

Morphometric Analyses of the Skin of Dogs with Atopic Dermatitis and Correlations with Cutaneous and Plasma Histamine and Total Serum IgE

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Abstract. In order to improve the diagnostic value of histopathologic examination of skin biopsy samples from dogs with atopic dermatitis and, perhaps, to identify any differences from the normal state that may predispose to this skin condition, we compared the anatomic and cellular morphology of skin from three standard sites in 21 normal and 15 atopic dogs. The standard sites were lateral neck, dorsal rump, and craniolateral abdomen. No differences between the two groups were found in the means of area or thickness of the stratum corneum or the remainder of the epidermis at any site. The area of sebaceous glands, but not apocrine sweat glands, was larger in the atopic group ($P \leq 0.05$ for the lateral neck skin and $P \leq 0.1$ for the dorsal rump skin). The mean number of non-metachromatic mononuclear cells in combined skin samples ($126 \mu\text{m}^2$) in atopic dogs (91.0 ± 28.7) was significantly greater ($P \leq 0.01$) than for the control normal dogs (65.3 ± 19.3); the mean number of mast cells in atopic dogs (12.39 ± 6.44) was similarly greater than in the controls (8.48 ± 5.14 ; $P \leq 0.1$). Eosinophils were significantly increased in atopic dog skin ($P \leq 0.01$), with the mean for all three sites combined of 0.81 ± 0.90 compared with a mean of 0.06 ± 0.15 for normal dogs. Numbers of circulating blood eosinophils were not significantly different in the atopic and normal group. Plasma histamine concentrations of 15.3 ± 7.8 for the atopics and 14.3 ± 4.1 for the normal dogs were not significantly different, although they were very high when compared to human values. Cutaneous histamine concentrations were always greater in the atopic dogs, although the difference was significant for only two of the three skin sites ($P \leq 0.05$). Plasma histamine concentrations did not correlate with those of cutaneous histamine. There was a very good correlation between mast cell numbers and cutaneous histamine concentrations ($r = 0.84$). Total serum IgE levels were not significantly different in atopic and normal dogs, although the means, 182.6 ± 112.4 (normal) and 195.4 ± 108.8 (atopic), are high when compared to serum concentrations in human beings. There was no correlation between serum IgE concentrations and those of plasma histamine. By confirming that numbers of cutaneous mast cells, non-metachromatic mononuclear cells, and eosinophils are significantly increased in the skin of atopic dogs in comparison to that of normal dogs, these data increase the validity of using such criteria for diagnostic purposes.

Key words: Atopic dermatitis; dog; histamine; IgE; morphometrics; skin.

Atopic dermatitis, also called allergic inhalant dermatitis, is a common canine disease.^{21,31} Firmly established diagnostic criteria for the disease are few, however; most investigations into this condition have been directed toward standardizing intradermal testing with allergens.^{2,22,38} Histopathologic examination of skin biopsy samples from atopic dogs, although frequently performed, have not been considered helpful in confirming the diagnosis.^{21,22} Few authors have described the histopathologic lesions seen in atopic dermatitis^{21,28,31,39} and fewer have attempted to quantify the change.^{28,31}

Similar diagnostic problems have occurred with human atopic dermatitis,¹³ and attempts to improve the usefulness of microscopic examination of skin have

resulted in several histopathologic and morphometric studies characterizing the microanatomical changes and cellular infiltrates in atopic dermatitis.^{17,20} Although it is far from certain that canine and human atopic dermatitis are completely analogous, there is sufficient similarity^{13,31} to make comparisons useful. Like the canine disease, human atopic dermatitis is characterized primarily by pruritus,¹³ for which histamine is, at least in part, responsible.¹² Cutaneous histamine levels have been found to be elevated in human beings with chronic atopic dermatitis,^{17,20} and in some patients, increased plasma histamine has been found.^{26,27} Serum IgE levels, which are usually elevated in human beings with atopic dermatitis, correlate with the severity of the disease.^{6,24} Eosinophilia, although uncommon in

human beings with atopic dermatitis that is uncomplicated by other atopic diseases,¹⁵ appears to be correlated with high IgE concentrations.^{6,24}

This investigation comprises a morphometric study of the microanatomy of the epidermis and adnexal glands and enumeration of some of the immigrant and resident cells of the skin, principally those associated with type 1 hypersensitivity, such as mast cells and eosinophils and mononuclear cells. Cutaneous histamine, plasma histamine, and serum IgE were also measured. By using these results, we hoped to improve the diagnostic information obtainable from histopathologic examination of skin from atopic dogs, to correlate skin and humoral mediators, and identify, if possible, any anatomical or cellular feature of the skin that may be a predisposing factor in atopic dermatitis.

Materials and Methods

Animals

Atopic dogs. Twenty dogs with atopic dermatitis were studied. These were accepted as having atopic dermatitis if they met the following criteria: 1) a clinical history and clinical signs appropriate to atopy;³¹ 2) absence of any other dermatological disease; and 3) a strongly positive reaction³¹ to at least one allergen, other than biting insects, on intradermal testing with 15 standard allergens (Hollister-Steir Canada, Mississauga, ONT). The dogs, which included both sexes and various ages and breeds, were required to have not received any medication for at least 4 weeks previously.

Normal dogs. Twenty-two, random-source dogs, including dogs of both sexes and various ages and breeds, including mixed breeds, were used. They had no evidence of skin disease on clinical or histopathologic examination and did not react strongly to any of the 15 standard allergens.

Skin samples

Skin samples were taken from three sites (lateral neck; dorsal rump at tail base; and cranio-lateral abdomen) from each dog using a standard 6-mm biopsy punch (Baker-Cummins, Dormer Labs Inc., Pointe Claire, PQ). Subcutaneous fat was stripped off before each biopsy was bisected, one-half processed for histamine and the remaining half fixed in 10% neutral buffered formalin prior to routine embedding in paraffin and sectioning at 6 μ . Sections from each sample were stained with hematoxylin and eosin, toluidine blue, and chromotrope 2R.

Morphometrics

Using a modular system for quantitative digital image analysis (MOP II, Carl Zeiss Inc., NY) and the hematoxylin and eosin-stained sections, the area of the stratum corneum and of the remaining epidermis over a standard 2-mm length of skin was calculated. The sebaceous gland area and apocrine sweat gland area (including the lumen) were calculated over the same length of skin. The thicknesses of stratum corneum and of the remainder of the epidermis were estimated by measuring five randomly selected sites and calculating the means.

Mast cells were enumerated in the toluidine blue-stained sections as previously described.⁷ Four sites were selected at

random in each section, cells in three adjacent high-powered fields (0.45 mm in diameter) from epidermis to subcutis, at each site, were counted, and the mean for each site was calculated. Non-metachromatic mononuclear cells, principally mononuclear leukocytes, were calculated similarly from the toluidine blue-stained sections. Eosinophils were counted in a similar way using the chromotrope-stained sections.

Cutaneous histamine estimation

Half the bisected skin sample was weighed and either frozen at -70°C until processing or processed within 1 hour. The skins were processed as follows. After slicing each sample as finely as possible, it was placed in 4 ml of 2% perchloric acid in siliconized test tubes and boiled for 5 minutes, replenished to 4 ml with more perchloric acid, and then cooled and filtered through gauze to remove hair and tissue debris. Samples were then stored at -70°C until analyzed for histamine content without dialysis in a Technicon Auto II one-channel spectrofluorometric autoanalyzer (Alpkem Corp., Clackamas, OR), using the basic assay method of Siragani-an.³²

Plasma histamine

Blood samples taken by venipuncture were centrifuged at $800 \times g$ for 3 minutes. Platelet-rich plasma was removed and centrifuged in a high-speed ($13,000 \times g$) microcentrifuge (Fisher Scientific Ltd., Don Mills, ONT) for 10 minutes to remove platelets. The supernatant was mixed with equal quantities of 0.4 normal perchloric acid and centrifuged in the microcentrifuge for a further 10 minutes to remove the precipitate. The supernatant was stored at -70°C until thawed for analysis of histamine content in the autoanalyzer.

Measurement of total serum IgE

Antiserum. Each ml of a commercially prepared goat anti-canine-IgE (Calbiochem-Behring, La Jolla, CA) was precipitated with 33% saturated ammonium sulphate and resuspended in 2 ml of phosphate buffered saline (0.01 M, pH 7.2), and dialyzed for 72 hours against phosphate buffered saline at 4°C . The antibody protein content was calculated using the Biorad Protein Assay system (Biorad, Richmond, CA) and diluted to give 3 mg/ml. The antiserum was tested against canine serum high in IgE. On double immunodiffusion, it gave a double line of precipitation that had identity with a known anticanine IgE antiserum of rabbit origin. The antiserum had no lines of identity with antisera to canine IgG, IgM, or IgA.

Iodination. The antiserum was iodinated using 0.5 mCi of carrier free ^{125}I iodine (ICN Biomedicals, Irvine, CA) for each 10 mg of antibody. The iodination was performed by the basic method of Markwell and Fox.¹⁹ Glass reaction tubes were precoated with Iodogen (Pierce, Rockford, IL) that had been dissolved in chloroform at 0.25 mg/ml and dried under a stream of nitrogen. The antiserum and ^{125}I iodine were allowed to react in these tubes for 15 minutes at room temperature. Bound and unbound iodine was separated on two, 10-cm Biogel P6 (Biorad, Richmond, CA) columns in series. Half-milliliter fractions were collected, and fractions containing the first peak of radioactivity were pooled.

Radioimmunoassay. The basic method of Nielsen and Wilkie²³ was used. The optimal dilution of the antiserum used against undiluted canine serum in the radial immuno-

Table 1. Area and thickness of morphologic skin features observed in a 2-mm length of skin from normal dogs and from dogs with atopic dermatitis.

Parameter	Skin Site	Normal Dogs		Atopic Dogs	
		Number of Dogs	Mean $\pm$ Standard Deviation	Number of Dogs	Mean $\pm$ Standard Deviation
Area (mm ²) of stratum corneum	Lateral neck	21	$(0.7702 \pm 0.4577) \times 10^{-2}$	13	$(0.8497 \pm 0.5357) \times 10^{-2}$
	Dorsal rump	19	$(0.7802 \pm 0.7947) \times 10^{-2}$	12	$(0.5692 \pm 0.5604) \times 10^{-2}$
	Cranio-lateral abdomen	19	$(0.9307 \pm 1.0973) \times 10^{-2}$	13	$(0.8659 \pm 0.5831) \times 10^{-2}$
Thickness (mm) of stratum corneum	Lateral neck	21	$(0.3457 \pm 0.2098) \times 10^{-1}$	13	$(0.3620 \pm 0.2205) \times 10^{-1}$
	Dorsal rump	19	$(0.3923 \pm 0.4140) \times 10^{-1}$	12	$(0.2690 \pm 0.1555) \times 10^{-1}$
	Cranio-lateral abdomen	18	$(0.4154 \pm 0.4271) \times 10^{-1}$	13	$(0.3654 \pm 0.2809) \times 10^{-1}$
Thickness (mm) of remainder of epidermis	Lateral neck	21	$(0.4490 \pm 0.1287) \times 10^{-2}$	13	$(0.5499 \pm 0.3502) \times 10^{-2}$
	Dorsal rump	19	$(0.4253 \pm 0.1662) \times 10^{-2}$	13	$(0.5447 \pm 0.2842) \times 10^{-2}$
	Cranio-lateral abdomen	19	$(0.3988 \pm 0.1149) \times 10^{-2}$	13	$(0.4792 \pm 0.3390) \times 10^{-2}$
Thickness (mm) of remainder of epidermis	Lateral neck	21	$(0.1884 \pm 0.0557) \times 10^{-1}$	13	$(0.2305 \pm 0.1075) \times 10^{-1}$
	Dorsal rump	19	$(0.1816 \pm 0.0986) \times 10^{-1}$	12	$(0.2150 \pm 0.0827) \times 10^{-1}$
	Cranio-lateral abdomen	18	$(0.1794 \pm 0.0788) \times 10^{-1}$	12	$(0.1850 \pm 0.0894) \times 10^{-1}$
Area (mm ²) of apocrine sweat gland	Lateral neck	19	$(0.6231 \pm 0.3527) \times 10^{-2}$	12	$(0.9157 \pm 0.8179) \times 10^{-1}$
	Dorsal rump	21	$(0.9118 \pm 0.6538) \times 10^{-2}$	12	$(1.0321 \pm 1.0845) \times 10^{-2}$
	Cranio-lateral abdomen	20	$(0.7818 \pm 0.5163) \times 10^{-2}$	12	$(0.6575 \pm 0.3026) \times 10^{-2}$
Area (mm ²) of sebaceous gland	Lateral neck	20	$(0.5747 \pm 0.2048) \times 10^{-2}$	13	$(0.8704 \pm 0.4472) \times 10^{-2*}$
	Dorsal rump	21	$(0.7487 \pm 0.2725) \times 10^{-2}$	12	$(1.2229 \pm 0.7843) \times 10^{-2†}$
	Cranio-lateral abdomen	19	$(0.7329 \pm 0.3671) \times 10^{-2}$	11	$(0.6431 \pm 0.2337) \times 10^{-2}$

* Statistically significant at $P \leq 0.05$.† Statistically significant at $P \leq 0.1$.

diffusion (Mancini) test was determined in previous experiments, and the radiolabeled antiserum was diluted to a concentration of approximately 1.5 mg/ml. The labeled antiserum was mixed in equal quantities with 2% agarose (Biorad, Richmond, CA) and 2% polyethylene glycol 4000 in phosphate buffered saline (0.01 M, pH 7.2). Multiple well Mancini plates (Miles, Rexdale, ONT) were used for the radial immunodiffusion. Three μ l of test serum were applied per well. After incubation for 24 hours in a moist chamber and extensive washing of plates for 72 hours with phosphate buffered saline, plugs of 1.0 cm in diameter were cut from around each well and counted in a Beckman 5505 gamma counter. Each plate had, as a control for retained radioactivity, a plug from around an empty well in an area of the plate where antigen and antibody could not have reacted. On each plate, six standards, which were doubling dilutions of the canine serum of known IgE concentration, were run. IgE concentration of test sera were calculated using the gamma radiation counts per minute for the appropriate sample and the graph of the standards for the plate on which it was tested.

Statistical analyses

Results were analyzed using Students' *t*-test to compare the means of the atopic and normal dog groups. The values were considered statistically significantly different if the probability (*P*) was less than 0.1. Correlations were calculated using regression analysis, and the correlation coefficient (*r*) calculated based on the assumption $y = a + bx$ where *x* and *y* are the independent and dependent variables, respectively.

Results

Microanatomical findings

The means and standard deviations for 1) area of stratum corneum, 2) thickness of stratum corneum, 3) area of nucleated layers of epidermis, 4) thickness of nucleated layers of epidermis, 5) area of sebaceous glands, and 6) area of apocrine sweat glands are compared between atopic and normal dogs at each of three skin sites in Table 1. The differences are significant only for sebaceous gland area, as indicated in Table 1.

Skin cells

The mean number and standard deviation of mast cells, non-metachromatic mononuclear cells, and skin eosinophils, per high-powered area (i.e., one high-powered field wide by three fields deep, going from epidermis to subcutis as described in Materials and Methods) for each site are given in Table 2. Statistical significances in the differences between the normal and atopic groups are given in Table 2. Mast cells were significantly increased in numbers in atopic skins at two of the sites ($P \leq 0.05$). The overall means and standard deviations for all three sites combined are given in Table 2. Eosinophils were in low numbers in atopic skins but were still significantly more numerous than in normal skin ($P \leq 0.02$). The ranges of eosinophil numbers per high-power field were 0 to 5 for the

Table 2. Numbers of cells in the skin of normal dogs and dogs with atopic dermatitis.

Cells	Skin Site (0.45 mm in diameter)	Normal Dogs		Atopic Dogs	
		Number of Dogs	Mean $\pm$ Standard Deviation	Number of Dogs	Mean $\pm$ Standard Deviation
Mast cells	Lateral neck	21	10.13 $\pm$ 5.08	13	11.85 $\pm$ 6.02
	Dorsal rump	21	7.40 $\pm$ 4.19	12	13.02 $\pm$ 8.19*
	Craniolateral abdomen	21	8.28 $\pm$ 5.56	12	12.33 $\pm$ 5.34*
	All sites combined	21	8.48 $\pm$ 5.14	13	12.39 $\pm$ 6.44†
Skin eosinophils	Lateral neck	20	0.063 $\pm$ 0.179	13	0.712 $\pm$ 0.889‡
	Dorsal rump	20	0.113 $\pm$ 0.401	13	1.000 $\pm$ 1.233‡
	Craniolateral abdomen	20	0.025 $\pm$ 0.077	13	0.712 $\pm$ 0.918‡
	All sites combined	20	0.063 $\pm$ 0.146	13	0.807 $\pm$ 0.90§
Non-metachromatic mononuclear cells	Lateral neck	21	61.9 $\pm$ 23.4	13	87.2 $\pm$ 40.6*
	Dorsal rump	21	68.1 $\pm$ 33.5	13	98.9 $\pm$ 46.8*
	Craniolateral abdomen	21	64.9 $\pm$ 15.1	13	87.2 $\pm$ 31.1*
	All sites combined	21	65.3 $\pm$ 19.3	13	91.0 $\pm$ 28.7§

* Statistically significant at $P \leq 0.05$.† Statistically significant at $P \leq 0.1$.‡ Statistically significant at $P \leq 0.02$.§ Statistically significant at $P \leq 0.01$.

normal skins and 0 to 7 for the atopic skins. Eosinophils were observed in 67% of the atopic skin samples but in only 12% of normal ones. Mononuclear leukocytes were significantly more numerous in atopic skin at all the sites ($P \leq 0.05$). The overall means and standard deviations for all three skin sites given in Table 2 are statistically different with a probability of $P \leq 0.01$.

Hematologic examination of the peripheral blood

The hemograms were unremarkable for both the normal and atopic dogs. Eosinophilia in our laboratory is considered to be numbers of circulating eosinophils in excess of 1.9×10^9 /liter. With values ranging from 0 to 1.07×10^9 /liter, none of the atopic dogs had eosinophilia. One of the normal dogs had an eosinophilia. The range for normals was 0 to 2.12×10^9 /liter.

Histamine

The histamine, in micrograms per gram of wet skin, for each skin site in each group is given in Table 3. The correlation coefficient (r) between cutaneous histamine (y) and cutaneous mast cell numbers (x) for atopic and normal dogs at each of the three sites is $r = 0.8401$. If $y = a + bx$ then the estimated values are $a = -3.791$, $b = 0.9029$.

Plasma histamine in nanograms per milliliter of platelet free plasma is given for each group in Table 4. The coefficient of correlation between cutaneous and plasma histamine of normal and atopic dogs is $r = -0.1093$.

Total serum IgE

The means and standard deviations of total serum IgE in the atopic and normal dog groups are given in Table 5. The correlation coefficient between total serum IgE and plasma histamine, using 24 pairs of values for atopic and normal dogs, is $r = 0.1282$.

The coefficient of correlation between mast cells and total serum IgE for atopic dogs (12 pairs of values) is $r = -0.0438$ and for atopic and normal dogs (33 pairs of values) is $r = -0.1453$.

Discussion

The stratum corneum is vital to the integrity of the skin. An intact and optimally hydrated stratum corneum is necessary for a soft and flexible skin. Dry, itchy skin with a reduced water-binding capacity is a feature of human atopic dermatitis.³⁶ Scratching and subsequent loss of loose squames probably accounts for the reduced thickness and numbers of cell layers

Table 3. Cutaneous histamine concentrations in normal and atopic dogs.

Cutaneous Histamine $\mu\text{gm/gm}$ Wet Skin	Normal Dogs		Atopic Dogs	
	Number of Dogs	Mean $\pm$ Standard Deviation	Number of Dogs	Mean $\pm$ Standard Deviation
Lateral neck skin	21	3.98 $\pm$ 1.56	13	5.01 $\pm$ 1.76
Dorsal rump skin	21	3.75 $\pm$ 2.34	13	9.30 $\pm$ 7.29*
Craniolateral abdominal skin	21	3.85 $\pm$ 2.23	13	8.27 $\pm$ 5.95*

* Statistically significant at $P \leq 0.05$.

Table 4. Plasma histamine concentrates in normal and atopic dogs.

Normal Dogs		Atopic Dogs	
Number of Dogs	Mean $\pm$ Standard Deviation Plasma Histamine ng/ml Plasma	Number of Dogs	Mean $\pm$ Standard Deviation Plasma Histamine ng/ml Plasma
11	14.3 $\pm$ 4.05	14	15.3 $\pm$ 7.75

observed in human atopic skin.³⁷ Measurement of the stratum corneum of atopic dog skin in the present study did not reveal any alteration from normal skin that might have reflected similar changes. Neither was the ichthyosis and xeroderma,¹ or hyperkeratosis,^{28,31} previously reported in canine atopic dermatitis, reflected in our findings. It is possible, however, that some hyperkeratotic stratum corneum was lost from atopic skins during fixation and processing. Washing the skin prior to histopathologic examination has been shown to significantly reduce the thickness of the stratum corneum in both normal and atopic human skin.³⁷

The epidermis is no longer considered simply a protective barrier but an important part of the immune surveillance system.³⁴ Increased area of skin without similarly increased thickness, reflects increased folding of the dermo-epidermal junction, whereas increased area and thickness occur together in acanthotic conditions.³⁴ Increased epidermal area is a feature of most of the lesions associated with human atopic dermatitis,³⁴ and some degree of acanthosis has been reported to occur in greater than 90% of canine atopic dermatitis skin samples.^{28,31} Although the atopic skins, in this study, had consistently greater mean areas and thicknesses, the differences were not sufficiently greater to be statistically and significantly different from normal. Our measurements did not indicate any significant increase in dermal-epidermal folding, and hence they support the contention that elongation of rete ridges is not common in canine atopic dermatitis.²⁸ We conclude that epidermal area, thickness, and contour are unlikely to be suitable measurements to aid in the diagnosis of this condition.

Hyperhidrosis has been reported in 10% of atopic dogs.^{18,31} This was not reflected in the present investigation in any consistent alterations in the area of sweat glands in the atopic dog skins. The sebaceous glands, however, were significantly larger in the atopic skins at two of the three sites examined. This increase

in sebaceous glands concurs with previous findings.²⁸ Increased sebaceous secretion may be partially responsible for the apparent excess wetness of the coat of atopic dogs.¹⁸ The role of increased sebaceous glands in predisposition to or in the pathogenesis of canine atopic dermatitis is unknown and contrasts with the situation in human atopic dermatitis in which unaltered or atrophied sebaceous glands are usually observed.³³

Atopic dermatitis is believed, at least in part, to be due to type 1 hypersensitivity. Mast cells are one of the key participants in this immune response. Mast cells in the chronic lesions of human atopic dermatitis have been reported to be almost twice as numerous as in normal skin.²⁰ The cutaneous histamine levels in chronic human atopic dermatitis were reported to be increased by approximately 75% above normal.¹⁴ We found mean numbers of mast cells to be increased in the atopic skins at all of the three sites examined, although the difference was not statistically significant at site 1. Similarly, mean cutaneous histamine concentrations were higher for the atopics at all three sites with similar statistical significances as for the mast cells. There was a very good correlation between histamine and mast cell numbers, and the coefficient of x was calculated as close to unity. This was somewhat surprising since we did not include atypical mast cells in our counts. Since these do not show metachromasia unless fixed in lead acetate and since most histopathologic examination of the skin is performed on routinely formalin-fixed material, inclusion of these mast cells in the counts would have been of no advantage to histopathologists. Atypical mast cells have been reported to account for about one-third of the total number of mast cells in dog skin.⁴ On this assumption, we would have expected b , the coefficient of x , to be closer to 1.5, unless the atypical mast cells are much less significant in the production of histamine in the skin.

The mean value for mast cells per site in normal dog

Table 5. Total serum concentrations IgE/ng/ml in normal dogs and dogs with atopic dermatitis.

Normal Dogs			Atopic Dogs			Statistical Significance
Number of Dogs	Mean $\pm$ Standard Deviation Total Serum IgE (μ g/ml)	Range	Number of Dogs	Mean $\pm$ Standard Deviation Total Serum IgE (μ g/ml)	Range	
21	182.57 $\pm$ 112.43	25–410	16	195.38 $\pm$ 108.79	85–550	Not Significant

skin calculated in the present study (8.5) is slightly higher than the value per high-power field previously reported.⁷ It is unclear in that previous study⁷ how the authors arrived at the per high-power value. Our results agree with theirs in that mast cells are most frequent in the deep dermis and much less frequent near the subcutis. Hence their result, presuming it represents the high-power field centered over the zone of most numerous mast cells, would include 75% of our total counts and would then closely match our results. Another author quotes a normal range of skin mast cells as 4–12, although no supporting evidence is given.³¹ The range of mast cell numbers, if calculated in the present study on a per high-power field basis in the zone of most numerous mast cells, would be slightly lower (3–9).

Mast cells have been reported as being increased in atopic dog skin in 35% of cases.³¹ The mean number of mast cells per site in atopic skin in this study was 50% greater than in the normal skins. In 75% of the atopic skins examined, mast cells were increased above the mean for the normal skin, and in 50% of the atopic skins, mast cells were greater than the mean plus the standard deviation for the normal skin. Hence increased numbers of mast cells was one of the most consistent findings in canine atopic dermatitis, and the good correlation with cutaneous histamine provided objective confirmation of our results.

The values for cutaneous histamine from the present study, for both normal and atopic skins, were approximately one-half those measured in human skin; however, the percentage increase over normal for atopic skin in this study was similar to that reported for the human skin.¹⁴ The reported range of values for total histamine per gram of skin, released by antigen from presensitized dogs,⁸ was also somewhat greater than the normal levels established in the present study, but this probably reflects the greater sensitivity and specificity of the histamine assay used in the present study over previous methods.^{8,14} It has been suggested that, for human beings, atopic mast cells have enhanced releasability of histamine upon immunologic and non-immunologic stimuli rather than simply greater quantities of histamine.^{27,29} This needs to be examined for atopic canine mast cells, to establish if histamine, as has been questioned,¹ is one of the mediators of pruritus in this disease.

Although eosinophils respond to chemotactic factors secreted by activated mast cells,³ eosinophils are rarely observed in human atopic dermatitis.²⁰ In the canine disease, eosinophils have been reported to be rare³¹ or in moderate numbers.²⁸ We found eosinophils to be present in two-thirds of the atopic skins, although in low numbers. Even so, this was statistically significantly greater than in the normal skins in which eo-

sinophils were extremely rare. More intensive investigations in the human disease have identified eosinophil-granule major basic protein deposits in atopic skin, and it has been suggested that these cells have a role in the disease despite the low numbers of intact cells.¹⁶ We conclude, similarly, that eosinophils are frequently present in skins from canine atopics but in such low numbers that they are not a useful diagnostic criterion for canine atopic dermatitis. Eosinophilia has been reported as a diagnostic criterion for canine atopic dermatitis,^{1,5} although this has been disputed.^{25,31} The present study also found no eosinophilia in the group of atopic dogs.

Non-metachromatic mononuclear cells were the predominant infiltrating cell in the atopic dog skins. These results agree with the previous studies.^{28,31} Further characterization of the lymphocytic cells will be valuable, when the appropriate canine reagents become available, to confirm whether they are, as in the human disease, principally of helper T cell phenotype.¹⁷

The plasma histamine concentration rarely exceeds 1 ng/ml in normal human beings, but it has been reported to be higher in some atopic dermatitis patients.^{27,29} While we did not find any significant difference in mean plasma histamine between the normal and the atopic groups, the normal values were very much higher than in human beings. Histamine is released from leukocytes during storage,⁹ but our samples were processed promptly to avoid this effect. Elevated plasma histamine concentrations are associated, in human beings, with elevated concentrations of serum IgE.²⁶ Since serum IgE is very high when compared to human beings, in normal as well as in atopic dogs,³⁵ it is possible that there is rapid release of some leukocyte histamine into the plasma.

Serum IgE is usually elevated in human beings with atopic dermatitis.^{6,24} In the present study, serum IgE was found to be very high in both normal and atopic dogs. The values obtained confirmed the findings in the two other studies that quantitated serum IgE in normal and atopic dogs.^{30,35} The method used by us to quantitate serum IgE was quite different from those used in the previous studies,^{30,35} although it must be noted that we used an IgE standard provided by one of the previous investigators.³⁵ There was no correlation between total serum IgE and plasma histamine concentrations. Serum IgE in human beings correlates with the severity of the skin disease, but we could not make this correlation for dogs. Neither did serum IgE correlate with cutaneous mast cell numbers, which might be considered a measure of disease severity. The correlation between serum and cutaneous IgE in normal dogs has been reported to be poor.¹⁰ The cause of the elevated serum IgE in normal and atopic dogs is

believed to be due to intestinal parasitism,³⁵ and it is clear from the present study and previous studies^{10,11,35} that most of the circulating IgE is not involved in the atopic skin disease. More intensive investigation into allergen-specific IgE and its action in the skin is needed to clarify these findings.

In the present study, standard sites for skin sampling were used rather than the most affected areas. This was done in order to make comparisons with normal samples more valid, since skin histology and morphology are very variable from site to site.^{7,39} While periorbital, auricular, and interdigital areas are often the most affected in atopic dermatitis,³¹ it is more difficult and less desirable to biopsy these sites. Consequently, we have undoubtedly missed some areas with more severe or chronic lesions. The advantage of our method, however, is that the findings may be applied more generally and may also apply to non-lesional or mildly affected skin.

In conclusion, while we have not identified any new morphologic characteristic that is diagnostically significant for atopic dermatitis, we have confirmed, by quantitation, some of the present histologic criteria, such as increased mast cell numbers and sebaceous gland area. In addition, this study has provided data in terms of cutaneous and serum histamine and total serum IgE, as well as measurements of epidermis, skin glands, mast cells, and some immigrant cells that can be used for future studies of canine atopic skin or normal skin.

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