Histological and Ultrastructural Evaluation of Cardiac Lesions in Idiopathic Cardiomyopathy in Dogs

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ABSTRACT

Described are pathological studies of eight dogs which died in congestive heart failure, with a clinical diagnosis of congestive cardiomyopathy. Examination revealed marked dilatation and enlargement of all four chambers of the heart. The ventricular walls were thin with small atrophic papillary muscles. Histological studies on the myocardium revealed scattered areas of myocardial necrosis, especially around the papillary muscles of the left ventricle and random scattered areas of fibrosis. Electron microscopic studies revealed fewer and disoriented myofibrils, myocytolysis, increased numbers of mitochondria with swelling and inclusions, increased glycogen granules and increased numbers of lysosomes, lipofuscin granules and lipid droplets. Mild Z-band abnormalities were found throughout the myofibers.

Key words: Cardiomyopathy, dog, ultrastructure, heart.

RÉSUMÉ

Les auteurs décrivent leurs observations pathologiques relatives à huit chiens chez lesquels ils avaient posé un diagnostic clinique de cardiomyopathie congestive et qui succombèrent à une défaillance cardiaque congestive. Les lésions macroscopiques se traduisaient par une dilatation et un agrandissement marqués des quatre chambres cardiaques. Les parois ventriculaires étaient amincies et leurs muscles papillaires, petits et atrophiés. L'histopathologie du myocarde révéla de la nécrose focale, surtout autour des muscles papillaires de la paroi du ventricule gauche, et des foyers épars de fibrose. La microscopie électronique permit de constater une diminution et une mauvaise orientation des myofibrilles, de la myocytolyse, une augmentation du nombre de mitochondries, lesquelles présentaient du gonflement et des inclusions, une augmentation du nombre des granules de glycogène, ainsi qu'une augmentation du nombre des lysosomes, des granules de lipofuscine et des gouttelettes lipidiques. La plupart des fibres musculaires du myocarde affichaient une légère anormalité de leurs bandes Z.

Mots clés: cardiomyopathie, chien, ultrastructure, coeur.

INTRODUCTION

Idiopathic cardiomyopathy has been described in the dog by several investigators (1,2,3,4,5,6). This disease appears to be similar to idiopathic cardiomyopathy in man. Dogs with cardiomyopathy have dyspnea, tachypnea and other clinical signs of heart failure. The most prominent functional disturbance is a rapid and irregular heart rate with atrial fibrillation. Ascites, hepatomegaly and splenomegaly are found with right-side heart failure (4). However, there is a paucity of light and electron microscopic investigations on cardiac lesions that occur in cardiomyopathy of dogs. Marked dilatation of all four chambers of the heart has been reported with scattered areas of endocardial and myocardial necrosis and fibrosis (4,6).

The objective of this study was to evaluate and describe the microscopic, histological and electron microscopic lesions in congestive cardiomyopathy in the dog.

MATERIALS AND METHODS

Tissues from eight dogs with congestive cardiomyopathy and tissues from three normal healthy control dogs were available for study. Criteria for clinical diagnosis of cardiomyopathy included left ventricular or biventricular enlargement, the presence of an abnormal electrocardiogram, presence of a gallop rhythm, absence of valvular disease and the absence of pulmonary disease. These criteria are considered essential in the establishment of a clinical diagnosis of cardiomyopathy.

The dogs were presented in terminal heart failure. At death, the entire heart was removed and the chambers washed free of blood. The total heart weight was recorded and both the tricuspid and mitral valve circumferences were measured. Both the heart weight/body weight ratio and the mitral valve circumference/tricuspid valve circumference ratio were determined. The weights and ratios were similarly determined for the hearts of 30 large breed dogs. The heart and tissues from other organs were fixed in cold, phosphate-buffered 10% neutral formalin. Sections were examined from the following areas of the heart: a) left ventricular free wall with papillary muscle, b) right ventricular free wall containing the lateral leaf of tricuspid valve with right atrium, c) interventricular septum with septal

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leaf of tricuspid valve and interatrial septum and d) left atrium. Sections of heart were routinely stained with hematoxylin and eosin (HE) and Masson's trichrome.

Immediately after death, a few selected areas of the myocardium from each chamber were cut into 0.5 mm cubes, placed in 3% glutaraldehyde in 0.2 M sodium cacodylate, washed in buffer and postfixed in 1.3% osmium tetroxide with s-collidine. The tissue was dehydrated with descending concentrations of ethyl alcohol, transferred to propylene oxide and embedded in Epon. Thick (0.5 μm) sections were stained with toluidine blue and evaluated by light microscopy to select areas of myocardium for electron microscopic examination. Ultrathin (60-80 nm) sections of selected areas of the heart were cut on an LKB ultramicrotome, stained with uranyl acetate and lead citrate and examined with a Philips 200 electron microscope. The significance of difference between control animal data and cardiomyopathy animal data was assessed by Student's t-test.

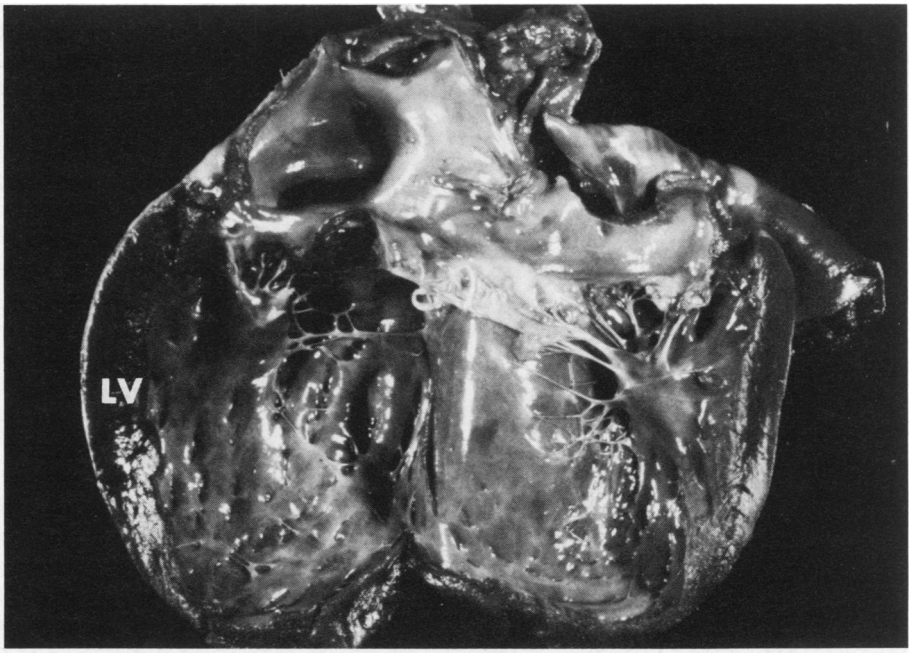

Fig. 1. Enlarged left ventricular chamber (LV) in a two year old dog with severe congestive cardiomyopathy with thin ventricular walls.

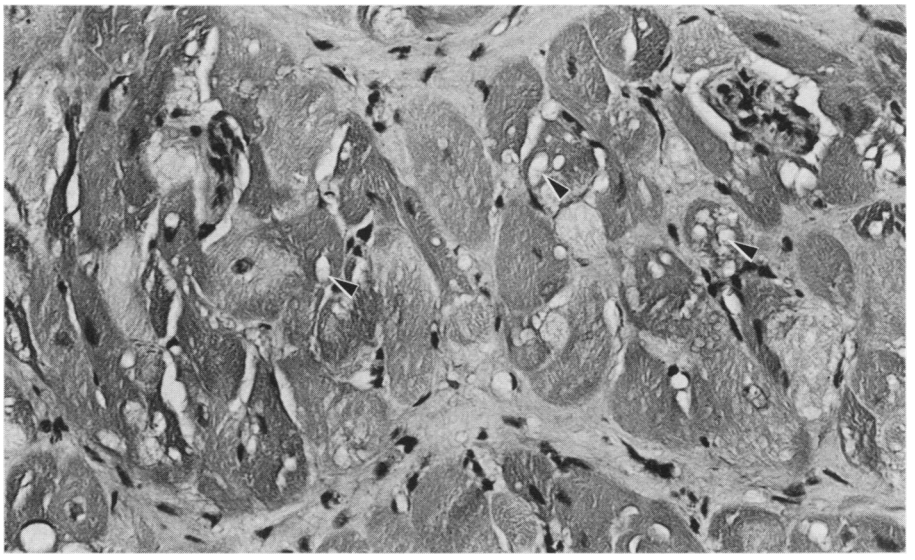

Fig. 2. Extensive vacuolation (arrowheads) of cardiac muscle cells in left ventricle and separation by collagen fibers in a dog with cardiomyopathy.

RESULTS

NECROPSY FINDINGS

The dogs were large breed and consisted of three Great Danes, two Doberman Pinschers, two Afghans and one St. Bernard. The most common findings in six of eight dogs were cardiomegaly, ascites, chronic passive congestion of the liver, pulmonary congestion and edema and occasionally hydrothorax.

Table I summarizes the mean cardiac weights and valve circumferences

Table I. Heart Weight and Valvular Circumference Measurement Ratios in Hearts of Normal Large Breed Dogs and Dogs with Congestive Cardiomyopathy

Group	Statistic	Body Weight (kg)	Heart Weight (g)	Mitral Valve Cir (mm)	Tricuspid Valve Cir (mm)	Heart/ Body Weight Ratio	Mitral/ Tricuspid Ratio
Normal Large Breed Dogs	Mean	40.76	257.4	106.5	128.0	6.53	0.8338
	Std	13.74	87.2	12.2	14.4	1.53	0.0627
	Stderr	2.51	15.9	2.2	2.6	0.28	0.0114
	N	30	30	30	30	30	30
Cardiomyopathy	Mean	55.97[a]	440.1[a]	128.4[a]	147.7[a]	7.86[a]	0.8808
	Std	6.88	110.3	15.3	24.8	1.67	0.1022
	Stderr	2.60	41.7	5.8	9.4	0.63	0.0386
	N	7	7	7	7	7	7

[a]$P \leq 0.05$, One tailed Student t on raw data.

in eight dogs with cardiomyopathy and in 30 dogs without cardiomyopathy. All dogs with congestive cardiomyopathy had large absolute heart weights (mean 440.1 g) when compared with those in our control group (mean 257.4 g). The mean heart weight/body weight ratio was increased in the dogs with cardiomyopathy (7.86) compared with the control dogs (6.53). Both the mitral valve circumference and tricuspid valve circumference were enlarged when compared to the control dogs. The mitral tricuspid valve ratio between dogs with cardiomyopathy and our control group was not significant.

Extreme dilatation of all four chambers of the heart was present in all eight dogs. This gave the heart a globoid appearance. The left ventricular free wall was thinner than normal in six of eight dogs. The papillary muscles were atrophied and shrunken in six dogs. The entire heart was pale and the left ventricular myocardium was flabby (Fig. 1). Both atrioventricular valves were normal with no changes seen in the valve leaflets. The circumferences of the mitral valves and tricuspid valves were greater than normal. White areas of scarring were seen in the subendocardium of the left ventricle in five of eight dogs. No thrombi were seen in the left atria, however multifocal areas of infarction were seen in the kidneys and spleens of two dogs.

LIGHT MICROSCOPIC OBSERVATIONS

Histopathological alterations were most often seen in the left ventricular wall. Scattered multifocal areas of fibrosis and multifocal areas of myocardial degeneration and necrosis were present in the wall of the left ventricle. The base of the papillary muscles had more fibrosis and myocardial degeneration and necrosis than other areas of the heart. The degenerating myocytes contained variable numbers of sarcoplasmic vacuoles of varying sizes (Fig. 2). Myofibers were thin and wavy in areas and varied in size. Strands of fibrous connective tissue extended from the endocardium through the myocardium to the epicardium in two dogs. One dog had a multifocal lymphocytic infiltration in the left atrium and seven dogs had scattered areas of fibrosis in both atria and in the right ventricle. No inflammation was seen in the myocardium of the ventricles.

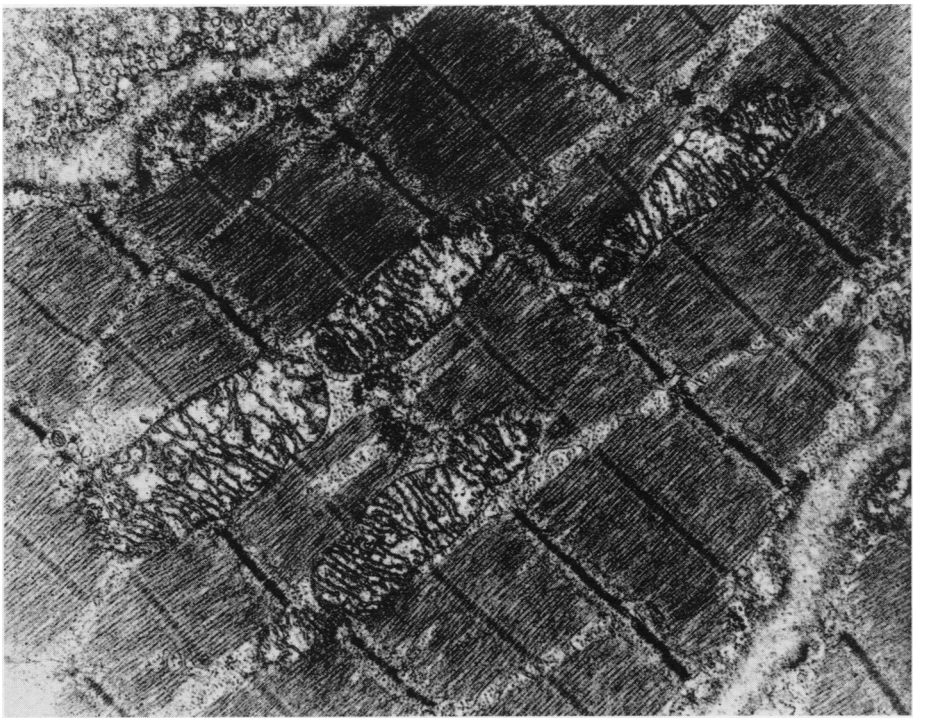

Fig. 3. Left ventricular myocardium from a normal control dog. X32,000.

TRANSMISSION ELECTRON MICROSCOPIC FINDINGS

The myocardium of the three control dogs in this study was similar to those reported for normal canine cardiac muscle (7). Figure 3 depicts a normal fiber characterized by an arrangement of myofibrils lined up uniformly with a single row of mitochondria between the myofibrils.

As observed under light microscopy in the myocardium of dogs with con-

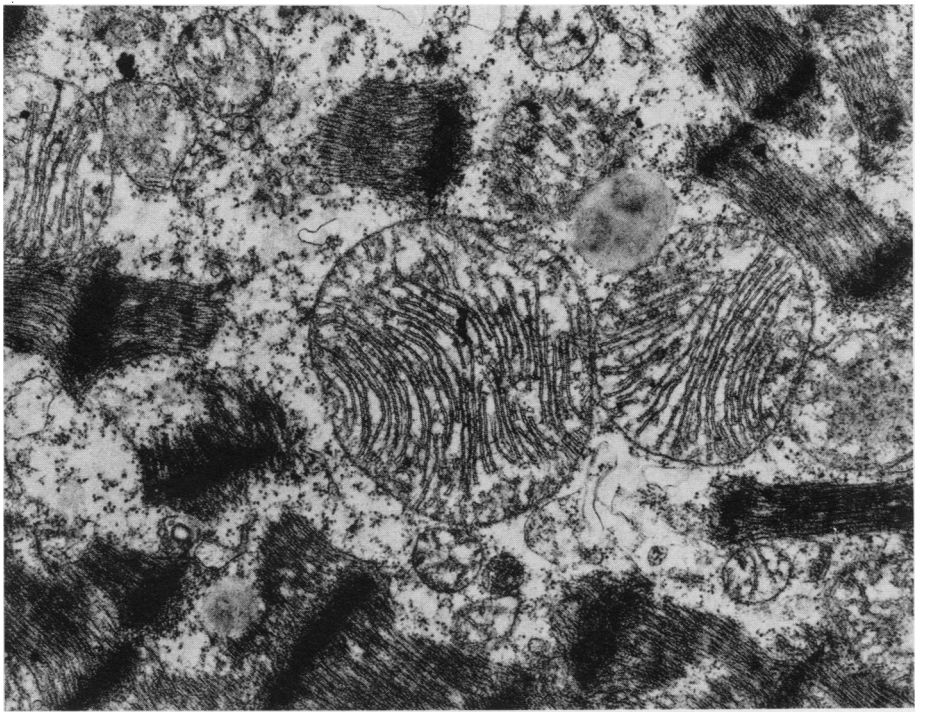

Fig. 4. Electron micrograph of cardiac muscle illustrating loss of myofilaments, disorientation of remaining myofibers and increased size of mitochondria. X15,000.

gestive cardiomyopathy, considerable amounts of collagen and many fibroblasts were seen in the interstitium between myocardial cells. Edema was also evident distending the interstitium.

The myofibrils in the myocytes were disrupted, with only short fragments of myofibrils remaining (Fig. 4) in degenerating cells. These myofibrils appeared as disoriented and loosely arranged in the cytoplasm. Some cells had no remnants of myofibrils remaining. This severe lesion varied and was seen in all four chambers of the heart (Fig. 5). Some adjacent myocytes had a normal appearance with minimal changes. Changes present in the Z-bands were an irregular widening and thickening (Fig. 6).

Mitochondria were prominent when seen between the remaining fragments of myofibrils. Many mitochondria were irregularly-shaped, some were large in size, and some were clustered together, pushing myofibrils apart. Occasionally, mitochondria were swollen with disruption of cristae and formation of myelin figures. Intramitochondrial inclusions consisting of spherical bodies with concentric rings were seen in two of eight dogs.

Numerous glycogen granules were present in degenerated myocardial cells (Fig. 7). Swelling of the sarcoplasmic reticulum was occasionally observed, but it was focal and was associated with damage of other subcellular structures. Vacuoles were seen widely scattered in myocardial cells undergoing degeneration. Large numbers of lipofuscin granules (Fig. 8) were seen in the sarcoplasm and in perinuclear spaces in degenerating cardiac myocytes.

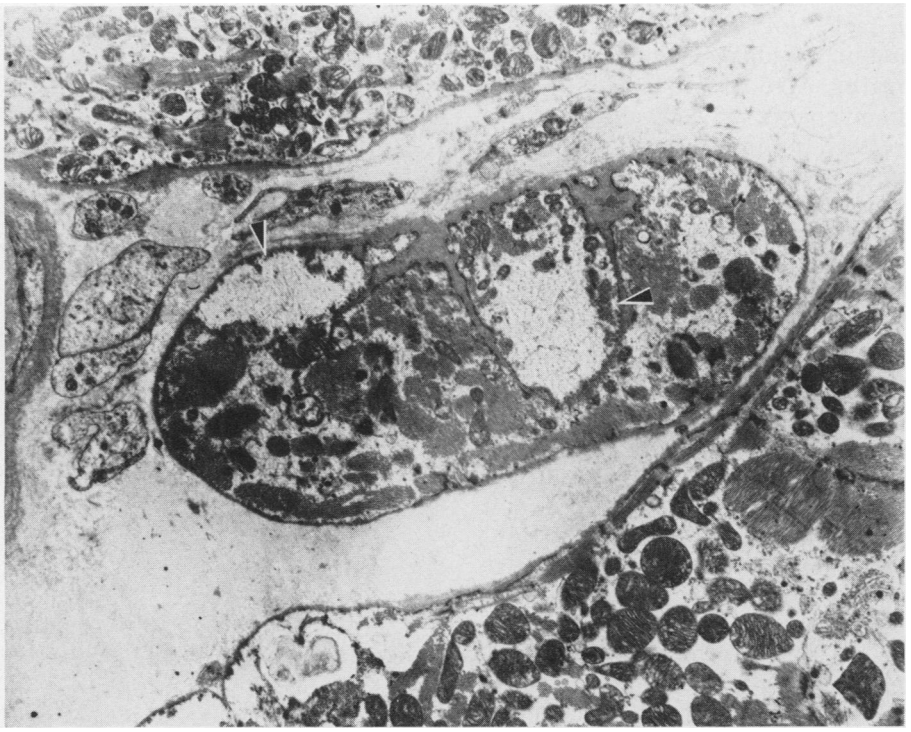

Fig. 5. This electron micrograph shows a cross sectional area of three myocardial fibers. Myocytolysis with loss of myofibrils and most organelles is seen in two destroyed fibers (arrowheads). X5,000.

DISCUSSION

The clinical findings in the study are similar to those previously reported for dogs with congestive cardiomyopathy. An unusual aspect of this disease process is that this clinical entity is restricted to large breed purebred dogs. The Great Dane was the most commonly affected, which is similar to previous studies (3,6).

Mean heart weights were moderately increased in the dogs with cardiomyopathy compared either with previously published values (6) or with values for our control group of dogs. The dilated mitral and tricuspid valve circumferences were slightly increased in dogs with cardiomyopathy compared with our control group of dogs. Pathologically this has been reported but not measured in previous studies (3,6). The heart weight/body weight ratio was slightly increased in dogs

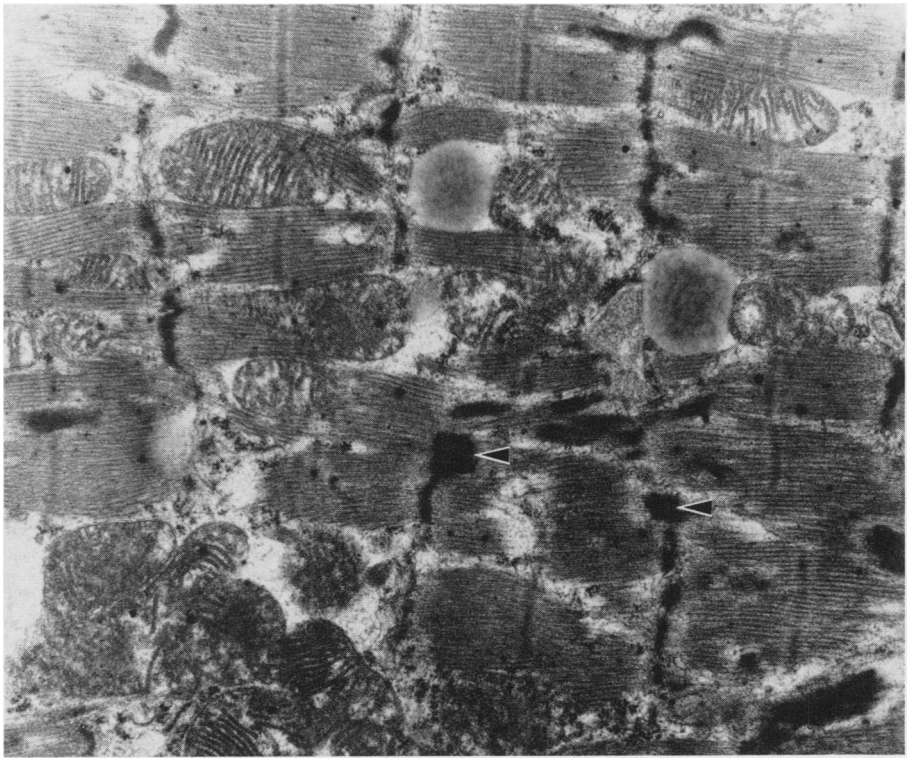

Fig. 6. Degenerating myofibrils are seen with mitochondria and lipid vacuoles. Early irregularities and thickening are seen in the Z-bands (arrowheads). X15,000.

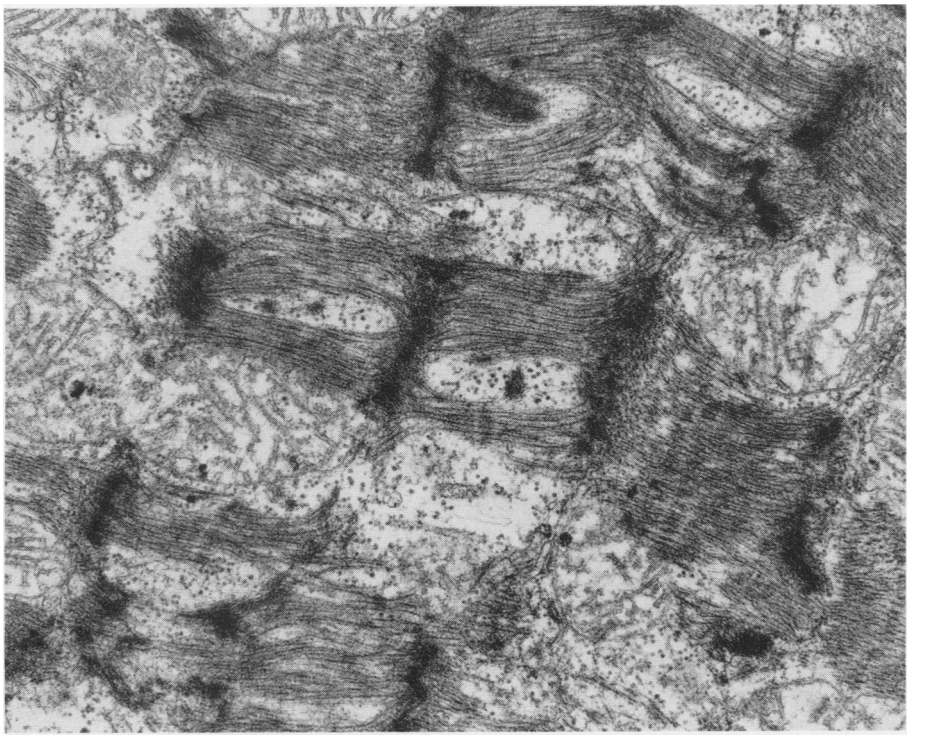

Fig. 7. Numerous cytoplasmic glycogen granules are seen in the cells and within degenerating myofibrils. X30,000.

with cardiomyopathy compared to previous reports (6) and our control group of dogs.

The gross heart lesions in the eight dogs with congestive cardiomyopathy examined included dilatation of all four chambers of the heart with thin ventricular walls. Microscopic examination of the hearts revealed patchy interstitial fibrosis and multifocal areas of myocardial degeneration and necrosis especially noted around the left ventricular papillary muscle region. These changes were similar to those found previously in dogs (2,3, 4,5,6), cats (8,9) and man (10,11,12, 13,14,15) with congestive cardiomyopathy. Thrombi were seen in two dogs in this study while intracardiac fibrin-platelet thrombi were common in human patients (14) and cats (8).

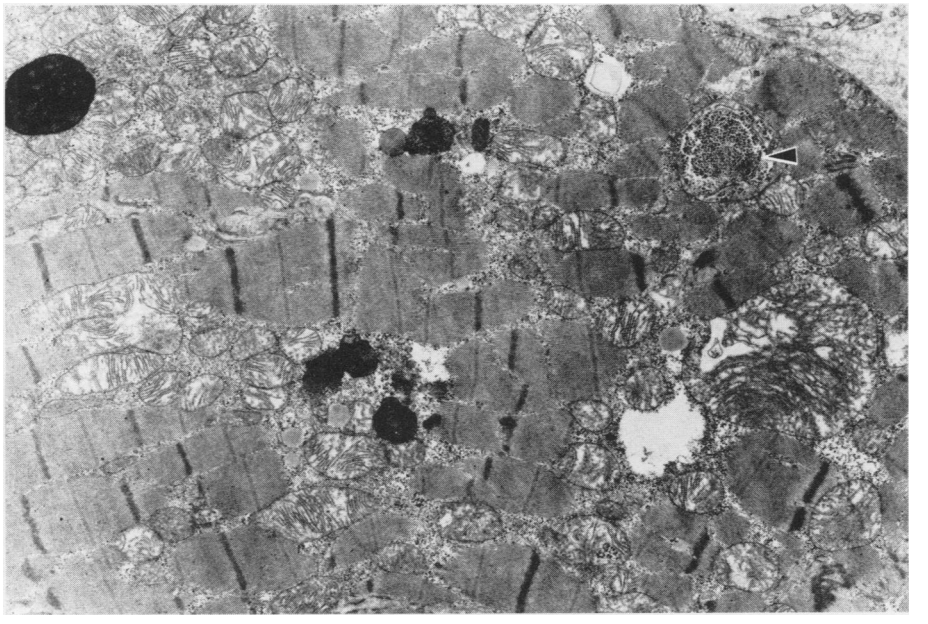

Fig. 8. Irregular-sized mitochondria with one containing numerous particles (arrowheads) and numerous large dense lipofuscin granules. Minor irregularities in Z-bands are seen. X4,500.

Ultrastructurally, the main changes in this study in the degenerating myocytes were disorientation and loss of myofibers with short fragments remaining. Irregular thickening of Z-bands and Z-band degeneration was present in these cells. Numerous glycogen granules were seen in the myocytes along with an increase of lipid vacuoles. Mitochondrial alterations were present in these degenerating myocytes.

Electron microscopic studies in man (10,11,13,14) revealed degenerating cardiac myocytes that have been described in various chronic heart diseases. These include increased numbers and various sizes of mitochondria, numerous glycogen granules, autophagosomic lysosomes and dilated sarcoplasmic reticulum and T system.

Other degenerative lesions which have been previously described in man in cardiomyopathy are cellular edema, loss of myofibrils, increased numbers of lipid droplets, lysosomes and lipofuscin granules (13). In addition, various mitochondrial alterations such as swelling, loss of cristae and myelin formations have been seen along with myofibrillar damage and Z-band alterations (13,14,15). These observations and changes are similar to those seen in the dog (3,6) and cat (9) with cardiomyopathy.

The ultrastructural changes in the dog and man are nonspecific degenerative cardiac changes which are not diagnostic of congestive cardiomyopathy and have been described in many chronic cardiac diseases (16). However, the ultrastructural lesions of cardiomyopathy may reflect the limited ability of the myocardium to respond to a variety of injurious agents.

The combination of gross examination, microscopic studies and ultrastructural changes presents a characterization of the structural changes occurring with idiopathic cardiomyopathy. The similar clinical and pathological patterns in man, cat and the dog suggest that congestive cardiomyopathy in the dog is similar to the same disease entity in man and the cat.

REFERENCES

1. **ETTINGER SJ, SUTTER PF comps.** Canine cardiology. Philadelphia: WB Saunders, 1970: 383-402.
2. **LORD PF.** Left ventricular volume studies on the diseased canine heart: congestive cardiomyopathy and volume overload. Am J Vet Res 1974; 35: 493-501.
3. **TILLEY LP, LIU SK.** Cardiomyopathy in the dog. Recent Adv Stud Cardiac Struct Metab 1975; 10: 641-653.
4. **ETTINGER SJ.** Diseases of the myocardium. In: Ettinger SJ, ed. Textbook of veterinary internal medicine. Philadelphia: WB Saunders, 1975: 953-976.
5. **LIU SK, MARON BJ, TILLEY LP.** Hypertrophy cardiomyopathy in the dog. Am J Pathol 1979; 94: 497-508.
6. **VAN VLEET JF, FERRANS VJ, WEIRICH WE.** Pathologic alterations in congestive cardiomyopathy of dogs. Am J Vet Res 1981; 42: 416-424.
7. **PRICE Z, EIDE B, PRINTZMETAL M, CARPENTER C.** Ultrastructure of the dog cardiac muscle cell. Circ Res 1959; 7: 858-865.
8. **TILLEY LP, LIU SK, GILBERTSON SR, WAGNER BM, LORD PF.** Primary myocardial disease in the cat: a model for human cardiomyopathy. Am J Pathol 1977; 87: 493-522.
9. **VAN VLEET JF, FERRANS VJ, WEIRICH WE.** Pathologic alterations in hypertrophic and congestive cardiomyopathy of cats. Am J Vet Res 1980; 41: 2037-2048.
10. **RODIN AG, HARRIS LC, NGHIEM Q.** Idiopathic nonobstructive cardiomyopathy. Arch Pathol 1971; 91: 62-69.
11. **OLSEN EGJ.** Pathology of primary cardiomyopathies. Postgrad Med J 1972; 48: 732-737.
12. **ROBERTS WC, FERRANS VJ.** Pathological aspects of certain cardiomyopathies. Circ Res 1974; 35 (Suppl. II): 128-144.
13. **ROBERTS WC, FERRANS VJ.** Pathologic anatomy of the cardiomyhopathies: idiopathic dilated and hypertrophic types, infiltrative types, and endomyocardial disease with and without eosinophilia. Hum Pathol 1975; 6: 287-342.
14. **ROBERTS WC, FERRANS VJ, BUJA LM.** Pathological aspects of idiopathic cardiomyopathy. Comp Path Heart Adv Cardiol 1975; 13: 349-367.
15. **IZUMI T, HATTORI A, HIGQUMA N, TAMURA K.** Cardiac myofibril disorientation and Z-band abnormalities in idiopathic cardiomyopathy. Arch Histol Jpn 1978; 41: 293-308.
16. **BISHOP SP, COLE CR.** Ultrastructural changes in the canine myocardium with right ventricular hypertrophy and congestive heart failure. Lab Invest 1969; 20: 219-229.