree of hypertension, more lesions being observed in the lungs of dogs infected with the most parasites. However, lesions occurred in only a few sections from the lungs of dog "J", an animal having both numerous parasites and an elevated pressure. Dog "P", with the highest pulmonary arterial pressure and 11 parasites, had only a moderate amount of tissue damage. Interstitial pneumonia, patchy in distribution and varying in severity, occurred in the lungs of all infected dogs.

No relationship could be established between renal lesions and systemic blood pressure. The mean femoral arterial pressures of dogs "G" and "F", animals with severe nephritis, was 65 and 85 mm. Hg. These pressures in the dogs with less severe nephritis varied from 75 to

TABLE 9

THE RELATIONSHIP OF PULMONARY LESIONS AND NUMBER OF PARASITES TO THE HEMODYNAMIC CHANGES IN THE PULMONARY CIRCULATION

Dog	No. Worms	Mean Pulmonary Arterial Pressure*	No. Sections Examined	Number of Sections Containing					
				Fibrosis	Intimal Villous	Prolifer. Diffuse	Thrombi	Arteritis	Worms
K	84	33	15	12	6	9	5	2	2
R	44	48	18	3	5	6		1	
H	34	30	10		5	3	2	3	2
J	30	37	15	1	4				
O	30	42	11	3	4	1			
G	27	12**	13	1	4	6	1		
N	23	28	14	4	6	4	1		
F	22	27	11	3	7	9	5	3	2
L	21	23	11	4	4	1	1		
S	15	33	12	4	4	3	4		
M	11	40	17	7	4	2	1		
P	11	73	12	4	1	1	2		
I	7	30	10	8		4			
Q	6	26	11		1	1			
X	6	6**	12		2				
Y	4		8						

* mm. Hg.

** R.V. pressure greater than P.A.

160 mm. Hg. and dog "O", with mild renal lesions, had a mean systemic blood pressure of 150 mm. Hg.

Effect of Treatment to Destroy the Adult Parasites

The dogs used in this study, three females and two males, consisted of one Boxer, one Cocker Spaniel, one Boston Terrier, and two mongrels. Three of the dogs were considered to be between the ages of three and four years and the other animals to be more than four years old.

Table 10 gives the results of the hemodynamic study in the treated dogs. In dogs #2, #3, and #4 pre-treatment pressures of the lesser circulatory system were elevated and little change occurred following treatment. Increased mean pressures were observed in all animals. While in dogs #3 and #4 both systolic and diastolic pressures were elevated, only the diastolic pressure was increased above the normal range in dog #2.

Pre-treatment pressures of the pulmonary circulation were normal in dog #5. Following treatment the mean pulmonary arterial and right ventricular pressures were increased to approximately twice the upper limits of the normal range. The pulmonary arterial diastolic pressure was elevated, as was the right ventricular end-diastolic pressure. Systolic pressures were at the upper range of normal.

Since in dog #1 pre-treatment pressures of the right ventricle greatly exceeded pulmonary arterial pressures, the pulmonary hypertension apparently following treatment cannot be attributed to the effect of the dead worms.

TABLE 10

FEMORAL ARTERY, RIGHT VENTRICULAR, AND PULMONARY ARTERIAL PRESSURES BEFORE AND AFTER TREATMENT TO DESTROY THE ADULT FORMS OF D. IMMITIS
(values mm. Hg.)

Dog	Pulmonary Artery			Right Ventricle				Femoral Artery		
	S	D	M	S	D	M	End.	S	D	M
1	*25	3	11	40	5	13	8	125	90	115
	a53	42	49	58	6	29	24	140	100	115
	b57	35	44	57	4	29	14	115	70	85
2	*43	24	32	49		18	5	160	105	130
	a44	33	39	51		24	10	165	120	145
	b44	25	31	55	5	21	10	160	105	135
3	*50	30	43	50	10	29	15			
	a			53	12	30	18	165	125	140
	b50	15	37	50	9	27	14			
4	*72	40	52	74	6	33	12	150	125	135
	a70	45	51	71	6	33	14	155	130	135
5	*16	3	9	19	4	7	8	135	90	100
	a42	26	37	42	6	24	13	117	60	85

S - systolic

D - diastolic

M - mean

End. - end-diastolic

* - pre-treatment

a - 3 weeks after treatment

c - 5 weeks after treatment

All determinations of systemic pressures in this group of dogs were normal and little change occurred following administration of the arsenical compound. No electrocardiographic abnormalities were detected.

At necropsy all five dogs were found to be infected with D. immitis, the number of parasites varying from 8 to 61. The pulmonary arteries and right ventricle of dog #4 contained 61 parasites, 40 of which were still viable, and a mass of degenerating worms from which it was impossible to separate individual parasites. All of the worms recovered from the other dogs were dead. The anterior venae cavae of dogs #1 and #2 contained a few worms and one parasite occurred in the right atrium of dog #4. Figure 24 shows the relationship of the hemodynamic changes to the number of nematodes. In this group of animals the more heavily parasitized dogs had higher pulmonary arterial pressures.

The gross lesions of these dogs were similar to those of the previous study. Cardiac hypertrophy was not obvious in any of the animals. The intima of the pulmonary artery had a granular appearance in the more heavily infected dogs and there was intimal thickening in the right ventricle of dog #3. One small infarct was present in the intermediate lobe of the right lung in dog #4 and small nodules could be palpated in the lungs of the more heavily infected animals. Dog #4 was infected with both S. lupi and D. caninum. In addition to congestion of

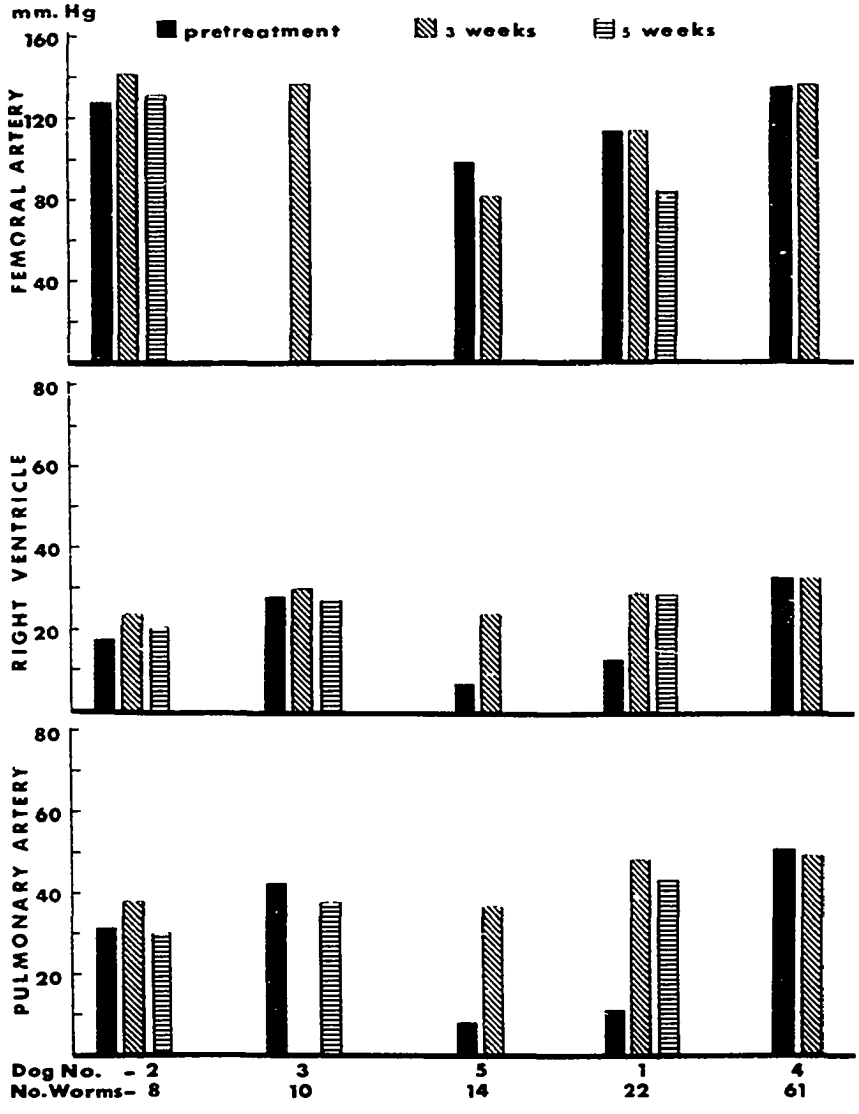


Figure 24. Mean pressures of the systemic and pulmonary circulatory systems in dogs treated to destroy the adult forms of D. immitis.

the lungs, liver, spleen, kidneys, and mesenteric veins in dog #5, there was severe pulmonary edema.

Microscopic lesions of the cardio-vascular and pulmonary systems of these dogs resembled those of the previous group, the only differences being in the greater number of arteries containing parasites and the increased frequency of arteritis. Table 11 shows the relationship of lesions to the number of worms and to hemodynamic changes. While no specific alteration could be related to degree of hypertension, lesions were more common in dogs with higher pulmonary arterial pressures.

Arterial thrombosis occurred in five sections of the lung in dog #5, the only animal which exhibited increased pressures following treatment. These thrombi were of recent origin and showed no evidence of organization (Figure 25). Except for these recent thrombi and severe pulmonary edema and congestion, the pulmonary lesions of dog #5 were similar to those of the other four animals.

As in the previous study, lesions of other organs were limited to the liver and kidneys. Inflammatory foci, often containing microfilariae, occurred in the livers of four of the dogs and microfilariae were present in the hepatic sinusoids of three animals. Mild interstitial nephritis and glomerulitis occurred in all dogs and microfilariae were present in capillaries of the kidney. These larvae were also observed at times in capillaries or larger vessels in the pancreas, parotid glands, intestines,

TABLE 11

THE RELATIONSHIP OF PULMONARY LESIONS AND NUMBER OF PARASITES TO THE HEMODYNAMIC CHANGES IN THE PULMONARY CIRCULATION OF TREATED DOGS

Dog	No. Worms	Mean Pulmonary Arterial Pressure *	Number Sections Examined	Number of Sections Containing					
				Fibrosis	Intimal Villous	Prolifer. Diffuse	Thrombi	Arteritis	Worms
4	61	52	16	2	4	5	4	5	3
1	22	49	18	5	3	10	8	7	11
5	14	37	14	1	3	7	5	1	3
3	10	43	10		2		1	1	1
2	8	39	8	1	2				

* mm.Hg.

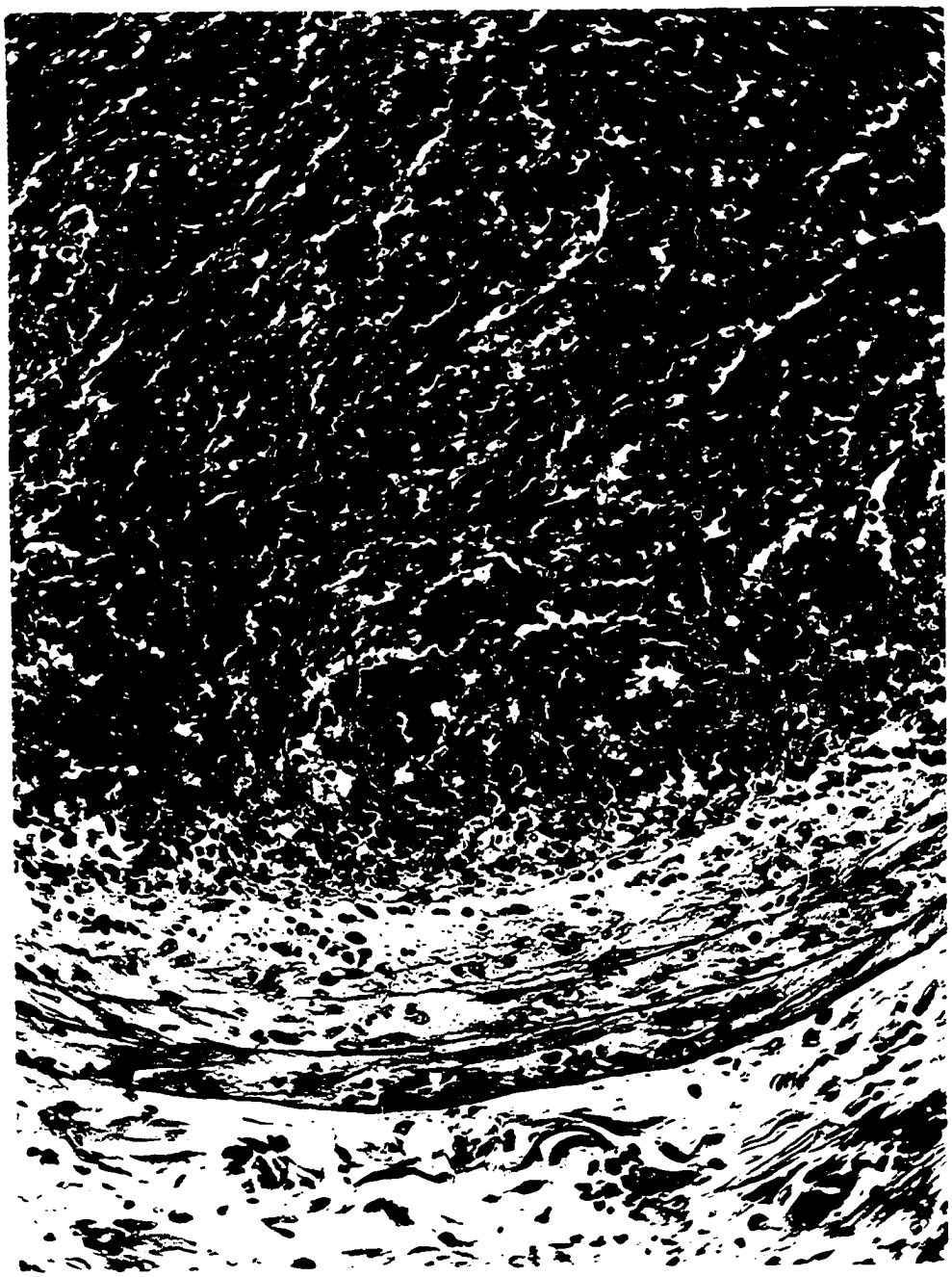


Figure 25. Thrombosis of an intrapulmonary artery. H&E, X500

adrenal glands, spleen, urinary bladder, esophagus, uterus, ovaries, brain, and spinal cord. The microfilaria in these locations were apparently viable and were not associated with inflammatory infiltrations.

Cardiac Hypertrophy

While hypertrophy of the heart was not apparent upon gross examination of the experimental dogs, in some animals critical measurement of these organs revealed alterations. Table 12 gives the body weight, cardiac measurements, and heart weight/body weight ratios occurring in the experimental animals. Evaluation of cardiac weights was made by utilizing information obtained from the 50 normal dogs. Both HW/BW ratios and regression data of heart weight on body weight were considered. The weight of the ventricles was evaluated on the basis of information obtained from the regression studies relating ventricular weight to the weight of the ventricular mass.

Heart size in the dogs of the control group was normal, all animals having HW/BW ratios inside the expected range of 5.08 to 8.78. When the heart and body weights of these dogs were applied to the regression data, the cardiac weights of all animals fell inside the 95 per cent confidence belt.

Heart size in only two infected dogs was abnormal. The heart of dog "F", a medium sized animal, weighed 157 gm. and the HW/BW ratio of 9.57 exceeded the normal range. When the heart and body weights were fitted to the regression data, the heart weight was found to fall

TABLE 12

BODY WEIGHT AND HEART MEASUREMENTS OF THE EXPERIMENTAL DOGS

Dog	Body Wt. kg.	Heart Wt. gm.	R.V. gm.	R.V. mm.	L.V. gm.	L.V. mm.	I.V.S. gm.	I.V.S. mm.	Atrium gm.	HW/BW (gm./kg.)
Controls										
V	8.3	49	12	4	20	8	11	7	6	5.90
U	9.3	75	17	5	33	7	14	7	11	8.01
T	9.4	56	13	3	25	8	12	8	7	5.95
E	11.1	63	15	4	27	10*	14	8	7	5.67
D	13.0	92	21	4	37	15*	23	12	11	7.10
C	13.6	100	22	5	43	15*	24	12	11	7.35
A	17.7	120	32	7	50	20*	28	11	10	6.77
W	19.7	131	32	7	57	11	30	9	12	6.62
B	22.0	129	30	4	53	16*	31	8	10	5.86
Infected										
5	5.7	49	13	4	21	10	10	10	5	8.59
3	9.3	63	16	3	26	8	15	11	6	6.77
X	9.8	69	14	3	31	11	15	10	9	7.06
4	12.0	95	25	4	40	11	19	10	11	7.91
2	12.9	89	21	5	39	10	20	15	9	6.89
1	13.9	121	33	6	50	10	25	10	13	8.66
I	14.5	102	22	4	48	9	18	10	13	7.00
L	14.5	119	24	3	59	10	23	12	13	8.20
P	14.8	102	22	4	46	10	22	9	12	6.92
M	15.0	86	17	4	37	10	20	10	12	5.70
O	15.8	125	28	5	57	12	27	13	13	7.87
G	15.9	136	33	5	56	22*	26	12	21	8.55
F	16.4	157	36	5	68	20*	35	10	18	9.57
R	16.4	116	32	5	47	12	25	10	12	7.07
Q	16.5	114	24	5	54	10	24	10	12	6.90
S	16.6	129	30	6	61	13	24	13	14	7.74
J	16.9	136	33	4	61	14	29	12	13	8.06
H	17.0	127	34	4	53	10	28	15	12	7.47
N	17.0	74	18	4	33	8	15	8	8	4.32
K	19.0	140	36	6	57	13	28	18	19	7.03
Y	21.6	137	31	5	64	15	26	18	16	6.31

* measured through a papillary muscle.

slightly above the 95 per cent confidence belt. The heart of dog "N", one of the heavier animals, weighed 74 gm. and the HW/BW ratio of 4.32 was smaller than any observed in the normal dogs. When the heart and body weights of this animal were applied to the regression data, the weight of the heart was found to fall slightly below the 95 per cent confidence belt.

Figure 26 compares the weights of the right ventricles of the control dogs to the expected weight and indicates that the right ventricles of dogs "A" and "W" exceed the normal values. While pulmonary hypertension was observed in dog "A", the pressures of the lesser circulatory system of dog "W" were within the normal limits.

Figure 27 compares the weights of the right ventricles of the infected dogs to the expected weights and to the degree of parasitism. The right ventricles of dogs "G", "H", "J", "K", "R", #1 and #4 exceed the 95 per cent confidence belt and thus are considered to be too heavy. These were the more heavily infected animals and all had some degree of pulmonary hypertension.

While the weights of the left ventricles of all dogs in both the control and infected groups fell within the 95 per cent confidence belt and thus can be considered of normal size, the weights of the left ventricles of dogs "G", "H", "J", "K", "R", #1, and #4 were at the lower limits of normal. In all dogs the weights of the interventricular septa and the thickness of all parts of the heart fell within the respective normal ranges.

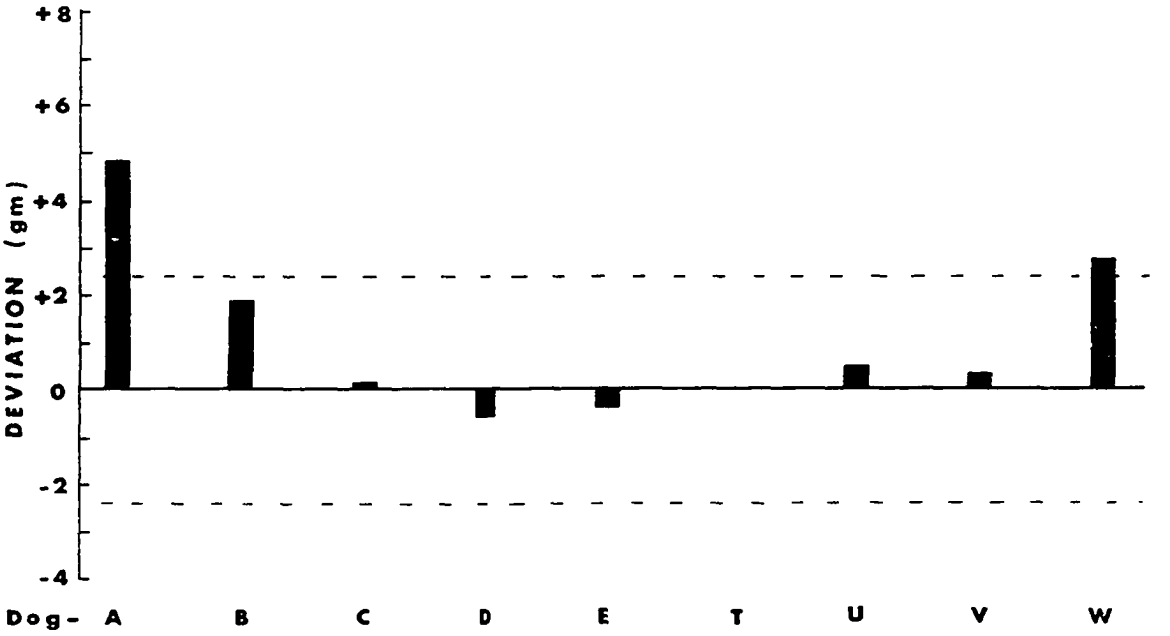


Figure 26. Right ventricular weights of the control dogs compared to the expected weights as calculated from the regression study. The broken lines indicate the 95 per cent confidence belt.

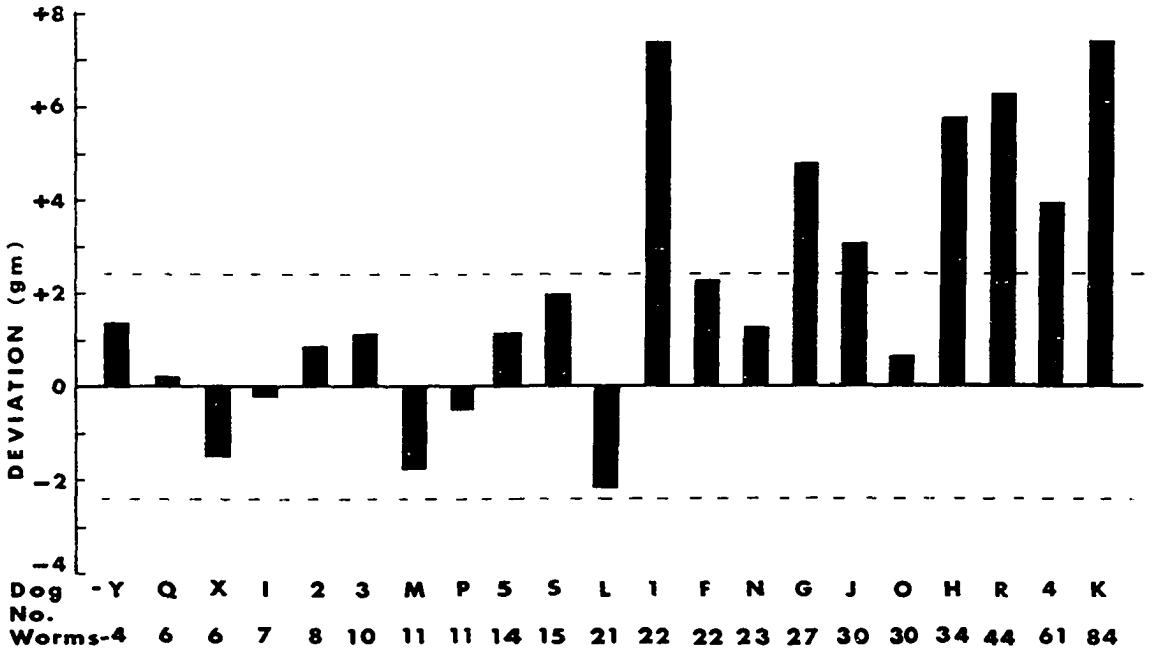


Figure 27. Right ventricular weights of the D. immitis infected dogs compared to the expected weights as calculated from the regression study. The broken lines indicate the 95 per cent confidence belt.

Since by chance alone the right ventricular weights of five per cent of the population may be expected to fall outside the 95 per cent confidence belt, further evidence of hypertrophy was obtained through determination of the length of the myocardial muscle fiber segment. The results of this study are shown in Table 13. Except for dog "A", in which there were pulmonary hypertension and a heavy right ventricle, uniform measurements were observed in the dogs of the control group. With two exceptions these measurements in the infected groups were greater than those of the controls. All measurements of dog "J" were comparable to those of the control dogs and the nuclear length of dog "R" was less than any in the control group; however, the increased length of the internuclear distance in dog "R" produced a muscle fiber segment length comparable to those of the other infected animals.

The mean nuclear length, mean internuclear distance, and mean length of muscle fiber segment of the infected and control dogs are shown in Table 14. Analysis of these data by Students "t" showed significant differences between the internuclear distances and between the lengths of muscle fiber segment in the two groups. Since dog "A" had an elevated pulmonary arterial pressure, it was considered to be abnormal and was not included in the control group.

TABLE 13

LENGTH OF RIGHT VENTRICULAR NUCLEI, INTERNUCLEAR DISTANCE, AND LENGTH OF MUSCLE FIBER SEGMENT OF THE CONTROL DOGS AND INFECTED DOGS WITH HEAVY RIGHT VENTRICLES

Dog	Nuclear Length	Inter-nuclear Distance*	Length Muscle Fiber Segment*
		Controls	
T	12.2	40.1	52.3
E	12.5	39.9	52.4
U	12.4	40.0	52.4
C	12.3	41.2	53.5
B	13.3	41.5	54.8
V	13.4	43.1	56.5
W**	12.1	44.6	56.7
D	12.5	44.4	56.9
A**	13.6	52.4	66.0
		Infected	
J	11.8	39.9	51.7
G	12.7	48.4	61.1
R	11.8	52.5	64.3
K	13.4	51.0	64.4
H	14.3	50.3	64.6
1	13.8	51.0	64.8
4	14.1	51.1	65.2

* Microns, average of 100 measurements

** Ventricles heavier than expected

TABLE 14

MEAN AND STANDARD DEVIATION OF RIGHT VENTRICULAR NUCLEAR LENGTH, INTERNUCLEAR DISTANCE, AND LENGTH OF MUSCLE FIBER SEGMENT

Dog	Nuclear Length		Internuclear Distance*		Length Muscle Fiber Segment *	
	Mean	S.D.	Mean	S.D.	Mean	S.D.
Controls (8)	12.58(a)	0.49	41.85(c)	1.94	54.41(e)	2.06
Infected (7)	13.12(b)	1.04	49.17(d)	4.25	62.30(f)	4.83

* Microns

a - b, T = 1.31, $.3 < P < .2$ (df = 14)

c - d, T = 4.08) $.01 < P < .001$ (df = 14)

e - f, T = 4.18)

Electron Microscope Study

Electron microscope study of the lungs of dog #4 revealed apparent thickening of the basement membranes of capillaries. Figures 28 and 29 compare a capillary in the lung of this animal, which had a mean pulmonary arterial pressure of 52 mm. Hg., to a capillary in the lung of an uninfected dog with normal pulmonary arterial pressure. When membrane thickness was determined at intervals of five mm., the average of 18 determinations was 0.17 microns in dog #4 and 0.11 microns in the normal dog.

Larval forms of D. immitis were observed in capillaries in the lung of dog #4. These microfilariæ had a cuticle which varied in thickness



Figure 28. Capillary in the lung of a normal dog. X22,630. B. M. - basement membrane; Alv. - alveolus; Cap. - capillary.

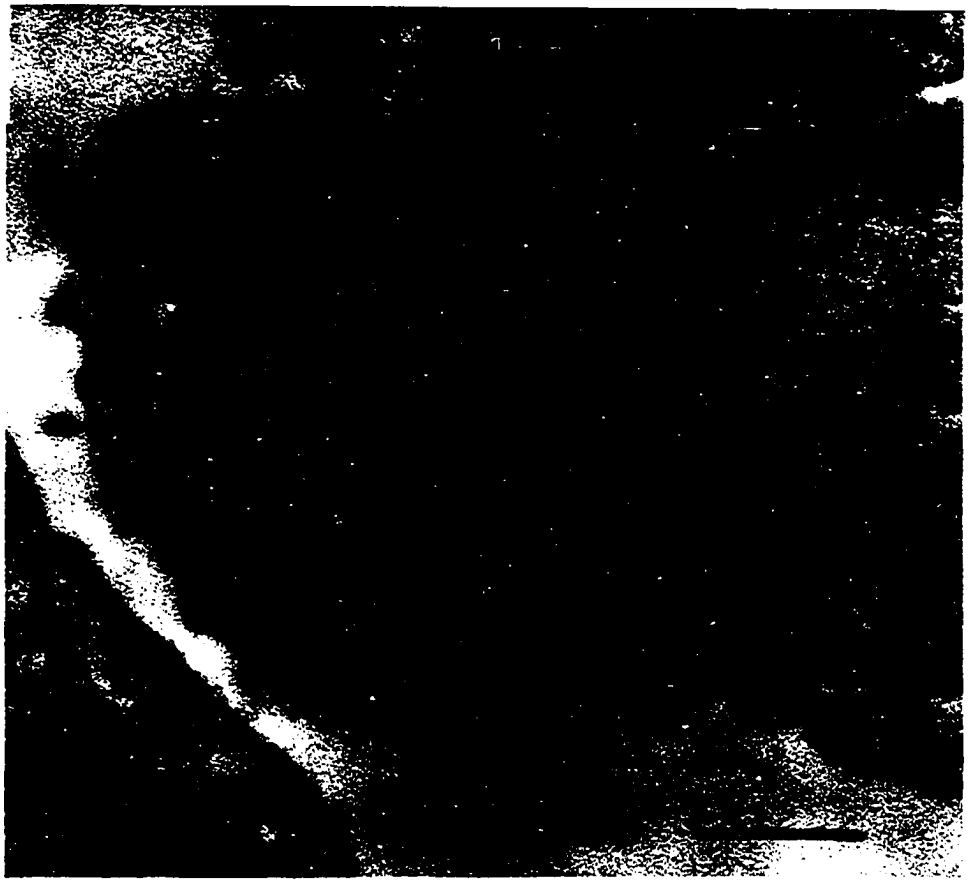


Figure 29. Capillary in the lung of a D. immitis infected dog with elevated pulmonary arterial pressure. X22,630. B. M. - basement membrane; Cap. - capillary.

from 0.09 to 0.13 microns and possessed a series of indentations which apparently encircle the body in a transverse manner. These indentations varied in depth and interval with bending of the body. The distance between indentations averaged 1.08 microns along the greater curvature and 0.7 microns along the lesser curvature of the larva (Figure 30). Averaging these values produces an interval distance of 0.89 microns. In another preparation it was possible to measure the interval between 20 indentations on an unbent larva. The average interval distance in this case was 0.90 microns.

At the anterior blunt end of the larva directly beneath the cuticle and approximately of equal width, was a dense layer which became thicker and filled the area between indentations on the lesser curvature when the larva was bent. Inside this dense layer was a laminated zone approximately 0.42 microns wide. These layers were not apparent in the more posterior regions of the larvae and probably represent ends of elongated cells. Cell membranes were in contact with the cuticle in a transverse section from this region (Figure 31).

A cutting artefact at the anterior end of the larva obscured finer details of structure. Neither body cavity nor tubular organs were apparent. The cephalic end contained a row of five large cells with faintly discernible nuclei and mitochondria (Figure 30). Further posteriorly, cell shape and arrangement became very irregular and intercellular vacuoles and irregularly shaped dense bodies were numerous (Figure 31).

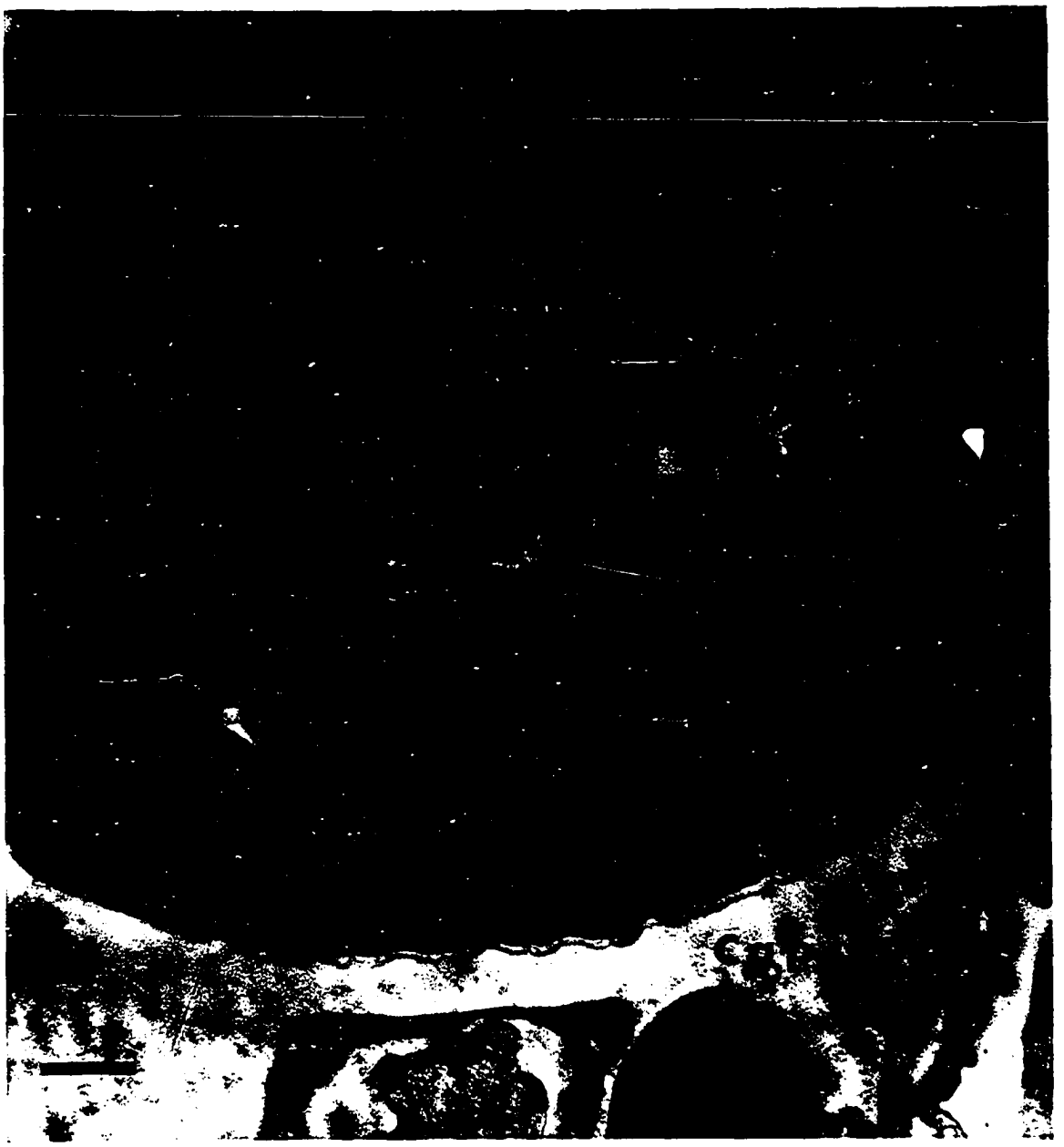


Figure 30. Longitudinal section of a microfilaria in a pulmonary capillary. X12,450. Cap. - capillary.



Figure 31. Transverse section of a microfilaria in a pulmonary capillary. X16,740. Cap. - capillary; Alv. - alveolus.

Periodic acid-Schiff staining of microfilaria showed the presence of PAS-positive material only in, or on, the surface of the cuticle (Figure 32a). These preparations also demonstrated the cuticular indentations when larvae were viewed at low illumination and the cuticle critically focused. The PAS-positivity was diastase labile and thus identified as glycogen. Lack of a positive PAS reaction in smears stained without prior oxidation demonstrated the absence of free aldehyde groups. Deoxyribonucleic acid (Feulgen, methyl green-pyronine, and acridine orange) occurred in the nuclei of somatic cells (Figure 32b) and ribonucleic acid (methyl green-pyronine and acridine orange) occurred in scattered irregular clumps in the central areas of the body. The latter was also observed in clumps and strands in Toluidine blue preparations. Acid mucopolysaccharides (Toluidine blue, alcian blue, Astrablau) were not identified. Oil red 0 failed to reveal the presence of lipids and only light general staining was observed with alkaline fast green (basic protein).

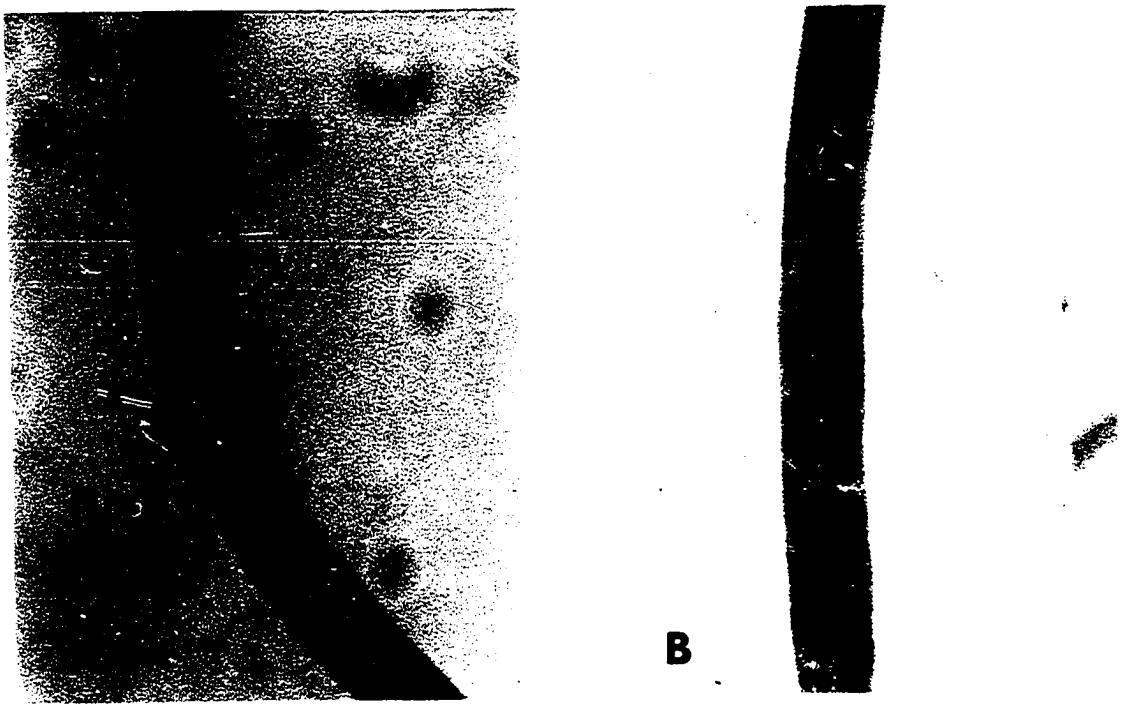


Figure 32. A - Glycogen on the cuticular surface of a microfilaria. PAS, X2,000. B - DNA localized in the nuclei of somatic cells of a microfilaria. Feulgen, X2,000.

CHAPTER IV

DISCUSSION

The prediction of cardiac hypertrophy in the dog by use of heart weight/body weight ratios is of doubtful accuracy in individual animals. These ratios have been studied by several workers and a wide range of values reported (Table 15). The mean HW/BW ratio of 6.94 in the 50 normal dogs of the present study (mean body weight of 15.11kg.) is lower than any documented in the literature; however, the age, sex, and breed of the animals studied probably account for the differences. Removal of the epicardial fat may also contribute to this variance. Cohn and Steele (1936), working with Wirehaired Fox Terriers, showed that the rapid increase in HW/BW ratio up to the age of 1.5 years is followed by a gradual decrease with age. Joseph (1908) observed mean HW/BW ratios of 7.43 in 58 male dogs and 7.61 in 60 females. Northup et al. (1957) working with 481 dogs, observed statistically significant differences between these ratios in large adult females and small adult females and between those of large adult males and large adult females. Equivocal differences were noted between large adult males and small adult males and the ratios were almost equal in immature males and

TABLE 15

CANINE HW/BW RATIOS REPORTED IN THE LITERATURE

	No. Dogs	Mean Body Wt. (kg.)	Mean HW/BW (gm./kg.)
Heinz (1905)	19	4.71	9.71
Cohn and Steele (1936)	63	6.2	8.81
Herrmann (1925)	200	11.37	7.98
Northup et al. (1957)	169*	10.08	7.74
Northup et al. (1957)	177**	8.86	7.56
Stewart (1911)	20	8.71	7.21

*Adult males

**Adult females

females. Herrmann (1926) studied the HW/BW ratio in 10 racing Greyhounds and observed a range of 11.3 to 17.3 with a mean of 13.4. Thus, higher HW/BW ratios occur in smaller dogs and in animals bred for strenuous exercise.

While weights of the component parts of the canine heart have been studied by several workers, variations in method of dissection render comparison of results difficult. Stewart (1911) studied the hearts of 20 small dogs and observed a mean right ventricular weight of 13.58 gm., a mean interventricular septal weight of 14.34 gm., and a mean left ventricular weight of 26.43 gm. The mean LV/RV ratio was 1.950 in this group of dogs. These data are comparable with those of the present study. Utilizing formalin-fixed hearts and a method of dissection in

which the interventricular septum was divided into right and left parts, Herrmann (1925) observed a mean left heart/right heart ratio of 1.398 in 200 dogs. The mean ratio was 1.393 in 80 males and 1.390 in 80 females. Platt (1952), using Herrmann's (1925) method of dissection, reported mean LV/RV ratios of 1.488 in 4 dogs with mild nephritis and of 1.761 in 13 dogs with severe chronic nephritis.

When dissected by the method used in the present study, the canine LV/RV ratio changes as heart size increases; from the regression study these ratios were 1.79 and 2.19 at ventricular mass weights of 40 and 210 gm. In order to determine whether this finding was related to the method of dissection, linear regression studies were made on data from 20 canine hearts randomly selected from those recorded by Herrmann (1925). These hearts were dissected after fixation in the method described by Lewis (1913) and LV/RV ratios remained constant at 1.388 and 1.394 for ventricular mass weights of 40 and 200 gm. Thus the method of dissection has a marked effect on cardiac component ratios.

The great variation in cardiac thickness observed in the 50 normal dogs of the present study was probably produced by lack of uniformity in the state of rigor mortis. While necropsies were performed on most animals immediately after death, in some cases one or two hours elapsed before it was possible to measure the hearts. It was noted that muscular thickness almost doubled after the occurrence of rigor mortis.

The pressures obtained in the hymodynamic study of the nine control dogs, after allowing for correction of intrathoracic pressure, were comparable to those reported in the literature (Hellems et al., 1948; Haddy et al., 1949, 1953; Alden et al., 1953a, 1953b; Haddy and Campbell, 1953). While the means of the pulmonary arterial systolic pressure and femoral artery mean pressures were slightly lower than those documented, the ranges were within the reported limits. The upper limits of the normal pulmonary arterial pressure are difficult to establish but from the reports of most workers can be set at approximately 45/20 (average 22) mm. Hg. Detailed anatomical studies were not carried out in all animals in any of these reports.

Except for the greater number of animals with elevated diastolic pressures, the pulmonary hemodynamics of the infected dogs in the present study are comparable with those reported by Wallace and Hamilton (1962) in a group of 27 D. immitis infected dogs. In these animals, which also showed no clinical signs of congestive heart failure, the systolic pressures ranged from 22 to 55 mm. Hg. and the diastolic pressures from 9 to 30 mm. Hg. Elevated systolic pressures were observed in eight dogs and elevated diastolic pressures in five animals. Both systolic and diastolic pressures were elevated in five dogs and in three animals there were elevated systolic and normal diastolic pressures. Elevation of only the diastolic pressure occurred in one dog; however, these pressures were not reported in 13 animals. The mean right atrial pressure varied from

0 to 5 mm. Hg. and the mean right ventricular systolic pressure for the group was 42 mm. Hg. Femoral artery systolic pressures varied from 118 to 220 mm. Hg.

While much variation was noted in the pulmonary arterial pressures occurring in individual dogs in the present study and in the work of Wallace and Hamilton (1962), Hyman, et al. (1963) reported spontaneous variations of only 5.2 mm. H_2O in the canine mean pulmonary pressure. A number of factors, in addition to progression of the disease, may be responsible for these differences. In the present study no record was kept of the vessel catheterized or of the site of measurement. These factors may produce minor differences in pressure readings, as would differences in positioning of the catheter in the vessel. Lack of uniformity in the plane of anesthesia could also induce hemodynamic variations, bradycardia resulting in lowering (Campbell et al., 1949) and anoxia in elevation (Motley et al., 1947; Liljestrand, 1948) of the pulmonary arterial pressure.

In one measurement on dogs "X", "G", "K", "P", and #1 a pressure gradient of at least 20 mm. Hg. occurred in the systolic pressure between the right ventricle and the pulmonary artery and the right ventricular mean pressures exceeded the pulmonary arterial mean pressures. This fall in pressure was not observed in subsequent measurements in dogs "N", "K", "P", and #1. It is probable that these pressure differences reflect mechanical occlusion of the pulmonary artery by a mass

of worms or the dampening effect of sharply bending the catheter, the presence of parasites often making catheterization difficult. Since these discrepancies were observed in the only measurements made on dogs "X" and "G", the readings on these animals are considered incorrect and must be disregarded. The marked increase in pressure which occurred in the second determination on dog "P" is unexplained.

While Peterson et al. (1951) reported sinus arrythmias in unanesthetized dogs, tachycardia with regular rhythm was observed in electrocardiographic examinations of all dogs in the present study. The mean electrical axes were 89 degrees in the eight normal control dogs, 89 degrees in the 21 infected dogs, and 101 degrees in the seven dogs suspected of having right ventricular hypertrophy. Much variation occurred in animals upon which serial tracings were made. Lombard and Withan (1955) observed similar changes in electrocardiograms on anesthetized dogs. Day-to-day variations were noted in spite of careful reproduction of electrode placement and body positioning. Detweiler et al. (1960) reported marked right deviation of the mean electrical axis in four dogs with right ventricular hypertrophy, one animal being infected with D. immitis. Wallace and Hamilton (1962) observed mean electrical axes of 154 degrees in a group of D. immitis infected dogs which had clinical signs of cardiac failure and of 97 degrees in heartworm infected dogs not in cardiac failure.

The intimal lesions of the pulmonary arteries in the present study are compatible with those described in the literature. These changes have been considered to be specific for dirofilariasis and to represent an attempt by the host to wall off the parasite (Adcock, 1961). Alterations somewhat similar in type were observed in rabbits subjected to multiple intravenous injections of thromboplastin (Gore et al., 1962), suggesting that thrombosis may be a factor in the development of such lesions in canine dirofilariasis. The absence of medial hypertrophy in muscular arteries and arterioles, as observed by Porter (1951), Hennigar and Ferguson (1957), and Adcock (1961), may be the result of selection of experimental animals, none of the dogs in the present study having clinical signs of congestive heart failure. Spencer (1962) described these changes in cases of chronic pulmonary hypertension. The arteritis observed in the present study is apparently due to the presence of parasites, living or dead, and is not related to the chronically elevated pulmonary arterial pressure (Old and Russel, 1950).

Alterations in other organs have an equivocal relationship to the parasitic infection. While microfilariae were often associated with inflammatory foci in the myocardium and lung, in some cases these larvae were numerous and evoked no inflammatory response. Degenerating larvae surrounded by inflammatory infiltrations were observed in the livers and kidneys of the infected dogs. The distribution of microfilariae in other tissues of the body was irregular and in these locations larvae

were rarely associated with inflammatory reactions. While microfilariae were commonly observed in the lungs, kidneys, livers, intestines, and brains, they were rarely recognized in spleens or lymph nodes. Difficulty in identification of larvae cut in cross section may be a factor affecting their apparent distribution in tissue sections. Dermatitis occurred in only two of the three dogs in which microfilariae were present in the skin. Monlux (1953) failed to find evidence relating microfilariae to the renal lesions in chronic canine nephritis.

In the present study, the degree of pulmonary hypertension could not be directly related to the number of adult parasites in the pulmonary circulation. The most heavily parasitized dog (84 worms) had only a moderate increase in pulmonary arterial pressure and one of the more lightly infected animals (11 worms) had the greatest elevation. Neither incompetence nor blockage of the pulmonary valves, except when artifactually produced, was observed. These findings are in accordance with the work of Wallace and Hamilton (1962). Thus, in dirofilariasis the physical presence of adult parasites plays a minor role in the production of pulmonary hypertension.

No specific lesion could be associated with higher pulmonary arterial pressures. Hypertension is known to follow thrombosis and embolism of the pulmonary arteries (Haynes et al., 1947; Harrison, 1951; Jaques et al., 1960). These lesions were present in 13 of the 21 infected dogs; however, Haynes et al. (1947) and Swenson and Choudhury (1962) demonstrated that one major branch of the pulmonary artery can be

completely occluded with little rise in blood pressure. In no dog was there evidence of sufficient thrombosis to reduce the pulmonary blood flow one half. Gore et al. (1962) did not observe increased pulmonary arterial pressure in experimental rabbits with severe proliferative and inflammatory intimal lesions of the intrapulmonary arteries. The administration of epinephrine, however, did result in increased pulmonary pressures in these animals.

In many dogs of the present study there was disproportion between the hemodynamic and arterial changes, lightly infected dogs exhibiting elevated pressures but only minimal lesions. Some degree of interstitial pneumonitis was observed in all of the infected dogs and may be the primary factor in the production of pulmonary hypertension. This hypothesis is supported by electron microscopic examination of the lungs of one dog in which thickened basement membranes were observed in capillaries; however, further study, utilizing more animals and confirmation by statistical analysis, is necessary to prove the theory.

Total cardiac size in the dogs of the present study was within normal limits. Dog "F", which had the highest HW/BW ratio, was a Greyhound in a low state of nutrition. Animals of this breed are noted for their large hearts (Herrmann, 1926). Dog "N", which had the lowest HW/BW ratio, was an obese female in the late stages of pregnancy. Gross and microscopic examination of the hearts of seven dogs indicated the presence of right ventricular hypertrophy. The ventricles were heavier

and possessed muscle fiber segments of greater length than those of normal dogs. Linzbach (1956) observed significant differences in the length of muscle fiber segments in human left ventricles above and below the critical weight of 200 gm. Electrocardiograms made on the dogs of the present study did not confirm the right ventricular hypertrophy. This may be explained by the slight increase in ventricular size and the great normal variation in canine electrocardiograms (Lombard and Withan, 1955). Interstitial pneumonitis probably accounted for both the pulmonary hypertension and right ventricular hypertrophy of control dog "A".

The microfilariae observed in electron microscopic study of the lungs of dog #4 can be only speculatively identified as larval forms of D. immitis since it was impossible to measure the total length of the larvae in electron microscope grids. However, the host did have adult forms of D. immitis in the right heart and no adult filariids were observed in subcutaneous locations. There is a paucity of information concerning the ultrastructure of nematode larvae. The present study indicates that the microfilariae of D. immitis possess neither body cavity nor internal tubular organs. A cutting artifact prevented observation of the hooks at the anterior end of the larvae (Taylor, 1959) but large cells did occur in the head (Taylor, 1960). Transverse striations, shown best by phase-contrast microscopy or silver impregnation methods, have been observed in the cuticle of D. immitis larvae. These striations were reported by Foshay (1947) to have an average interval distance of 0.9 microns and

Taylor (1959) observed the width between striations to be 0.6 microns. The interval between cuticle indentations observed in the present study corresponds to the striation interval reported by Foshay (1947); however, indentations were present at the extreme cephalic tip of the larvae.

The limited histochemical study of the larvae indicated that the nuclei of the body wall contain DNA and that RNA is present in the central areas of the body. While the distribution of these substances would be better demonstrated in sections of the microfilariae, Rothstein (1958) made similar observations using acridine orange in the vital staining of D. immitis larvae. The nuclei of somatic cells stained yellow and irregular red areas were observed. In the present study PAS-positivity, indicating the presence of 1,2-glycol groups, occurred only in, or on the surface of the cuticle. Acid mucopolysaccharides and lipids were not identified. Alkaline fast green preparations did not indicate high concentrations of basic protein. Further histochemical investigation is indicated.

CHAPTER V

SUMMARY

Pathophysiologic and anatomic alterations of canine dirofilariasis were investigated in a group of 16 infected dogs which had no clinical signs of congestive heart failure. The hemodynamic changes occurring in the pulmonary circulation following treatment to destroy the adult parasite were studied in five additional animals. Normal values were established for weights of the various parts of the heart by analysis of data obtained from the hearts of 50 normal dogs. The ultrastructure of the microfilariae of D. immitis was investigated.

Pulmonary hypertension occurred to some degree in all of the experimental dogs. Elevated pulmonary arterial mean pressures were observed in 16 dogs; diastolic pressure was elevated in 13 and systolic pressure in only 6 animals. While right ventricular mean pressures were elevated in all dogs, systolic pressures of only 11 animals exceeded the normal range. No evidence of blockage of the pulmonary artery by adult parasites was obtained. Destruction of the adult parasites produced elevation of pulmonary arterial pressure in only one of five dogs.

While gross lesions were minimal, marked and characteristic microscopic alterations were observed. Intimal fibrosis of diffuse or

villous type involved both the main pulmonary artery and its intrapulmonary branches. Medial hypertrophy was not detected in arteries or arterioles. Arteritis was associated with the presence of both living and degenerating parasites and thrombosis of arteries and arterioles occurred commonly. Other lesions included endothelial proliferation in arterioles and capillaries, interstitial pneumonitis, and pulmonary fibrosis. Direct relationship could not be established between hemodynamic alterations and either the physical presence of the parasite or the arterial lesions. It is suggested that lesions of the capillary and arteriolar beds are responsible for much of the hypertension. Focal collections of inflammatory cells were associated with the presence of degenerating larvae in the lung, myocardium, kidney, and liver.

Right ventricular hypertrophy was detected in the hearts of seven infected dogs by gross and microscopic examination. No electrocardiographic abnormalities were observed.

Electron microscopic study of the microfilaria revealed neither a body cavity nor internal tubular organs. The cuticular indentations had a similar interval distance to that of the previously described transverse striations.

The occurrence of pulmonary hypertension and right ventricular hypertrophy in D. immitis infected dogs not in cardiac failure warns against the use of such dogs as experimental animals in critical research.

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