Postmortem Findings in Four Litters of Dogs With Familial Canine Dermatomyositis

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Postmortem evaluations were performed on 20 juvenile to young adult collie and collie–Labrador retriever crossbred dogs with dermatomyositis and 10 neonatal collies. Cutaneous, muscular, and vascular lesions were present in the juvenile and adult dogs and were most severe in areas of the head and distal extremities. In more severely affected dogs, lesions were more generalized, including myositis of esophageal muscle and arteritis of skin, muscle, bladder, and spermatic cord. Although viruses were not isolated from muscle, crystalline viral-like structures were present in cytoplasm of endothelial cells within skeletal muscle. The dogs with dermatitis and myositis consistently had lymphoid hyperplasia, especially of peripheral lymph nodes. More severely affected dogs were smaller than less severely affected littermates, and the more severely affected males had reduced weight of testicles and prostate glands, compared with body weight. The reduced weight of genital organs correlated positively with reduced fertility. A few lymphoid aggregates were present in or around thyroid glands of 6 of the 20 dogs. There was no histologic evidence of glomerular disease in any of the dogs. The neonatal collies had no evidence of dermatomyositis. (Am J Pathol 1986, 123:480–496)

DERMATOMYOSITIS, inflammation of the skin, muscle, and sometimes vasculature, has been reported in adults and children.[1–5] The cause or causes of dermatomyositis are not known, but it is considered to be one of the autoimmune diseases[6] for which the pathogenesis involves both cell-mediated[7] and humoral[8] immunity. Infectious agents, including viruses, have been implicated in the causation of dermatomyositis, but no infectious agent has been proven unequivocally as a cause.[9–13] We recently described a familial disease in collie dogs that resembles dermatomyositis in children.[14–16] The purpose of this study was to evaluate the extent of myositis and the internal organ involvement in dogs of different ages and with different degrees of severity of dermatomyositis and to compare further canine and human dermatomyositis.

Materials and Methods

Complete necropsies were performed on 20 dogs with familial canine dermatomyositis between 5 months and 1.25 years of age. These dogs were from four litters (designated I, II, III, and IV) produced at Washington State University and described below. Three newborn dogs from Litter III and 7 newborn dogs from Litter IV were also necropsied.

The colony was initiated with 2 male collies (A and C) and 1 female collie (B) affected with dermatomyositis and 1 female Labrador retriever (D) without dermatomyositis. The disease in collies A, B, and C has been described previously.[15,16]

Litter I consisted of 7 dogs obtained from a breeding of collies A and B. One dog from this litter died within 12 hours of birth and necropsy results are presented elsewhere.[14] Four collies from this litter (312, 314, 315, and 316) were euthanatized at 1.25 years of age by an intravenous injection of sodium pentobarbi-

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tal and underwent necropsy. Two collies from this litter remain in the colony (311 and 313). Body weight, length of femur from greater trochanter to lateral condyle, length of skull from between upper central incisors to occipital condyle, and the weights of the following organs were obtained: right ventricle, left ventricle, interventricular septum, right and left gonads, prostate, right and left kidneys, right and left adrenal and thyroid glands, pituitary gland, and brain. Samples from all body organs including intercostal, temporalis, masseter, extensor carpi radialis, biceps femoris, diaphragm, flexor digitorum superficialis, longissimus, gastrocnemius, triceps brachii, and longus colli muscles, were placed into 10% neutral buffered formalin (10% NBF). In 3 dogs the hyopharyngeus or thyropharyngeus muscles and the spinalis et semispinalis and iliocostalis muscles were also collected. Tissues were processed in an automatic tissue processor, sectioned at 6 μ, and stained with hematoxylin and eosin (H&E). In addition, kidney was sectioned at 2 μ and stained with the Jones method. Portions of skin, temporalis muscle, spleen, thymus, mesenteric lymph node, and small intestine were collected aseptically for viral detection. The methods of evaluation for virus included viral isolation in cell culture, negative contrast electron microscopy, and enzyme-linked immunosorbent assay (ELISA) for rotavirus (Abbott Laboratories, Chicago, Ill). The cell cultures used for virus isolation included Crandell feline kidney cells, Vero monkey kidney cells, and Madin–Darby canine kidney cells.

Litter II consisted of 5 dogs from a breeding of collie A and the female Labrador retriever D. One dog from this litter was stillborn, and necropsy results are presented elsewhere.[14] The 4 remaining dogs from Litter II (421, 422, 423, and 424) were euthanatized at 1.1 year of age by an intravenous injection of sodium pentobarbital and underwent necropsy. Body and organ weights and femur and skull lengths were obtained as listed for Litter I. Samples of all body organs, including the previously listed muscles, were collected for histopathologic and virologic study and processed as described above for Litter I.

The clinical, electrodiagnostic, genetic, immunologic, serologic, and skin and muscle biopsy results obtained from dogs in Litters I and II have been presented elsewhere.[14-16]

Litter III consisted of 12 dogs obtained from a second breeding of collies A and B. Three female pups died, 1 at 2, 1 at 3, and 1 at 5 days of age. Sections of lung, liver, and intestine were aseptically collected for bacteriologic study from the pups that died at 2 and 3 days of age. Fluid from a subcutaneous limb abscess was aseptically collected for bacteriologic study from the pup that died at 5 days of age. Prior to death, the pups were treated with fluids and antibiotics for bacterial infection. Portions of most body organs were collected in 10% NBF and were processed and stained as described above. Selected sections were stained with Brown and Brenn. The remaining 9 pups (31 through 39) were evaluated from birth to 7.5 months of age. In 2 female pups (31 and 33) and 2 male pups (35 and 38) subcutaneous abscesses developed within the first week of life. These pups and the 5 uninfected pups were treated with antibiotics. The cutaneous and muscular lesions in Dogs 31–39 are presented elsewhere.[17] Tissues and samples collected from Litter III dogs for virologic examination are listed in Table 1. The methods of evaluation for virus are as previously described. At 7.5 months of age each dog was euthanatized by an intravenous injection of sodium pentobarbital, and a postmortem examination was performed. Body and organ weights and measurements were obtained as for Litters I and II. In addition, skull width was measured. Samples of all body organs, except eyes, were collected in 10% NBF and were processed as for Litters I and II. The eyes were removed immediately after death, fixed in Zenker's solution for approximately 3 hours, washed in cold running water for approximately 12 hours, and transferred to 70% ethanol. The lateral and medial calottes were removed, and the central portion of each eye was processed and embedded in paraffin. Sections were made from three levels in each eye, with the deepest through the optic disc, and each section was stained with H&E. In addition, portions of flexor digitorum superficialis and gastrocnemius muscles from Dogs 34, 35, 36, and 37 were frozen in isopentane (2-methylbutane) chilled in liquid nitrogen and were stored at −70 C for subsequent histochemical evaluation. Also, small sections of the flexor digitorum superficialis and gastrocnemius muscles from Dogs 34, 35, 36, and 37 were minced and placed in 2.5% glutaraldehyde and 0.5% dimethyl sulfoxide in 0.1 M cacodylate buffer for electron microscopy. An additional 3 × 6 × 6-mm section of each of the two muscles was stretched in a muscle biopsy clamp and placed in the glutaraldehyde fixative. The stretched muscle was removed from the biopsy clamp between 15 and 30 minutes after immersion, was immediately returned to the fixative, and 4 hours after originally being placed in the glutaraldehyde fixative, was minced into 1-mm cubes. The specimens were rinsed in 0.1 M cacodylate buffer with 2 M sucrose, postfixed in 1% osmium tetroxide, dehydrated in graded ethanols, and embedded in Epon–Araldite. Sections of 1 μ were stained with hot toluidine blue, and areas were selected for thin sectioning. Thin sections were stained with uranyl acetate and lead citrate and were examined in an electron microscope.

Litter IV consisted of 11 dogs obtained from a breed-

Table 1—Results of Virus Isolation and Electron Microscopy for Virus

Dog	Ages (weeks) 2.5	5.0	9.5	10.5	11.5	11.5	12.5	13.5	27
31	Feces isolation (+) Feces EM (−)	Tonsil and nares isolation (−)	Buffy coat isolation (−)	ND	ND	ND		Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
32	Feces isolation and EM (−)	Tonsil and nares isolation (−)	ND	ND	Buffy coat isolation (−)	Skin isolation (++) Muscle isolation (−)	ND	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
33	Feces isolation (+) Feces EM (−) Buffy coat isolation (−)	Tonsil and nares isolation (−)	ND	ND	Buffy coat isolation (−)	Skin and muscle isolation (−)	ND	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
34	Feces isolation (+)	Tonsil and nares isolation (−)	ND	Buffy coat skin, and muscle isolation (−)	ND	ND	ND	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
35	Feces isolation (+) Feces EM (−) Buffy coat isolation (−)	Tonsil and nares isolation (−)	ND	ND	Buffy coat isolation (−)	ND	Skin and muscle isolation (−)	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
36	Feces isolation and EM (−)	Tonsil and nares isolation (−)	ND	ND	Buffy coat isolation (−)	ND	Skin and muscle isolation (−)	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
37	Feces isolation (+) Feces EM (−)	Tonsil and nares isolation (−)	Buffy coat isolation (−)	ND	ND	ND	ND	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
38	Feces isolation (+) Feces EM (−) Buffy coat isolation (−)	Tonsil and nares isolation (−)	ND	Buffy coat, skin, and muscle isolation (−)	ND	ND	ND	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)
39	Feces isolation (+) Feces EM (−)	Tonsil and nares isolation (−)	ND	Buffy coat isolation (−)	ND	ND	ND	Feces, nares, and tonsil isolation (−)	Feces, nares, and tonsil isolation (−)

+, positive for enterovirus; −, negative for virus; ++, positive for calicivirus; EM, transmission electron microscopy performed; ND, isolation or identification techniques not done.

ing of male collie C and an affected female collie (311) from Litter I. Seven of the 11 collies were killed with an intraventricular cardiac injection of sodium pentobarbital and underwent necropsy at 1 day of age. Samples of feces were collected for viral detection as previously described. Samples of all body tissues were collected in 10% NBF and were processed as for Litters I, II, and III. Four of the remaining dogs were given physical examinations, and blood samples were collected every 2 weeks. Three of the 4 collies remaining in Litter IV (541, 543, and 544) were euthanatized with an intravenous injection of sodium pentobarbital and necropsied at 5 months of age. The fourth dog was clinically normal at 2.5 months of age and was removed from the colony, but this dog subsequently developed dermatomyositis at 4 months of age. Samples of thymus and mesenteric lymph node and temporalis, extensor carpi radialis, flexor digitorum superficialis, and tibialis cranialis muscles were collected for viral detection as previously described. Samples of temporalis, extensor carpi radialis, flexor digitorum superficialis, tibialis cranialis, and gastrocnemius muscles were frozen in isopentane (2-methylbutane) chilled in liquid nitrogen. The muscles from the Litter III dogs (34, 35, 36, and 37) and Litter IV dogs (541, 543, and 544) that were stored in liquid nitrogen were sectioned with a cryostat

and stained for adenosine triphosphatase (ATPase) with incubation at pH 9.4.[18] The lesser diameters of 100 Type I and 100 Type II myofibers were measured with an image analysis system (Bioquant, Nashville, Tenn). Mean myofiber diameter and SD were calculated. The variability coefficient was determined by dividing the SD by the mean myofiber diameter and multiplying by 1000. The percentage of Type I and Type II myofibers was determined by counting the number of Type I and Type II fibers within 16 2-mm grids of an ocular micrometer. A two-tailed *t* test was used for evaluating differences between means of myofiber diameters in control dogs and dogs in Litter III. A one-tailed *t* test was used for evaluating differences between means of myofiber diameters in control dogs and dogs in Litter IV. A one-tailed paired *t* test was used to evaluate differences between mean myofiber diameters and percentage of Type I and Type II myofibers in areas of muscle with and without myositis in dogs of Litter IV. A two-tailed *t* test was also used for evaluating differences in means of percentage of Type I myofibers in control dogs and dogs in Litters III and IV. The arcsin transformation was used on the percentage of Type I and Type II myofibers before performance of he *t* test.[19] Three adult female (Siberian husky, Samoyed-crossbred, springer spaniel) and two adult male (golden retriever and German shepherd-crossbred) dogs were used as controls for muscle histopathology and ATPase staining. Four of the control dogs weighed more than 15 kg. The springer spaniel female weighed 14 kg. Samples of the temporalis, extensor carpi radialis, flexor digitorum superficialis, tibialis cranialis, and gastrocnemius muscles were collected from controls. Portions were placed in 10% NFB for histopathologic study. Frozen muscles were collected, processed, and stained as previously described. Two portions of temporalis and extensor carpi radialis from Dogs 541, 543, and 544 of Litter IV were collected for electron microscopy as previously described.

Body and organ weights and measurements were obtained for Litter IV as listed for Litters I and II. All body organs, including the above mentioned muscles, except spinalis et semispinalis and iliocostalis, were collected and processed as for Litters I, II, and III. In addition, the following muscles were collected from one or more of the 3 dogs of Litter IV: deltoideus, fibularis longus, flexor digitorum superficialis, flexor carpi ulnaris, flexor digitorum ulnaris, extensor carpi ulnaris, extensor digitorum communis, extensor digitorum lateralis, semitendinosus, extensor digitorum longus, tibialis cranialis, vastus lateralis, gracilis, pectineus, sternomastoideus occipitalis, ulnar head of flexor digitorum profundus, flexor hallucis longus, and supraspinatus.

Alloantisera, produced by littermate cross-immunization,[20] were used to histocompatibility type the parents and the dogs of the 4 litters for the A and B alleles.[21] The typing procedure consisted of the standard two-stage microcytotoxicity test. Homozygous typing cells (HTCs) from dogs homozygous at the DLA-D locus[22] were used in the mixed lymphocyte culture (MLC) assay to type for D alleles as described.[23] Briefly, one-way MLCs were carried out with the HTCs as irradiated stimulators for the responder lymphocytes of experimental dogs. Lymphocytes were collected by Ficoll-Hypaque density gradient centrifugation and cultured in triplicate for 7 days at 37 C and 7% CO_2/air atmosphere. Radioactivity was determined after labeling with ^{3}H-thymidine. The relative response was used to discriminate positive and negative MLC responses.[23]

Results

Gross Lesions

Cutaneous lesions in the dogs of Litters I, II, and III have been presented elsewhere.[14-16] The distribution of skin lesions in the dogs of Litter IV was similar to that

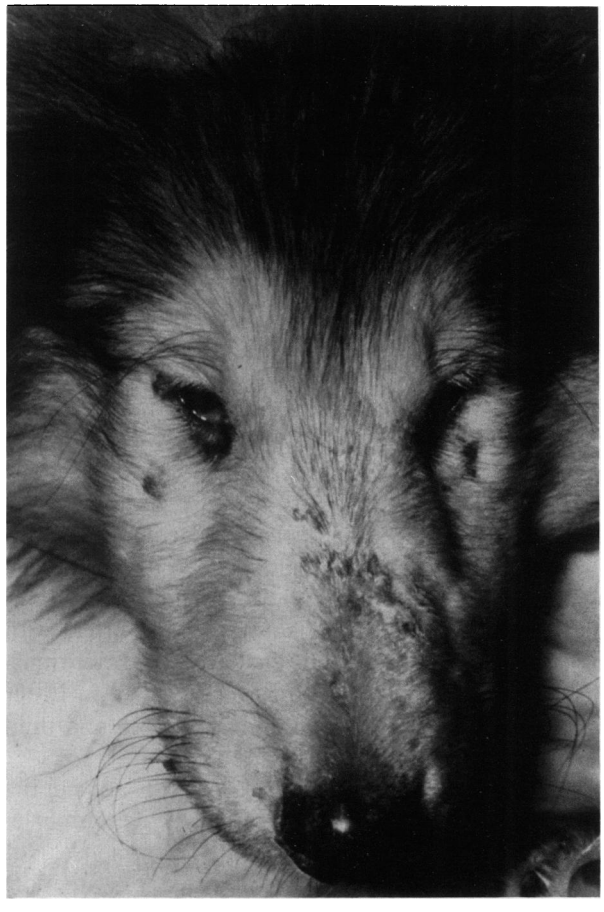


Figure 1—Ten-week-old male collie (316) with dermatomyositis. Note crusting on the bridge of nose and in periocular areas.

Table 2—Distribution of Grossly Visible Muscle Lesions in Dogs of Litters I, II, and IV

	Litter I				Litter II				Litter IV		
	312	314	315	316	421	422	423	424	541	543	544
Temporalis	+	+		+					+	+	+
Masseter	+	+								+	+
Sterno occipitalis mastoideus									+	+	+
Longissimus									+	+	+
Iliocostalis										+	+
Spinalis et semispinalis										+	+
Deltoideus											+
Triceps brachii											+
Extensor carpi radialis	+			+						+	+
Extensor digitorum lateralis	+			+						+	+
Extensor digitorum communis	+			+						+	+
Extensor carpi ulnaris	+			+						+	+
Flexor carpi ulnaris									+		+
Semitendinosis									+	+	+
Semimembranosis											+
Biceps femoris											+
Gracilis										+	+
Pectineus										+	
Vastus lateralis										+	+
Gastrocnemius	+									+	+
Flexor digitorum superficialis	+								+	+	+
Tibialis cranialis										+	+
Fibularis longus										+	
Flexor hallucis longus				+						+	+
Extensor digitorum lateralis										+	
Age at necropsy (months)	15	14	15	15	13	13	12	13	5	5	5

+, grossly visible lesions present.

in the dogs of Litters I and III. Briefly, lesions were present on face, ears, distal extremities over bony prominences, and the tip of the tail. The 3 dogs were severely affected, and, therefore, as in other more severely affected dogs, the cutaneous lesions were more generalized. Lesions consisted of alopecia, erythema, hypopigmentation, hyperpigmentation, scaling, ulceration, and crusting (Figure 1). The dogs in all 4 litters with more severe skin lesions tended to have more severe muscle lesions. The one exception was Dog 314 in Litter I, which had a severe secondary pyoderma and mild muscle changes.

Table 2 lists the grossly visible muscle lesions in the dogs of Litters I, II, and IV. Grossly visible muscle lesions in Litter III have been presented elsewhere.[17] Not every muscle in every dog was dissected, but an effort was made to evaluate a consistent group of muscles from all dogs. Lesions in many additional muscles were noted in more severely affected dogs. The three moderately to severely affected dogs in Litter I (312, 314, and 316) had muscle lesions. Lesions were present most often in muscles of mastication and extensor muscles below the elbow (Table 2). Figure 2 depicts the atrophy of muscles of mastication in Dog 312. Litter II dogs, which were least affected by dermatomyositis, had no grossly visible muscle lesions. Muscle changes were more severe in Litter IV dogs. Litter IV dogs were younger at necropsy (5 months of age) than the other dogs. Gross muscle lesions consisted of pale tan and slightly soft muscle in contrast to the reddish-brown of normal muscles. As previously reported for Litter III dogs,[17] muscle lesions were more severe in the anterior and superficial portions of the temporalis and masseter muscles (Figure 3). The superficial portions of other muscles, especially those in the limbs below the elbow and stifle, were also more severely affected (Figure 4). Un-

Figure 2—Skull of Dog 312 severely affected with dermatomyositis. No musculature has been dissected from the skull. Note marked atrophy of muscles of mastication.

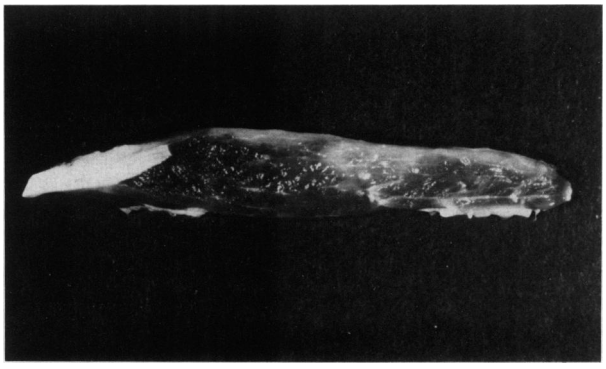


Figure 4—Extensor digitorum longus muscle from a 5-month-old collie (543). Note the superficial tan areas of myositis.

commonly a deep portion of a muscle was involved. Muscle lesion distribution, therefore, tended to coincide with cutaneous involvement.

The most consistent gross lesion other than dermatitis and myositis in dogs of all four litters was lymphoid hyperplasia of the peripheral lymph nodes, the severity of which corresponded to the severity of skin lesions. Two more severely affected dogs, 37 of Litter III and 544 of Litter IV, also had splenic lymphoid nodular hyperplasia. Dog 314 of Litter I had an extremely thin and small thymus. Another organ with gross lesions in two litters of dogs was the esophagus. Dog 312 of Litter I had moderate esophageal dilation, Dog 541 of Litter IV had a slightly dilated esophagus, and Dogs 542 and 543 of Litter IV had pale tan esophageal muscle. Only 1 dog, 314 of Litter I, had grossly visible cardiac lesions. This dog had firm tan muscle in the right atrium. Miscellaneous gross lesions included cysts in the thyroid glands of Dog 314 of Litter I and Dogs 32 and 37 of Litter III and in the pituitary gland of Dog 422 of Litter IV.

Figure 3—Temporalis muscle from a 5-month-old collie (543). The anterior portion is at the top and the posterior portion is at the bottom. Note the pale tan areas in more anterior and superficial areas.

Organ Weights and Measurements

More severely affected dogs in Litter I (312 and 314) weighed 45% and 64% as much as their least affected Littermate, Dog 315, and the moderately affected Dog 316 weighed 83% as much as Dog 315. Testicular weight as percentage of body weight was also substantially less in the severely affected Dog 314 (0.034%) and moderately less in the moderately affected Dog 316 (0.056%), compared with the mildly affected Dog 315 (0.074%). Prostatic weight to body weight was also only 0.035% in Dog 314 and 0.044% in Dog 316, compared with 0.056% in Dog 315. These results correlated with reduced fertility observed with more severely affected dogs.[15] Dog 314 had a thin-walled fluid-filled cyst (1 × 1 cm) in the left thyroid gland, which caused a discrepancy in weight between left and right thyroid glands. In Litter II, Dog 423 was 89% of the weight of its littermate, 424, although both dogs were minimally affected with dermatomyositis. Testicular weight to body weight was 0.084% in Dog 423 and 0.068% in Dog 424. Prostatic weight was 0.019% in Dog 423 and 0.046% in dog 424. The reason for the small prostate gland in

Dog 423 was not determined. The most severely affected male in Litter III (37) attained a body weight of 61% of that of his heaviest littermate (38). In addition, Dog 37 had smaller ratios of testicle to body weight (0.026%) and prostate to body weight (0.009%), compared with the ratios of testicle to body weight (0.039%) and prostate to body weight (0.026%) of Dog 38. Dog 35 had one testicle 41% of the weight of the opposite testicle. All dogs in Litter IV were severely affected, but because they were necropsied at 5 months of age, body and gonadal weight comparisons with less severely affected dogs and with older dogs of other litters were not done. Discrepancies of organ to body weight ratios occurred in the female dogs, but could not be unequivocally correlated with disease severity. Ovaries of Litter II Dogs 421 and 422 were heavier than ovaries of other female dogs probably because corpora lutea were present only in 421 and 422. Dog 312 of Litter I had no corpora lutea, and the severity of dermatomyositis may have prevented the onset of an estrous cycle. Female dogs in Litters III and IV had not had their first estrous cycle probably because of their young age. Other incidental alterations of organ weights were in Dogs 31 and 32 of Litter III. Each dog had one thyroid gland that was about 70% smaller than the opposite gland. Dog 33 had one kidney that was about 30% smaller than the opposite kidney.

Histopathologic Lesions

Histologic evaluation of skin of Litter I dogs revealed variably severe, active chronic dermatitis. Two of the most severely affected dogs, 312 and 314, had *Demodex canis* within hair follicles. Moderately affected Dog 316 had arterial walls replaced by acidophilic, homogeneous to granular material, pyknotic cells, and small mononuclear cells (arteritis). In Litter II dogs, skin lesions consisted of nonspecific dermatitis in Dog 421, and lesions were not seen in Dogs 422, 423, and 424. Dermatohistopathology results of Litter III dogs are presented elsewhere.[17] Litter IV dogs had severe dermatitis and folliculitis with *Demodex canis*. Dogs 541 and 543 also had arteritis similar to that in dogs of Litters I and III.[17]

Muscles that were histologically evaluated in all dogs in all four litters are listed in Table 3. Numerous other muscles were evaluated histologically, but not in each dog. The severity of the muscle disease is tabulated by dog and by muscle (Table 3). The rank of muscle by degree of severity (of those evaluated in all dogs) was temporalis, masseter, flexor digitorum superficialis, gastrocnemius, longissimus, triceps brachii, biceps femoris, intercostal, and esophageal. Lesions were not observed in the diaphragm in any dog. The extensor carpi radialis was not collected at necropsy in Dogs 31–39 because the right and left muscles had undergone biopsy previously. However, this muscle was frequently involved in dermatomyositis. The pale tan, slightly soft areas seen grossly were more severely inflamed than reddish-brown muscles. Inflammation was sometimes present in muscles that were grossly normal. Even though gross muscle lesions were not seen in Litter II dogs, each dog had minimal to mild histologic lesions in muscles of mastication. Histologic detail of muscle lesions has been reported elsewhere.[14] Briefly, multifocal collections of lymphocytes, plasma cells, macrophages, and fewer neutrophils or eosinophils were present within the endomysium and perimysium. Myofibers in these areas were often absent, fragmented, or vacuolated, or contained internal nuclei. Occasional myofibers had bluish-purple cytoplasm and vesicular nuclei with a large prominent nucleolus (regenerative). Myofibers varied slightly

Table 3—Severity of Histologic Lesions in Muscles in Dogs of Litters I, II, III, and IV

	Litter I				Litter II				Litter III									Litter IV			Severity/ muscle
	312	314	315	316	421	422	423	424	31	32	33	34	35	36	37	38	39	541	543	544	
Temporalis	4	2	1	3	1	2	2	1	3	2	2	3	1	2	4	2	1	4	3	4	47
Masseter	4	3	1	2	1	0	0	0	0	2	0	1	1	1	4	0	0	3	3	4	30
Longissimus	0	0	0	1	0	0	0	0	0	0	0	0	0	0	2	0	0	2	2	4	11
Triceps brachii	1	0	0	0	0	0	0	0	1	0	0	1	0	1	1	1	0	1	1	3	11
Extensor carpi radialis	0	0	0	0	0	0	0	0	ND	ND	ND	ND	ND	ND	ND	ND	ND	3	3	3	9
Flexor digitorum superficialis	3	0	1	0	0	0	0	0	0	2	1	1	1	0	4	1	1	0	0	3	18
Gastrocnemius	3	0	0	1	1	0	0	0	0	0	0	0	0	1	2	0	0	0	2	2	12
Intercostal	0	0	0	0	0	0	0	0	0	0	0	0	1	0	3	0	0	0	0	2	6
Diaphragm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Biceps femoris	0	0	0	0	0	0	0	0	1	0	1	1	0	0	0	0	0	1	1	3	8
Esophageal muscle	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	3	0	4
Severity/dog	15	5	3	7	3	2	2	1	5	7	4	7	4	5	20	4	2	14	18	28	

ND, not done due to previous biopsy; 0, no lesions; 1, minimal lesions; 2, mild lesions; 3, moderate lesions; 4, severe lesions.

to moderately in size, and the fibers were small in areas of severe inflammation. Generally, perifascicular atrophy was not a prominent feature. Increased perimysial and endomysial collagen was present in more chronic cases. In 1 dog, Dog 541 of Litter IV, mineralization of myofibers was seen. Vascular lesions were present in muscles of Dogs 312 and 316 (LitterI) and 541 (Litter IV). Vascular lesions in Dog 312 were principally in temporalis and masseter muscles, in which very little muscle remained. The arterial lumens were usually small, and the tunica intima was thickened by eosinophilic fibrillar material admixed with ovoid and spindle-shaped cells and few pyknotic and karyorrhectic cells (Figure 5). Dog 316 had an artery in the temporalis muscle with a focally thickened intima and a few lymphocytes in the tunica media. In Dog 541, an artery in the masseter muscle had a small lumen, and the wall was thickened by eosinophilic homogeneous material. A few pyknotic cells were also present. Sections of nerves within muscles severely involved with inflammation occasionally were incorporated by perimysial inflammatory cells. A small nerve in the temporalis muscle of Dog 316 of Litter I had fewer axons than expected, and lightly amphophilic fibrils replaced some of the axons.

Muscle histochemistry data from dogs in Litters III and IV and from control dogs are presented in Table 4. Areas of myositis were not seen in ATPase-stained sections from control or Litter III dogs. Myositis was present in extensor carpi radialis of Dogs 543 and 544 and the tibialis cranialis of Dog 543 of Litter IV. Areas of inflamed and noninflamed muscle were evaluated separately. Use of the two-tailed t test revealed there was no significant difference at $P > 0.05$ in mean diameters of Type I myofibers and of Type II myofibers in the gastrocnemius and flexor digitorum superficialis muscles between control and Litter III dogs. Use of the one-tailed t test revealed that both Type I and Type II myofiber diameters were significantly smaller ($P < 0.05$)

Table 4—Results of Muscle Histochemistry in Litters III and IV

	Muscle and myofiber type									
	Temporalis		Extensor carpi radialis		Flexor digitorum superficialis		Tibialis cranialis		Gastrocnemius	
	I	II	I	II	I	II	I	II	I	II
Litter III (n = 4)										
Diameter										
Mean	ND	ND	ND	ND	43.17	39.33	ND	ND	55.47	52.93
SD	ND	ND	ND	ND	10.20	6.74	ND	ND	10.90	10.55
Percent										
Mean	ND	ND	ND	ND	52.94	ND	ND	ND	42.94	ND
SD	ND	ND	ND	ND	5.73	ND	ND	ND	10.19	ND
Litter IV (n = 3)										
No myositis										
Diameter										
Mean	21.25	24.92	37.34	33.11	31.80	30.19	34.50	32.04	30.06	33.66
SD	6.18	7.21	5.55	2.08	3.51	1.57	11.50	6.56	1.97	2.13
Percent										
Mean	16.25	ND	23.85	ND	52.91	ND	30.74	ND	37.60	ND
SD	5.82	ND	6.99	ND	7.11	ND	4.60	ND	10.92	ND
Myositis										
Diameter										
Mean	NP	NP	31.46*	22.54	NP	NP	34.87†	31.58	NP	NP
SD			9.16	8.99			NA	NA		
Percent										
Mean	NP	NP	28.84	ND	NP	NP	NA	NA	NP	NP
SD			5.29	ND						
Controls (n = 5)										
Diameter										
Mean	40.19	40.99	45.02	44.03	46.59	42.92	46.39	48.92	46.33	45.85
SD	8.46	7.55	6.04	5.73	11.06	10.22	5.15	8.03	3.26	3.96
Percent										
Mean	22.34	ND	26.26	ND	65.47	ND	31.66	ND	38.85	ND
SD	2.30	ND	6.46	ND	5.77	ND	6.31	ND	11.77	ND

* N = 2 dogs.
† N = 1 dog.
Arcsin transformation was used on percentage data.
ND, not done; NA, not applicable; NP, myositis not present.

in the temporalis, extensor carpi radialis, flexor digitorum superficialis, tibialis cranialis, and gastrocnemius muscles between control and Litter IV dogs, with the exception of Type I myofibers in extensor carpi radialis and tibialis cranialis muscles, where there was no significant difference. Use of the one-tailed paired t test revealed both Type I and Type II myofibers were significantly smaller ($P < 0.005$) in areas with myositis than in areas without myositis in the extensor carpi radialis of Dogs 543 and 544 and the tibialis cranialis of Dog 543 of Litter IV. No differences in percentage of Type I or Type II myofibers were detected at $P > 0.05$ in these muscles. Use of the two-tailed t test revealed no significant difference at $P > 0.05$ in the percentage of Type I myofibers in the gastrocnemius muscle between controls and Litter III dogs, but there was a significant difference at $P < 0.05$ in the percentage of Type I myofibers in the flexor digitorum superficialis muscle. In Litter IV dogs, there was no difference detected in the percentage of Type I fibers at $P > 0.05$ between controls in any of the 5 muscles without inflammation or the extensor carpi radialis muscles with inflammation. In addition, generally there was greater variability in the size of myofibers in areas of myositis than in areas without myositis. Perifascicular atrophy was seen in the extensor carpi radialis in Dog 544 (Figure 6).

In Dog 312 of Litter I, the dilated esophagus had clusters of neutrophils in the surface epithelium, and lymphocytes and plasma cells were scattered in the lamina propria and around glands. Perivascular neutrophils, eosinophils, lymphocytes, and macrophages were also present. Unequivocal changes were not seen in the esophageal musculature. Dog 32 in Litter III had one myofiber surrounded by few lymphocytes and macrophages. Both layers of esophageal musculature in Dog 543 (Litter IV) had fragmented and vacuolated myofibers, internal nuclei, and interstitial collections of lymphocytes, plasma cells, and macrophages (Figure 7). Some myofibers were basophilic and had vesicular nuclei with a prominent nucleolus (regeneration). Dog 544 in Litter IV had several small acidophilic esophageal myofibers surrounded by lymphocytes.

Four dogs had mild inflammatory lesions in the heart (314 in Litter I, 424 in Litter II, and 33 and 36 in Litter III). In Dog 314, lesions seen grossly consisted of ac-

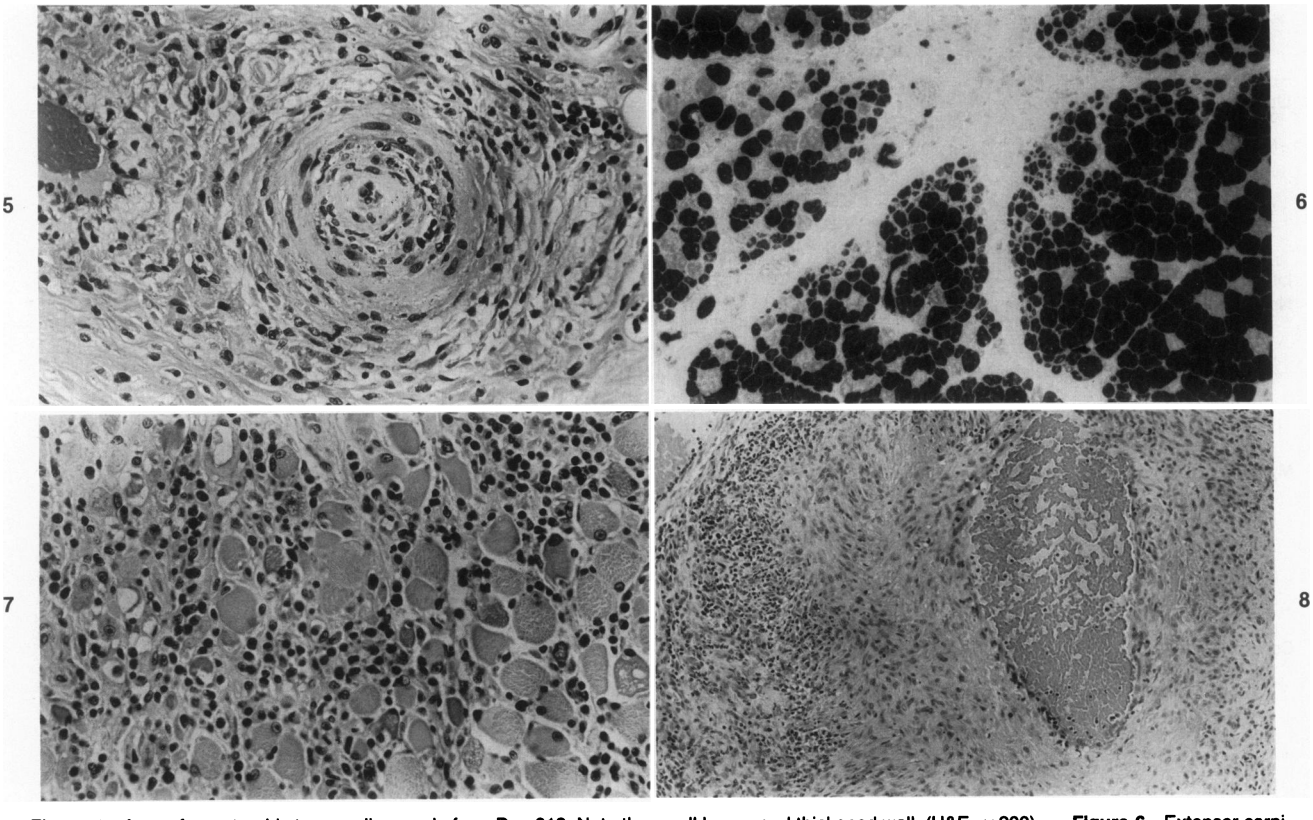


Figure 5—Artery from atrophic temporalis muscle from Dog 312. Note the small lumen and thickened wall. (H&E, ×200) **Figure 6**—Extensor carpi radialis from Dog 544. Note the smaller myofibers at the periphery of the fascicles (perifascicular atrophy). (ATPase, ×75) **Figure 7**—Section of esophagus with myositis from Dog 543. Note the fragmented myofibers and the lymphocytes, plasma cells, and macrophages. (H&E, ×200) **Figure 8**—Section of spermatic cord with arteritis from Dog 543. The wall is thickened by spindle-shaped cells. Neutrophils are prominent, especially in the adventitia. (H&E, ×75)

tive chronic endocarditis and myocarditis of the right atrium, which were judged a result of septicemia secondary to severe pyoderma. Dog 424 had mild neutrophilic and histiocytic epicarditis of the right atrium, and one myofiber in the left atrium was surrounded by several lymphocytes. A small cluster of lymphocytes was present in the right atrial myocardium of Dog 33, and one small focus of lymphocytes was present in the interventricular septum of Dog 36.

Lymphoid hyperplasia in the peripheral lymph nodes was confirmed histologically in all dogs, especially those dogs with more severe dermatitis. Lymphoid hyperplasia of spleen was also confirmed in Dog 544 (Litter IV) and Dog 37 (Litter III). Mitotic figures were present in germinal centers of tonsil and spleen and in cortex and medulla of thymus in all dogs except Dog 314 in Litter I, severely affected with pyoderma, in which severe thymic atrophy was present. Several lymphoid follicles were present in thymus from Dog 34 in Litter III.

Histologic lesions in genital organs varied. In severely affected Dog 312 of Litter I, there were ovarian follicles, but no corpora lutea. In severely affected Dog 314 of Litter I, no spermatozoa and only a few germinal cells were present in the testicles, and there were no spermatozoa in epididymides (testicular hypoplasia). Moderate numbers of plasma cells were in the peritubular areas of the epididymides. Dog 316 (moderately affected, in Litter I) had large perivascular clusters of lymphocytes in an epididymis and many spermatozoa in epididymal tubules. Dog 315 (the least affected necropsied dog in Litter I) had many spermatozoa in testicles and epididymides. Significant lesions were not seen in genital organs of Litter II dogs. In Litter III dogs, lesions were not seen in the genital organs of the females except for one large medullary ovarian cyst lined by flattened cuboidal cells. Dog 35 had unilateral testicular hypoplasia, and Dog 37 had bilateral testicular hypoplasia. In Litter IV dogs, 541 had follicles in ovaries and a minimal to mild neutrophilic endometritis and plasmacytic metritis. In Dogs 543 and 544, differentiation to spermatozoa was not seen in the testicles. The vascular portion of the spermatic cord of Dog 543 had an artery with a greatly thickened wall due to eosinophilic homogeneous material, spindle-shaped cells, necrotic cells, and neutrophils (Figure 8). A few periglandular lymphocytes and plasma cells were present in the epididymis of Dog 544.

Small cysts lined by ciliated cuboidal to columnar stratified to pseudostratified epithelial cells were found in or adjacent to the parathyroid glands of Dogs 314, 315, 316 (Litter I); 31, 34, 39 (Litter III); 541 and 543 (Litter IV). Dog 423 (Litter II) had a parathyroid cyst lined by stratified squamous epithelium. Cysts, lined with ciliated epithelium, similar to those in the parathyroid glands, were present in the thyroid glands of Dogs 312, 314 (Litter I); 421 (Litter II); 31, 37, 38, 39 (Litter III); and 541 (Litter IV). Lymphoid aggregates were seen in or adjacent to the thyroid glands in Dogs 314, 315 (Litter I), 32, 37, 38, and 39 (Litter III). Cysts were also found in the pituitary glands of 316 (Litter I); 422, 423 (Litter II); 31, and 32 (Litter III).

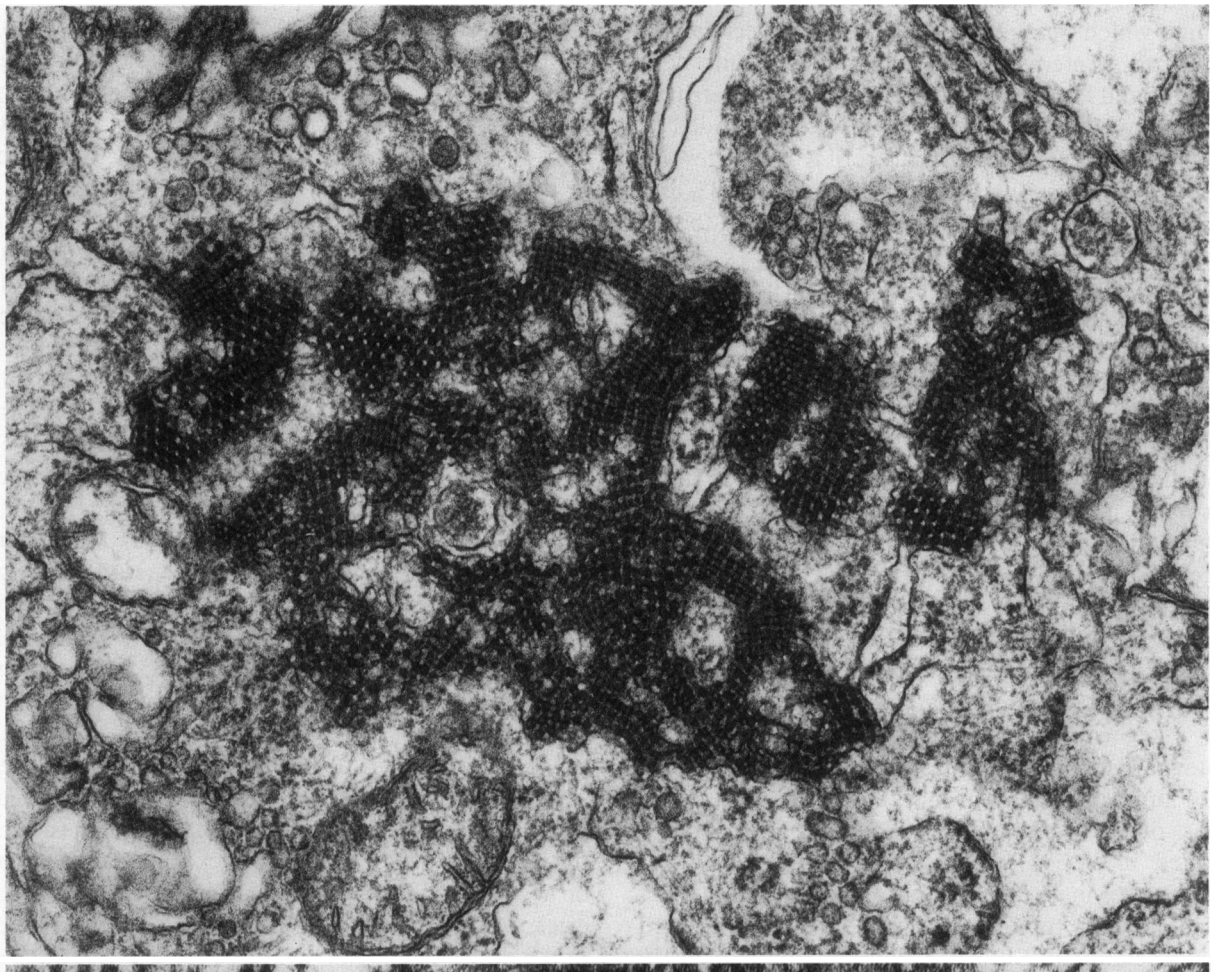
Inflammation was not present in any of the intraocular structures of the eyes of any of the dogs. Dogs in Litters III and IV were evaluated for collie eye anomaly (CEA). Dogs 31, 541, and 543 had a moderate degree of CEA (optic disc colobomas, choroidal hypoplasia, and a few retinal folds in each eye). Dogs 32, 37, and 38 had slight degrees of CEA (areas of choroidal hypoplasia and/or a few small retinal folds in each eye). Dogs 33, 34, 35, 36, 39, and 544 had no evidence of CEA. All dogs except 312, 421, 422, 423, 424, 31, 38, 541, 543, and 544 had small retinal cysts at the pars ciliaris retinae (peripheral cystoid degeneration). Dogs 32, 314, 315, 316, 541, 543, and 544 had mild conjunctivitis. Dogs 312, 314, 315, 316, 423, 36, 37, 38, 39, 541, 543, and 544 had subepithelial lymphoid follicle hyperplasia of anterior or posterior surfaces of their nictitating membranes.

Histologic lesions in other organ systems included several clusters of clear vacuoles in a cerebellar nucleus of Dogs 312, 316, 541, 543, and 544. Arteries in the urinary bladder of Dog 37 were thickened, primarily by an accumulation of neutrophils, plasma cells, lymphocytes, and fibroblasts in the adventitia. Several myocytes in the tunica media had pyknotic nuclei. Two dogs in Litter I with the most severe skin lesions, 312 and 314, had either plasmacytic perivasculitis or synovitis associated with carpal and stifle joints. Corresponding to previously reported radiographically evident reduced bone density,[15] osteoporosis was identified in 3 dogs of Litter I (312, 314, and 316).

Examination of H&E-and Jones-stained kidney sections revealed slight focal thickening in glomerular capillary walls and tubular basement membranes, but differences were not detected among dogs in Litters I, II, III, and IV.

Electron Microscopy

Evaluation of electron micrographs from the flexor digitorum superficialis and gastrocnemius muscles from Dogs 34, 35, 36, and 37 revealed that one cell in the perimysium of the flexor digitorum superficialis of Dog 37 contained irregularly shaped, crystalline structures ($0.6 \times 2.0\ \mu$) consisting of numerous, slender, electron-dense parallel arrays that tended to intersect at right angles. There was no evidence that the structures were within endoplasmic reticulum. Evaluation of electron



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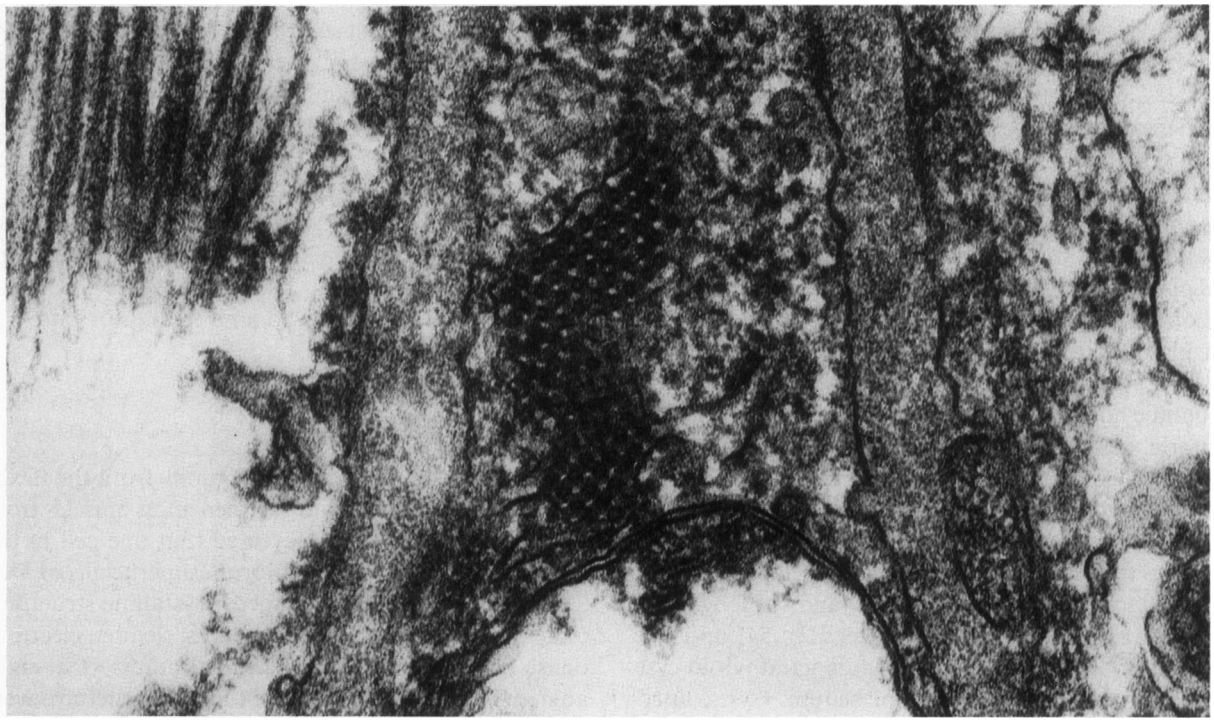

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micrographs of temporalis and extensor carpi radialis muscles of Dogs 541, 543, and 544 of Litter IV revealed that cytoplasm of endothelial cells within both muscles in each of the 3 dogs contained crystalline structures that were irregularly shaped and varied from 0.2 × 0.4 to 1.6 × 2.0 μ. The structures were within endoplasmic reticulum and consisted of clusters of parallel arrays of individual circular bodies (Figures 9 and 10). The individual circular bodies measured about 25–30 nm.

Virology

Results of the virologic studies of Litter III dogs are presented in Table 1. Viruses consistent with enterovirus (picornavirus) were isolated from feces from 7 of the 9 dogs at 2.5 weeks of age. A virus with the properties compatible with calicivirus was isolated from a skin biopsy specimen from Dog 32 at 11.5 weeks of age.

Viruses were not isolated from tissues collected at necropsy from dogs in Litters I, II, III, or IV. However, rotavirus was detected by ELISA in Dogs 314 and 315 of Litter I and 424 of Litter II. Negative-contrast electron microscopy revealed a few viral particles compatible with parvovirus, coronavirus, or rotavirus in intestinal scrapings from Dogs 312, 314, 315, and 316 of Litter I and Dogs 421 and 422 of Litter II.

Neonatal Pups

The 3 pups in Litter III that died by 5 days of age all died of septicemia. The pup that died at 2 days of age was small (181 g) and had subcutaneous edema and hemorrhage from fluid therapy. She had neutrophilic bacterial pneumonia and necrotizing bacterial myositis. *Streptococcus canis* and *Streptococcus pyogenes* were the pathogens isolated from liver and lung, respectively. The pup that died at 3 days of age weighed 289 g and had aspiration pneumonia and multifocal myositis of head and hind leg musculature. Hemorrhagic colitis was also present. *Klebsiella* sp was the pathogen isolated from both liver and lung. The pup that died at 5 days of age weighed 220 g. Abscesses were present in right rear and left front legs. Myocarditis, pneumonia, hepatitis, and myositis of head, tounge, lip, diaphragm, and shoulder were present. *Streptococcus* sp was the pathogen isolated from the material aspirated from an abscess.

Necropsy of the 7 pups of Litter IV euthanatized at birth and prior to suckling revealed that 2 pups had a thin white rim (compatible with marginal hepatic necrosis[24]) along the edge of a liver lobe. Virus was not isolated from the pups; however, the colonic scrapings contained particles compatible with coronavirus on negative-contrast electron microscopy. Histopathologic evaluation revealed that 3 pups had areas on the margins of a liver lobe in which hepatocytes were pyknotic, karyorrhectic, or were replaced by fibroblasts, collagen, and basophilic, granular material compatible with mineral (marginal hepatic necrosis).[24] Extramedullary hematopoiesis was present in liver and spleen of all pups and occasionally in other organs. One pup had ectopic thyroid in the thymus.

Results of the DLA typing studies are presented in Table 5.

Discussion

Familial canine dermatomyositis is a disease of juvenile collies. Gross or histologic lesions of dermatomyositis are not present in newborn dogs. In our studies, the cutaneous lesions usually developed between 7 and 11 weeks of age,[14,15] followed by myositis between 12 and 20–23 weeks of age.[15,17] In another study of canine dermatomyositis, dermatitis more frequently developed between 3 and 6 months of age, and myositis was detected after dermatitis was diagnosed.[25] Gross muscle lesions in the 4 litters of dogs in this study were most severe in the dogs that underwent necropsy at 5 months of age. In dogs 7.5 months of age, active gross muscle lesions were noted, but they were less extensive than in the younger dogs. By 1.25 years of age, severely affected dogs had extensive atrophy and some fibrosis of more severely involved muscles (temporalis and masseter), but gross evidence of active myositis was less commonly observed. In dogs with active muscle lesions, the muscles were pale tan and soft. In the temporalis and masseter muscles, there was reduced muscle mass, especially anteriorly and superficially; however, myositis also was histologically evident in grossly normal muscles. The dogs with more severe dermatitis generally had more severe myositis, and the severely affected dogs had vasculitis. The diameters of Type I and of Type II myofibers did not vary between control dogs and Litter III dogs, but with the exception of Type I myofibers in 2 muscles, both Type I and Type II myofibers were smaller in Litter IV dogs. The difference in size of fibers between control and Litter IV dogs may have been due to age and body weight differences. Young dogs have smaller myofibers than adult dogs,[26] and dogs weigh-

Figure 9—Electron micrograph of temporalis muscle from Dog 544. Note the viral-like crystalline structure within endoplasmic reticulum. (Uranyl acetate and lead citrate, ×49,000) **Figure 10**—Electron micrograph of extensor carpi radialis from Dog 543. Higher magnification of viral-like crystalline structure. (Uranyl acetate and lead citrate, ×75,000)

Table 5—Results of DLA Histocompatibility Typing in Parents and Dogs in Litters I, II, III, and IV

Dog	DLA-A-B Haplotype	DLA-D Phenotype	Disease Severity
Parents			
A	2/5 3/4	15/bl	Mild to moderate
B	2/5 3/bl	2/bl	Mild to moderate
C	2/5 3/bl	Not Done	Moderate
D*	9/6 bl/bl	1/bl	Normal
Litter I			
311	3/4 3/bl	bl/bl	Minimal
312	3/4 2/5	2/15	Severe
313	3/4 3/bl	2/15	Mild
314	3/4 2/5	15/bl	Severe
315	2/5 2/5	2/bl	Mild to moderate
316	3/4 2/5	2/15	Moderate
Litter II			
421	2/5 9/6	1/bl	Minimal
422	2/5 9/6	1/bl	Minimal
423	3/4 9/6	bl/bl	Minimal
424	2/5 9/6	1/bl	Minimal
Litter III			
31	2/5 2/5	NI	Mild to moderate
32	2/5 3/bl	NI	Moderate
33	3/4 2/5	NI	Minimal to mild
34	3/4 2/5	NI	Moderate
35	2/5 2/5	NI	Mild
36	2/5 2/5	NI	Mild
37	3/4 2/5	NI	Severe
38	2/5 3/bl	NI	Mild to moderate
39	2/5 3/bl	NI	Minimal to mild
Litter IV			
541	2/5 3/bl	Not done	Severe
543	2/5 3/4	Not done	Severe
544	2/5 3/bl	Not done	Severe

* Dog D was not DLA histocompatibility typed. The haplotype listed is an estimation based on haplotypes of offspring and sire of offspring.

bl, blank; NI, not interpretable because of hyporesponsive cells. Dogs were not available for repeat study.

ing less than 15 kg have smaller myofibers than dogs weighing over 15 kg.[27] Both Type I and Type II myofibers were smaller in areas of myositis than in areas without myositis, which corresponded to the atrophy grossly evident in areas of more severe inflammation. With one exception, there were no differences in percentages of Type I or Type II myofibers between control dogs and dogs in Litters III and IV. In addition, the percentage of Type I and Type II myofibers was the same in noninflamed and inflamed muscle, indicating, as previously reported,[14] that there is no selective involvement in Type I or Type II myofibers in dermatomyositis. Both Type I and Type II myofibers are affected in human dermatomyositis, and there is more myofiber diameter variation in areas of myositis.[5]

The onset and progression of dermatomyositis have been positively correlated with the elevation of circulating immune complexes (CICs) and serum IgG[17]; however, the reason for the elevations in these parameters has not been identified. The fact that elevated CIC and IgG levels develop first or concurrently with dermatitis suggests a causative role. In addition, the severity of the disease correlates with the degree of elevation of CICs and IgG, and, in mildly affected dogs, the CICs and IgG return to normal levels as the disease resolves.[17] Although many dogs outgrow the condition and are left with mild to moderate hyperpigmentation, hypopigmentation, and alopecia of skin and mild to moderate atrophy of muscles of mastication, other dogs maintain a more active chronic course and progressively develop more severe dermatitis and myositis. The dogs that have a progressive course frequently have developed demodicidosis, a disease associated with immune deficiency.[28] The dogs with progressive disease also developed pyoderma, septicemia, or mega-esophagus with secondary aspiration pneumonia. One male dog with severe secondary pyoderma had an extremely small thymus and slightly large adrenal glands, perhaps caused by stress. Other than the thymic atrophy in Dog 314, there was no gross or histologic evidence to suggest that the dermatomyositis affected dogs were immunodeficient. On the contrary, generalized lymphoid hyperplasia, especially of peripheral lymph nodes, generally occurred in dogs with dermatomyositis.

Distribution of skin and muscle lesions tended to coincide. Inflammation was more severe in skin and muscles of head and distal extremities, and it has been suggested that perhaps immune complexes tend to deposit in cooler areas of the body, in areas of previous inflammation, or in areas of increased local pressure.[17] Alternatively, other factors associated with humoral or cellular immunity may influence development of inflammation in the cooler portions of the body affected with dermatomyositis. One possible exception to the pres-

ence of lesions in cooler regions of the body was the esophageal muscle; however, ingested food and water are cooler than body temperature and could possibly influence, at least periodically, local esophageal temperature. The distribution of myositis in canine dermatomyositis is different from the distribution in humans, in which the more proximal muscles are involved.[5] The reason for this difference is not known.

It is interesting to note that there was no histologic evidence of glomerulonephritis in any of the dogs, even though elevated levels of CICs were present for at least several months. Deposition of immune complexes in various tissues is influenced by a variety of factors. The glomerulus is frequently a site of immune complex deposition, because, in part, of its filtering capabilities. Changes in blood flow, presence of vasoactive substances, immune complex size, antigen/antibody ratio, antibody avidity, antigen charge, and alterations in the mononuclear phagocytic system all may play a role in the localization of immune complexes and degree of damage evoked by the complexes.[29] Therefore, in dogs with dermatomyositis, any of these factors may influence the deposition or lack of deposition of immune complexes in tissues, including the glomerulus. Alternatively, the complexes could be deposited in the kidney of dogs with dermatomyositis without histologic evidence of the deposition. In people with systemic lupus erythematosus, a disease frequently associated with immune complex glomerulonephritis, four types of renal lesions have been noted.[30] In the first type, minimal or mesangial lupus nephritis, the glomeruli may be histologically normal or have a mild increase in mesangial cellularity. In these histologically normal glomeruli, immunoglobulin and immune complexes are demonstrable by immunofluorescence.[30] The three other types of renal lesions are histologically visible in at least some of the glomeruli.[30] It is possible, therefore, that some of the dogs with dermatomyositis may have had early but not histologically detectable renal lesions. However, renal disease has not been clinically documented in dermatomyositis-affected dogs.[16] Nonspecific renal lesions have been associated with human dermatomyositis,[5] as has myoglobinuric nephrosis in patients with rapidly developing myositis.[5] Renal lesions are usually absent in children.[1,31]

Arterial lesions varied. In the dog with severe atrophy of muscles of mastication (312), the arterial lumens were small and the intima thickened. These changes could have been secondary to the muscle atrophy. Other arterial lesions were more active, and necrosis was present in the vascular walls. Dogs with more active lesions had elevated CIC levels. In the childhood form of dermatomyositis, arteritis, frequently of intestinal submucosal vessels, may cause intestinal ulceration and perforation.[2] Vascular lesions within intestine were not seen in dogs.

Primary lesions in dogs with dermatomyositis were in skin, muscle, including esophageal muscle, and vessels. Vasculitis has led to neurologic dysfunction in children.[2] Neuropathy has also been reported, especially in the terminal branches of peripheral nerves.[2,32,33] Alterations of small nerves in muscles severely involved with inflammation were also seen in dogs. In more severely affected dogs, bone was osteopenic, but contractures were not seen in any of the dogs. Contractures and osteoporosis have been reported in children with dermatomyositis.[34] Calcification of muscle and subcutis is a sequel to dermatomyositis in people.[5] One area of muscular mineralization was seen in an affected collie. The generalized lymphoid hyperplasia in dogs may have been due, in part, to the cutaneous and muscular inflammation. Also, the lymphoid tissue may have been hyperplastic due to its excessive production of IgG, which was present in elevated concentrations in the serum.[17]

Moderate enlargement of groups of lymph nodes and hepatosplenomegaly were noted in 10 of 13 children.[34] The significance of the epicarditis in one dog, and minimal focal myocarditis in 3 dogs is not known. The electrocardiograms performed previously on some of the dogs were considered within normal limits.[15] Inflammation of cardiac muscle has led to cardiac failure in children.[31] In some children, only small inflammatory foci are present in the heart.[2] Other lesions of internal organs in dogs included the reduction in weight of genital organs associated with reduced fertility in more severely affected male dogs. The degree of inflammation of skin and muscle in these dogs appeared to be great enough to stunt growth and development and may have interfered with reproductive capabilities. The significance of the small lymphoid aggregates in or around the thyroid glands of some of the dogs is unknown. It is possible that the thyroid lesions could progress with time, although there was no evidence of severe lymphocytic thyroiditis or hypothyroidism in any of the dogs, nor was there evidence of inflammation of other endocrine organs. Conjunctivitis developed in some of the more severely affected dogs coincident with the dermatitis. Similarly, conjunctivitis has been reported in human dermatomyositis.[35] Retinopathy has been rarely reported in children.[36] Other than the alterations associated with CEA, retinal lesions were not seen in dogs. There was no correlation between degree of CEA and severity of dermatomyositis. The small cysts in the peripheral retina were judged to be incidental findings, although they are usually seen in older animals, in which they are considered to be degenerative changes associated with aging.[37] Similarly, cysts in endocrine or-

gans were judged to be incidental findings.[38] Pulmonary lesions were not seen in dogs, and they are rare in children.[39]

In our studies to date, all dogs having one affected parent and living to at least 5 months of age have developed dermatomyositis. In previous reports,[14,15] Dog 422 in Litter II was judged, on the basis of cutaneous and muscle biopsies, normal. However, mild myositis of temporalis muscle was detected in this dog at necropsy. This information further supports a dominant mode of inheritance of dermatomyositis in dogs.

The significance of the isolation of a picornavirus, more specifically an enterovirus, from feces of 7 of the Litter III dogs at 2.5 weeks of age is unknown. The dogs had no evidence of enteritis at the time of viral isolation. Picornaviruses, specifically caliciviruses, have been isolated from dogs with enteritis.[40] Human picornaviruses have also been isolated from feces of normal dogs with low neutralizing antibodies against the viruses.[41,42] Experimentally, picornaviruses (coxsackievirus B1) have induced polymyositis in mice,[43] and picornaviruslike structures have been associated with polymyositis in humans.[44,45] It is also interesting that a calicivirus was isolated from an area of dermatitis from Dog 32 of Litter III at 11.5 weeks of age. Whether the calicivirus was involved in the causation of the lesions remains to be determined. Attempts to isolate viruses from skin, muscle, buffy coat, tonsil, and nares in the other 8 dogs in Litter III were unsuccessful. At necropsy, with the exception of intestinal scrapings, viruses were not isolated from tissues of any of the dogs. The presence of parvo-, corona-, or rotaviruses in the intestine of Litter I and II dogs at necropsy was not expected. The dogs did not have evidence of enteritis, but were housed in kennels close to each other, so that viruses could have been transmitted easily. Previous studies indicated that several of the dogs in Litters I and II had antibody titers to canine calicivirus, coronavirus, and parvovirus.[16] The titer to parvovirus was thought to have been vaccine-induced. Dogs may shed enteric viruses in feces without having clinically evident enteritis.[46] The significance of identifying coronaviruslike particles in the feces of the 7 newborn pups that had not suckled is unknown. Coronavirus was not isolated from other tissues, and none of the pups in the litter were weak or born dead. The 4 pups not euthanatized at birth were normal clinically until the onset of dermatomyositis. There are numerous structures with an apparent peripheral fringe that may appear similar by electron microscopy to coronavirus particles.[47] These structures may have been present and yielded a false-positive result in the newborn pups. Crystalline structures were identified within cytoplasm of endothelial cells in sections of temporalis and extensor carpi radialis in all 3 dogs of Litter IV and in the cytoplasm of a cell in the perimysium of the flexor digitorum superficialis of Dog 37 of Litter III. The crystalline structure in Dog 37 was different from those in dogs of Litter IV in that the parallel arrays were linear and not composed of multiple circular bodies. Also in Dog 37 the crystalline structures were not within the endoplasmic reticulum. The structures in dogs of Litter IV resembled aggregates of picornavirus.[48,49] The reasons the crystalline structures were present in Litter IV and not Litter III dogs may include the observations that 1) the Litter IV dogs were more severely affected with dermatomyositis, 2) the temporalis and extensor carpi radialis muscles were more severely affected than the flexor digitorum superficialis or gastrocnemius muscles, and 3) Litter IV dogs were younger and had more active myositis than Litter III dogs. There are nonviral crystalline structures, such as ribosomal crystals, that resemble viral aggregates[50]; however, the crystalline structures in collies resembled viruses much more than they did ribosomal crystals. Additional ultrastructural and virologic studies on control and dermatomyositis affected dogs will be necessary to identify unequivocally the crystalline structures in these collies. The crystalline structures are similar to those reported in human dermatomyositis.[51-53] In human dermatomyositis the crystalline structures are present in cytoplasm of muscle, but the structures in dogs are in endothelial cells. However, some tubular cytoplasmic inclusionlike structures have been reported in endothelial cells in human dermatomyositis.[12,13] In most instances viruses are not isolated from patients in which crystalline or tubular structures are present[13,52]; however, a coxsackievirus Type A9 was isolated from an 11-year-old girl in whom the crystalline structures were present.[53] Also, coxsackievirus has experimentally induced polymyositis in mice.[54] It has been suggested that cytoplasmic crystalline structures in people may be defective viruses.[52] It has also been suggested that viral infection may cause myositis by triggering the immune system.[54]

The loss of 3 pups from Litter III to septicemia was also unexpected. The 4 other pups with subcutaneous abscesses probably would have died without antibiotic therapy; and if prophylactic antibiotics had not been given to the 5 uninfected pups, they might have developed infections as well. In a long-term study with a population at risk of 2700 dogs, deaths due to inflammation or degeneration accounted for only 1.6% of the perinatal mortality in colony-raised dogs.[55] Most of the perinatal deaths due to inflammation were related to umbilical infections.[55] In the pups in this study, the umbilicus was normal in the pups that died. The death of 3 of 12 pups, or 25%, due to infection was much greater than the 1.6% reported for colony-raised dogs. Also the

infection (subcutaneous abscesses) in 4 of the pups that lived was much greater than expected. If an immunologic defect is present in dogs that allows them to develop not only dermatomyositis, but demodicidosis, the defect may also predispose to the development of infectious diseases in general.

An extension of Green and Woodrow's method for detecting MHC disease associations[56] was used to evaluate DLA-A and -B haplotypes with dermatomyositis. No correlations between haplotypes and dermatomyositis were found in any of the 4 litters.[57] Preliminary HTC typing of Litters I and II indicated that animals with the DLA-D15 phenotype usually had moderate to severe clinical disease, whereas animals that did not express D15 usually had mild or minimal disease. Additional animals need to be tested before arriving at any conclusions. There is a significantly increased frequency of HLA-B8 in white patients, but not black or Latin Americans, and of HLA-DR3 with juvenile dermatomyositis.[6,58]

In summary, the principal lesions in human and canine dermatomyositis are in skin, muscle, and vessels. Lesions in other organs are less common. Similar lesions in both people and dogs have been identified in small nerves, bone, lymph nodes, heart, and conjunctiva. Both Type I and Type II myofibers are involved in dermatomyositis in man and dogs; and perifascicular atrophy, although not common in dogs, is also present. In addition, crystalline viral-like structures have been identified ultrastructurally in both groups. The major difference between human and canine dermatomyositis is in the distribution of muscle lesions. In humans, proximal muscles are more severely affected, whereas in dogs, the distal muscles are more severely affected.

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