Biochemical and Acoustical Parameters of Normal Canine Skin

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Abstract—**The scanning laser acoustic microscope (SLAM) at 100 MHz and backscattering acoustic technique (BAT) at 10–40 MHz were used to examine normal canine skin. Skin specimens from four animals and from four locations on the animal were analyzed biochemically and morphologically as well as acoustically. At 100 MHz, the mean ultrasonic speed obtained with the SLAM was 1632 ± 34 m/s and the mean attenuation coefficient was 66 ± 12 Np/cm. Using BAT, the mean integrated attenuation coefficient at 25 MHz was 13 ± 4 Np/cm. While the speed values fall within the range of values previously reported for skin from 1 to 10 MHz, the values for the attenuation coefficient using either SLAM or BAT are considerably higher than would be predicted from literature values at 1–10 MHz. Thus, the frequency dependence of the attenuation coefficient is a stronger function of frequency than the data at lower frequencies would suggest. The biochemical analyses yielded a collagen concentration of 20 ± 2 percent of the wet weight or 65 ± 12 percent of the dried defatted weight and a water concentration of 60 ± 3 percent of the wet tissue. Specimens appeared normal morphologically and sizes for structures in the skin were estimated. An analysis of variance of the SLAM data and the biochemical data showed significant animal-to-animal differences and some differences due to location on the animal.**

INTRODUCTION

EFFORTS to evaluate the ultrasonic propagation properties of diseased or experimentally modified tissue involve a comparison of the measurements to those of normal tissue. Although a considerable amount of attention has been focused on the development of a database for normal parenchymal tissues, and for liver in particular [1]–[3], very little attention has been given to the skin.

We have used the scanning laser acoustic microscope (SLAM) [4] and backscatter acoustic techniques (BAT) [5] to evaluate healing of skin wounds. The measurements were made at 100 MHz and at 10–40 MHz, respectively, which are higher frequencies than those for which published information on skin is currently available.

In this paper, we examine normal dog skin at these frequencies. Because of the anteroposterior differences noted in biological studies [6], duplicate skin samples from four locations (head to tail) were interrogated acoustically and morphologically as well as biochemically for collagen and water concentration. The variability of normal skin from different dogs was also analyzed. In addition, we present a detailed compilation of ultrasound data currently available on skin and compare it to our data at these higher frequencies.

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METHODS

To characterize the ultrasonic speed and attenuation coefficient in normal skin, specimens of skin from four locations on both sides of the back of each of four 21–25 kg mongrel dogs were obtained. One specimen was located over the scapula and one just proximal to the iliac crest. The other two were equally spaced between these two locations. Pairs of samples were obtained from each side of the spine at all four locations. The four locations will be referred to as I, II, III, and IV, with location I near the head and location IV near the tail (Fig. 1). All analyses were done without knowledge of the site from which the samples were taken. Specimens from each location were aliquoted for biochemical, morphological, SLAM, or BAT measurements (Fig. 1). Standard paravertebral incisions had been made on these animals, but were 2–3 cm away from the normal skin specimens. The results of the wound healing analyses are reported elsewhere [7].

Tissue water and total collagen concentrations were determined for all skin specimens as described previously in detail [4]. An analysis of variance (ANOVA) was performed to determine the effect of variation among dogs and the location on the animals from which the specimens were taken. The specimens were also fixed, embedded, and examined histologically [4]. The diameter of major structures in the skin such as collagen fiber bundles, hair follicles, and sebaceous glands were measured on a Leitz microscope using a calibrated eyepiece micrometer.

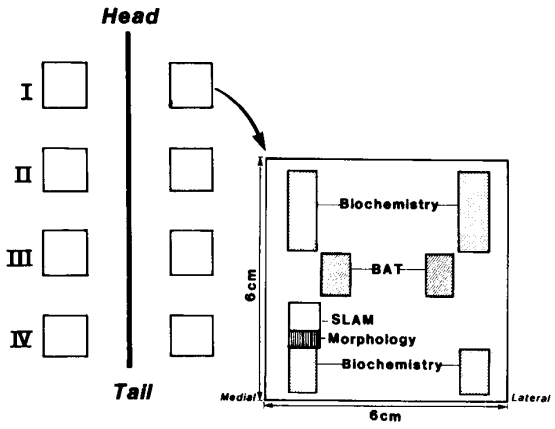

Fig. 1. Diagram showing locations on the dogs from which skin specimens were taken.

A scanning laser acoustic microscope (Sonomicroscope 100^R, Sonoscan Inc., Bensenville, IL) operating at 100 MHz was used to determine the ultrasonic speed and attenuation coefficient in the tissue specimens as described in detail elsewhere [4], [8]–[10]. Frozen sections (50, 100, and 150 μm in thickness) were cut parallel or perpendicular (cross section) to the plane of the epidermal surface. Parallel sections were taken at depths ranging from 100 to 500 μm; hence, for this section type, measurements were taken in the papillary and reticular dermis. For the cross sections, speed measurements were taken in the reticular dermis (300–700 μm from the epidermal surface), whereas attenuation measurement sites were more randomly selected in both papillary and reticular dermis. Hair shafts were avoided because of their very high attenuation.

Speed was calculated from the magnitude of the horizontal shift in the vertical lines on the interference mode of the SLAM. The normalized fringe shift was determined in the spatial frequency domain from the phase shift between the raster lines of the interference image. For each of three thicknesses of skin, at least one, and in some cases two or three, ultrasonic speed determinations were made at 30 C. The attenuation coefficient was measured utilizing the insertion loss method, which involves the comparison of two signal amplitudes received from the acoustic image, one with and one without a specimen of known thickness inserted in the sound path. The slope of the insertion loss versus thickness curve, determined by a least squares analysis, yields the attenuation coefficient in dB/mm.

An ANOVA was performed to determine the effect of variation among dogs, the location on the animal, the section type (parallel versus perpendicular), and the specimen thickness. Details of the statistical analyses are described elsewhere [10]. Significance was defined as $p < 0.05$.

BAT measurements were taken over a frequency range of 10–40 MHz. A broad-band transducer (Panametrics model V3182) was used with transmitter/receiver electronics having a bandwidth of 1–100 MHz [5]. Specimens were carefully mounted with pins to a rubber block and immersed in saline containing 0.2 g/l ethylenediaminetetraacetic acid to inhibit collagenase at 22–24 C. A 1.9 cm focal length transducer with a focal beam width of 0.2 mm (-6 dB level) was used. *C* scans were made at depths of 0.5 and 1.0 mm (i.e., in the reticular dermis) in planes parallel to the epidermal surface. A 500 ns gate centered at each depth was used to acquire a sufficient number (30 acquisition sites, each separated by 0.3 mm) of records to average.

For BAT, the attenuation coefficient was calculated from the ratio of frequency spectra from each of the two scan planes interrogated. Each spectrum was a *C*-scan average, i.e., the average of the 30 spectra from a particular depth [5]. The 95 percent confidence interval for the attenuation coefficient estimates from each location was ± 2.5 Np/cm (2.2 dB/mm) over the 10–40 MHz frequency range [11]. The attenuation coefficient over this frequency range was then averaged to determine the integrated attenuation coefficient [12]:

$$\text{integrated attenuation} = \frac{1}{f_2 - f_1} \int_{f_1}^{f_2} \alpha \, df$$

where f is frequency and α is the attenuation coefficient. An ANOVA was performed to investigate the effect of variation among dogs and location.

Results

The mean collagen concentration for the 32 normal canine skin samples was 20 $\pm$ 2 percent of the wet weight or 65 $\pm$ 12 percent of the dried defatted weight, and the water concentration was 60 $\pm$ 3 percent of the wet tissue. An analysis of variance (ANOVA) indicated that there was a significant dog-to-dog variation explaining 61 percent of the variance in the collagen concentration (whether based on wet weight or dried defatted weight). For the collagen concentration based on wet weight, the location on the animal was significant, but explained only 10 percent of the variance. There was no consistent pattern, however, among the dogs for variation due to location. For collagen concentration based on dried, defatted weight, significance was not shown for location.

Morphological examination of the fixed and embedded specimens revealed that all of the tissues were consistent with normal canine skin [13]. In fixed perpendicular sections, the thickness of the epidermis (from the basal layer up to but not including the stratum corneum) typically ranged from 30 to 50 μm. The papillary dermis ranged from 100 to 200 μm and the reticular dermis was greater than 2000 μm. Individual hair follicles ranged from 60 to 175 μm in diameter, but occasionally clusters were observed with a total diameter of 500 μm. The hair shafts for the main hairs were 70–120 μm and smaller accessory hairs (15–40 μm diameter) often surrounded a main hair within a single follicle. Sebaceous glands ranged in diameter from 50 to 200 μm. Collagen fiber bundles in the papillary dermis were typically 5–25 μm, while those in

the reticular dermis were more densely packed and ranged from 20 to 70 μm. The larger fiber bundles often appeared to be comprised of several discrete bundles. While these skin sections generally were very heterogeneous, the hair follicles and sebaceous glands penetrated to depths greater than 1000 μm: hence, all major structures of the reticular dermis were uniformly represented at the depths that most acoustic measurements were made (300–1000 μm).

Ultrasonic speed values were obtained with the SLAM at 100 MHz for normal skin from four dogs. An ANOVA revealed that there was a significant difference from animal to animal so a separate ANOVA was done for each dog. The results of the ANOVA did not, however, indicate a significant difference in ultrasonic speed for perpendicular sections versus parallel sections of skin so these data were combined.

The ultrasonic speed values from SLAM measurements for the four locations from each dog are given in Table I. Each mean value was determined from 10 to 29 speed measurements (both section types and duplicate locations were combined). All four dogs showed some variation in speed for the different locations, but there was no consistent pattern in the location variation. The location on the dog from which the skin specimen was taken did, however, explain 9–15 percent of the variance in speed and was considered statistically significant in three of the four dogs. The overall mean and SD for all of the speed data (300 speed values total) in the normal skin was 1632 + 34 m/s.

The ultrasonic attenuation coefficient was also determined for the skin specimens. A Student's t test was performed on the SLAM data to determine if there were significant differences between results for perpendicular and parallel sections. The results of this analysis indicated that the attenuation coefficient was not different for the two section types, so the attenuation data from the two section types were combined and an ANOVA was then done for each dog to determine the effect of specimen location on the attenuation coefficient. Although the effect of location was not shown to be statistically significant for any of the dogs, in three of the dogs, specimen location explained more than 30 percent of the variance in attenuation coefficient. In the fourth dog, location explained only 6 percent of the variance.

The mean attenuation coefficients for all locations for each dog are presented in Table II. The attenuation coefficient varied somewhat according to specimen location for each dog, but no consistent patterns were observed. If all data were combined, then the overall mean and SD attenuation coefficient values (60 total) for normal canine skin was 66 ± 12 Np/cm (57 ± 10 dB/mm) at 100 MHz.

BAT measurements are presented (Table III) in terms of the integrated attenuation coefficient, which is the average value of the attenuation coefficient over the frequency range of 10–40 MHz. The data for all duplicate locations for dogs *C* and *D* and for location III for dogs *A* and *B* are tabulated. Because of changes in protocol, comparable data for the remaining locations were not available. An ANOVA for regional and/or dog variations did not reveal any statistically significant differences. The overall mean and standard deviation for the integrated attenuation coefficient values was 13 ± 4 Np/cm (11 ± 3 dB/mm) at 25 MHz, which corresponds to the middle of the frequency range considered.

TABLE I
ULTRASONIC SPEED IN NORMAL CANINE SKIN FROM FOUR LOCATIONS

	I	II	III	IV
Dog *A*[a]	1631 ± 27	1669 ± 64	1640 ± 19	1636 ± 18
Dog *B*[a]	1621 ± 36	1599 ± 34	1624 ± 25	1640 ± 37
Dog *C*	1629 ± 23	1622 ± 28	1625 ± 28	1644 ± 31
Dog *D*[a]	1640 ± 26	1654 ± 25	1627 ± 29	1629 ± 24

I–IV represent locations from head to tail along the back, respectively. Values are the mean ±S.D. in m/s.
[a]indicates dogs for which the specimen location was statistically significant.

TABLE II
ULTRASONIC ATTENUATION COEFFICIENT IN NORMAL CANINE SKIN FROM FOUR LOCATIONS (100 MHz)

	I	II	III	IV
Dog *A*	64 ± 6	68 ± 13	56 ± 6	59 ± 5
Dog *B*	72 ± 14	72 ± 6	58 ± 15	83 ± 12
Dog *C*	75 ± 16	64 ± 12	74 ± 6	51 ± 7
Dog *D*	69 ± 15	62 ± 6	64 ± 12	63 ± 10

Values are the mean ±SD in Np/cm.

TABLE III
INTEGRATED ULTRASONIC ATTENUATION COEFFICIENT (Np/cm) FOR NORMAL CANINE SKIN USING BAT (10-40 MHz)

	I	II	III	IV
Dog *A*	—	—	15	—
			18	
Dog *B*	—	—	14	—
			14	
Dog *C*	13	18	8	19
	16	12	14	9
Dog *D*	17	3	9	14
	15	14	11	10

Integrated attenuation coefficients for skin from the left side of the animal are listed first.

DISCUSSION

The mean collagen concentration determined in this study agrees with the literature value previously reported for dog skin [14]. An ANOVA indicated that the variations in the biochemical data were primarily due to differences between dogs, and this was also true for all of the SLAM data. In addition, the location on the dog from which the skin specimen was taken explained some of the variation in the percent collagen data (based on wet weight) and the speed data for three of the four dogs. The effect of location, however, was not statistically significant for the SLAM attenuation coefficient data. Using the

BAT attenuation data, an ANOVA for regional and/or dog variations did not reveal any significant differences.

The mean ultrasonic speed from SLAM measurements at 100 MHz for normal dog skin was 1632 ± 34 m/s, which agrees with the value predicted by the equation of O'Brien [15]. The mathematical relationship between speed and tissue collagen concentration, namely, $c = 1588 + 32 \ln C$ where C is the collagen concentration as a percentage of wet weight and c is the ultrasonic speed, predicts an ultrasonic speed of 1684 m/s for skin with 20 percent collagen. Although this relationship was developed using literature values of speed and collagen concentrations for nine soft tissues excluding skin, it fits our experimentally derived skin data well.

Previous reports of ultrasound speed data for skin and the frequency at which they were measured are presented in Fig. 2. (No statement is being made about the frequency dependence of the speed measurements: the graph is merely a convenient way to present the body of data.) Speed values of 1710 and 1720 m/s were reported by Cantrell *et al.* [16] and Goans *et al.* [17], respectively, using porcine skin. Most of the other speed values reported, however, fall between 1500 and 1550 m/s. The speed values and species are as follows: 1498 [18] and 1518 [19], human; 1540, 1543, 1543, 1536 [20], young mouse; and 1512, 1511, 1510, and 1515, old mouse [20]. Speed values of a 0.6 percent calf skin collagen solution (1495 m/s) [21] and mouse tail tendon (1733 m/s) [22] which is 30 percent collagen are included for reference. All of these speed data were obtained at temperatures between 20 and 24°C. Thus, our speed values for dog skin using the SLAM at 100 MHz (1599–1669 m/s) fall within the ranges reported for skin (Fig. 2).

The attenuation data obtained with the SLAM and BAT are plotted as a function of frequency along with other values for skin from the literature (Fig. 3). One of the lowest frequencies reported was 0.97 MHz using fresh human or dog skin [23]. Nakaima *et al.* [24] refrigerated human or dog skin before interrogation at 0.57, 0.97, 1.7, 2.9, and 4.8 MHz. Dussik and Fritch examined fresh human skin at frequencies of 1, 3, and 5 MHz [25]. Young mouse skin had a higher attenuation coefficient than did old mouse skin at all frequencies examined (2.25, 5, 7.5, and 10 MHz) [20]. In spite of the variety of tissue preparations, temperatures (20 to 40°C), and measurement techniques used, the literature values from 1 to 10 MHz for skin suggest a power law dependence yielding a straight line relationship on a logrithmic plot (Fig. 3).

Data at frequencies higher than 10 MHz were first reported by Cantrell *et al.* They obtained an attenuation per unit frequency for normal porcine skin of 4.3 dB/cm · MHz over the frequency band of 7–13 MHz [16].[1] The integrated attenuation coefficient obtained with BAT over 10–40 MHz was 13 ± 4 Np/cm (plotted at 25 MHz) and the SLAM data at 100 MHz ranged from 51 to 76 Np/cm. (These data are also plotted in Fig. 3 for comparison to

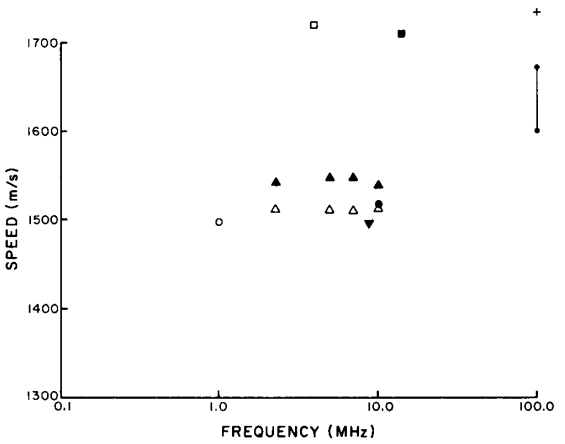

Fig. 2. Range of speed values for dog skin obtained with the SLAM compared to published values on skin. Data are presented as a function of frequency merely to visually present the data. Literature values: young pig (■ [16]), adult pig (□ [17]), human (○ [18], ● [19]), young mouse (▲ [20]), old mouse (△ [20]), mouse tail tendon (+ [22]), and 0.6 percent calf skin collagen (▼ [21]). Normal canine skin data range obtained with the SLAM reported herein: (●---●).

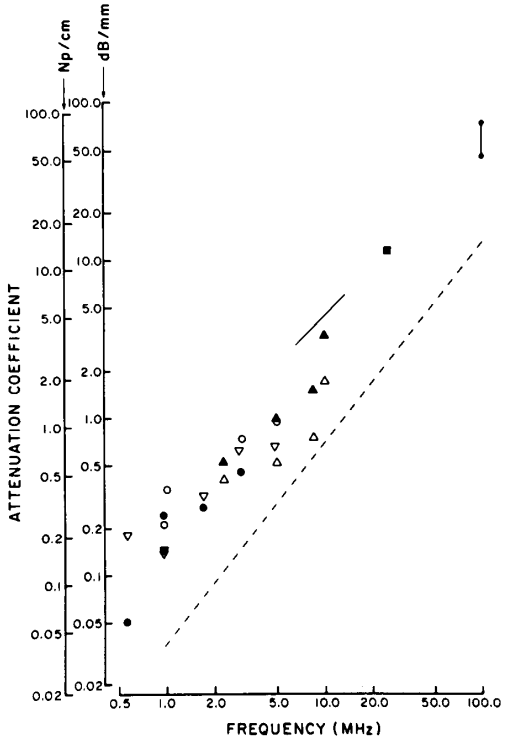

Fig. 3. Attenuation coefficient versus frequency from 1 to 100 MHz. Literature values: fresh human skin (○ [23] at 0.97 MHz and [25]), refrigerated human skin (● [24]), pig skin (–, 7–13 MHz [16][2]), young mouse skin (▲ [20]) and old mouse skin (△ [20]), fresh canine skin (▼ [23]), refrigerated canine skin (▽ [24]). Normal canine skin data reported herein obtained with the BAT (■) and data range reported herein with the SLAM (●—●). For comparison to normal parenchymal tissue, normal bovine liver is shown (---), 1–100 MHz [2].

[1]Note that the data in [16, Table III] must be multiplied by 2π (Cantrell, personal communication).

[2]This curve depicts the slope given, but the curve may shift up or down due to unknown reflection coefficients (Cantrell, personal communication).

the literature values.) Given these data above 10 MHz, the frequency dependence of the attenuation coefficient appears to be a stronger function of frequency than the data at lower frequencies would have suggested.

For comparison, the attenuation coefficient for normal bovine liver over the 1–100 MHz frequency range [2] is also plotted in Fig. 3. While there is no reason *a priori* to believe that attenuation data should go with the first power of frequency, a power law equation with a slope of 1.27 and an intercept at 1 MHz of 0.043 Np/cm fitted the liver data well. The skin data, however, show a much stronger frequency dependence, which may be related to the greater heterogeneity of the tissue and to the sizes of the various structural elements in skin. In addition, the normal skin values are about five times greater than that for liver over this frequency range.

Earlier studies using the SLAM at 76 MHz on kidney slices also showed a strong frequency dependence with a square law (or greater) frequency dependence at 222 MHz [26]. Estimates of the contribution of scattering to attenuation generally suggest an increased contribution of scattering at higher frequencies over the range of 1 to 10 MHz [3], [27], and at frequencies as high as 100 MHz may significantly contribute to the attenuation. In addition, the wavelengths at higher frequencies are the same size as collagen bundles in the dermis, contributing to an increase in scattering.

The fact that both the collagen concentration and the SLAM measurements showed animal-to-animal variation demonstrates the importance of doing biochemical determinations on the tissue specimens that are to be interrogated acoustically. Relying on published literature values may, therefore, be misleading, particularly if they are from different animals or species. In addition, differences among the SLAM measurements were found depending on the location on the animal from which the specimen was taken. These regional differences argue for the need to examine control tissue samples at a site near any diseased or wounded experimental skin site.

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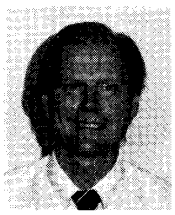

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