Effects of sample site and size, skin tension lines, surgeon, and formalin fixation on shrinkage of skin samples excised from canine cadavers

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Objective—To assess the effects of sample size and location, skin tension lines, surgeon, and formalin fixation on the extent of shrinkage that occurs in excised canine skin samples.

Animals—Cadavers of 4 adult purpose-bred mixed-breed hound dogs with grossly normal skin.

Procedures—54 circular areas of skin (2-, 4-, and 6-cm-diameter samples from each of 9 body regions on each side) were excised by 1 of 2 surgeons from each cadaver. The diameter of each sample was measured in 4 orientations (parallel to previously reported tension lines, perpendicular to tension lines, in a dorsoventral orientation, and in a craniocaudal [or rostrocaudal] orientation) at 3 time points (before and immediately after excision and after 24 hours of formalin fixation).

Results—216 samples were measured in all 4 orientations at all 3 time points. For all samples, mean ± SE decrease in diameter after fixation, compared with pre-excision findings, was 6.2 ± 0.7 mm. No significant correlations were found between percentage of skin shrinkage and surgeon, body side or region, or measurement orientation in relation to skin tension lines. The mean sample diameter immediately after excision differed significantly from that before excision (mean diameter decrease, 5.5 ± 0.7 mm). Overall, sample diameter immediately after excision and after formalin fixation did not differ.

Conclusions and Clinical Relevance—The extent of shrinkage of skin samples from hound cadavers that occurred immediately after excision was notable. A better understanding of the effectors of excised skin sample shrinkage is needed, especially when histopathologic findings provide guidelines for surgical margins. (*Am J Vet Res* 2014;75:1004–1009)

Cutaneous tumor excision and tumor margins in canids have been extensively researched, and those findings have been used to dictate surgical planning. Unfortunately, the effects of excision and formalin fixation on shrinkage of canine skin samples are not completely understood and are therefore rarely taken into account in such studies. Shrinkage of human skin samples after excision and formalin fixation has been reported and the changes are considerable.[1–4] In veterinary patients, skin shrinkage could potentially have a crucial role in the determination of surgical margins and the failure or success of tumor excisions. For example, if skin shrinkage of the healthy skin around an excised tumor is greater than that within the tumor, pathologists may conclude that the surgical margins were clean but close, when in fact the tumor-free margins at the time of surgery were much greater. Consequently, recommendations of unnecessarily large margins may be made and some tumors may be inappropriately deemed nonresectable. To understand the effects of pathological processes on skin sample shrinkage, factors that contribute to shrinkage in healthy skin samples must be determined. The purpose of the study reported here was to assess the effects of sample size and location, skin tension lines, surgeon, and formalin fixation on the extent of shrinkage that occurs in excised canine skin samples. We hypothesized that skin samples would shrink significantly immediately after excision but not significantly after 24 hours of formalin fixation. Furthermore, we hypothesized that a greater degree of skin shrinkage would occur in a direction parallel to the skin tension lines.

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Materials and Methods

Specimen preparation—Four apparently healthy young adult purpose-bred mixed-breed hound dogs that were euthanized (IV injection of pentobarbital so-

dium) for reasons unrelated to this study were used to obtain skin specimens. Prior to euthanasia, physical examinations were performed to ensure that the dogs had no grossly evident skin lesions (ie, cutaneous masses, lichenification, or visibly evident pyoderma) and that physical variables (ie, heart rate, respiratory rate, rectal temperature, oxygen saturation as measured by pulse oximetry, arterial blood pressure, and hydration status) were within reference limits. All skin samples were collected within 4 hours after euthanasia.

For the purposes of sample collection, each cadaver was placed in lateral recumbency in a physiologically normal position. The uppermost lateral aspect of each cadaver was divided by use of a permanent marker into 9 regions as follows: head (rostral to caudal base of ear), neck (caudal base of ear to thoracic inlet), forelimb proximal to the elbow joint, forelimb distal to the elbow joint, trunk, hind limb proximal to the stifle joint, and hind limb distal to the stifle joint. The trunk was further divided into 3 dorsoventral regions of equal width (**Figure 1**). Within each of the 9 regions, 3 circular areas of skin (simulating excisional margins) were marked by use of a permanent marker. Each of the 9 regions contained a 2 -, 4-, and 6-cm-diameter outlined area. The positioning of the 3 circular areas was chosen at random within each of the 9 regions by one of the authors (JRW). Premade flexible plastic templates were used to ensure accuracy in marking the areas. An arrow was drawn on each circular area to indicate lines of skin tension as previously described elsewhere.[5,6] After obtaining specimens from 1 side of each cadaver, the marking process was repeated on the other side.

Two individuals (JRW and RCM) surgically removed each skin sample by use of sharp dissection with No. 10 scalpel blades. To approximate the recommended surgical technique performed for cutaneous mast cell tumor removal in dogs, the excisional depth included subcutaneous fat and 1 deep fascial plane for all samples.

Skin measurements and data collection—After removal, the dorsal and cranial pole of each skin sample was marked with a different suture material. Data recorded for each sample included the dog, surgeon, anatomic region, pre-excision diameter (as determined by the diameter of the plastic template), and order of removal (first through third) within each body region. For measurement purposes after excision, samples were placed onto a flat dry surface covered with an impermeable drape and measured with a ruler. The diameter of each specimen was measured in 4 orientations as follows: parallel to previously reported skin tension lines, perpendicular to skin tension lines, in a dorsoventral orientation, and in a craniocaudal (or rostrocaudal) orientation (**Figure 2**). Measurements were made to the nearest millimeter. One investigator (DAU) performed all measurements. The 4 measurements for each sample were completed within 10 minutes after removal from the cadaver. Specimens were placed in resealable plastic bags containing neutral-buffered 10% formalin in a 1:10 ratio of tissue mass to formalin and stored for 24 hours at room temperature (approx 25°C). Following storage, the specimens were remeasured in the same 4 orientations. Thus, for each sample, 4 diameter measurements were obtained at each of 3 time points: before excision, immediately after excision, and after a 24-hour period of fixation in formalin.

Statistical analysis—For each sample at each time point, the diameter measurements obtained in the 4 orientations did not differ, and a mean value was calculated. As data analysis progressed, it became evident that changes in skin sample diameter varied only on the basis of body region; thus, for a given body region, data for all samples (regardless of diameter) from both sides

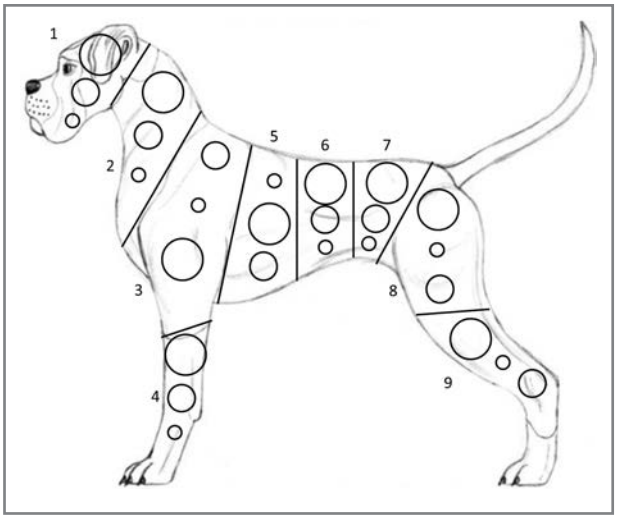

Figure 1—Diagram of the left side of a canine cadaver illustrating anatomic divisions to create 9 body regions and locations of each of 3 skin sample sites within each region. A similar pattern was used on the other side of the cadaver, and samples were collected to assess the effects of sample size and location, skin tension lines, surgeon, and formalin fixation on the extent of shrinkage that occurs in excised canine skin samples. Regions were designated as follows: 1, head (rostral to caudal base of ear); 2, neck (caudal base of ear to thoracic inlet); 3, forelimb proximal to the elbow joint; 4, forelimb distal to the elbow joint; 5, 6, and 7, trunk divided into 3 dorsoventral regions (cranial, middle, and caudal, respectively) of equal width; 8, hind limb proximal to the stifle joint; and 9, hind limb distal to the stifle joint. The circular areas of skin that were excised were 2, 4, or 6 cm in diameter; for regions 4 and 9, the circular regions were contoured to the curvature of the dorsal aspect of the limbs. The excisional depth included subcutaneous fat and 1 deep fascial plane for all samples.

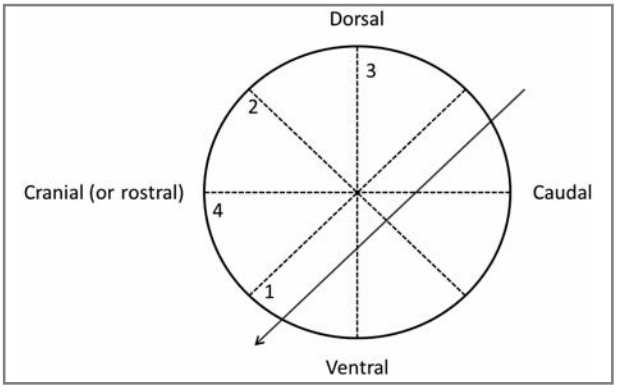

Figure 2—Diagram to illustrate the diameter measurements made in 4 orientations for cadaveric canine skin samples after excision and after formalin fixation. The solid line and arrow was applied to indicate the direction of tension lines in the skin sample. Diameter measurements (dashed lines) were made as follows: 1, diameter parallel to skin tension lines; 2, diameter perpendicular to skin tension lines; 3, diameter in dorsoventral direction; and 4, diameter in craniocaudal (or rostrocaudal) direction.

of all cadavers at a given time point were combined and a mean value was calculated. Excision-related skin shrinkage was determined by calculating the difference between sample diameter before and immediately after excision. The effect of formalin fixation on skin shrinkage was determined by calculating the difference between sample diameter immediately after excision and after the 24-hour period of formalin fixation. Total skin shrinkage was determined by calculating the difference between sample diameter before excision and after the 24-hour period of formalin fixation. The percentage shrinkage was calculated as the initial sample diameter minus the sample diameter after formalin fixation divided by the initial sample diameter × 100%.

Data analysis was performed with cadaver (1 through 4) as a random effect and analyzing the fixed effects of surgeon, body side, body region, pre-excision diameter, and the 3 time points of data collection. To ensure that no effect of time of removal on skin shrinkage occurred, data for the skin samples removed in the first hour after euthanasia were compared with data for those removed in the fourth hour after euthanasia. Correlations between the aforementioned variables and the measurement of the skin sample in each orientation were assessed by use of statistical software.[a] Correlations were assessed with a repeated-measures ANOVA and were considered significant at a value of $P \leq 0.05$.

Results

Each of the 2 surgeons collected 108 samples (27 from each dog). For each dog, all samples from 1 side were collected by one surgeon and all samples from the other side were collected by the second surgeon.

Fifty-four skin samples were obtained from each of the 4 canine cadavers; the total number of samples was 216. Seventy-two samples of each size (2, 4, or 6 cm in diameter) were collected. Twenty-four samples were removed from each of the 9 body regions. Immediately after excision and after a 24-hour period of formalin fixation, the diameter of each sample was measured in 4 orientations (ie, 864 diameter measurements were obtained at each of those time points; **Table 1**).

For each sample at each time point, the diameter measurements obtained in the 4 orientations did not differ, allowing a mean diameter value to be used for further analysis. No significant correlations were found

Table 1—Mean ± SE diameter (mm) of skin samples removed from 9 body regions (3 samples/region) on both sides of 4 healthy adult dog cadavers with grossly normal skin as measured before excision, immediately after excision, and after a 24-hour period of formalin fixation.

Pre-excision sample diameter (mm)	Time point	Body region 1	2	3	4	5	6	7	8	9
20	Before excision	20	20	20	20	20	20	20	20	20
	Immediately after excision	18.1 ± 0.38	17.6 ± 0.60	17.8 ± 0.47	18.5 ± 0.38	17.4 ± 0.44	16.5 ± 0.42	18.3 ± 0.30	17.2 ± 0.37	17.1 ± 0.29
	After formalin fixation (24 h)	18.7 ± 0.42	18.8 ± 0.61	17.7 ± 0.42	17.6 ± 0.38	17.6 ± 0.43	17.2 ± 0.34	18.4 ± 0.23	17.1 ± 0.40	17.9 ± 0.25
40	Before excision	40	40	40	40	40	40	40	40	40
	Immediately after excision	36.2 ± 0.56	36.8 ± 0.85	33.5 ± 0.75	33.8 ± 0.61	36.1 ± 1.01	33.4 ± 0.44	38.5 ± 1.06	35.8 ± 0.70	33.5 ± 0.95
	After formalin fixation (24 h)	35.3 ± 0.64	36.4 ± 1.21	34.0 ± 0.83	32.9 ± 0.61	34.8 ± 1.09	32.7 ± 0.43	37.4 ± 1.16	35.0 ± 0.54	32.8 ± 0.95
60	Before excision	60	60	60	60	60	60	60	60	60
	Immediately after excision	54.4 ± 0.87	54.9 ± 1.46	49.4 ± 1.42	51.1 ± 0.63	49.7 ± 0.72	50.6 ± 0.52	50.0 ± 1.09	51.7 ± 0.81	49.8 ± 0.85
	After formalin fixation (24 h)	53.1 ± 0.84	52.4 ± 1.17	46.4 ± 1.42	49.7 ± 0.72	47.4 ± 0.69	48.0 ± 0.37	48.3 ± 0.97	50.2 ± 0.82	48.3 ± 0.61

Dogs were euthanized by means of IV injection of pentobarbital sodium for reasons unrelated to the study. Body regions were designated as follows: 1, head (rostral to caudal base of ear); 2, neck (caudal base of ear to thoracic inlet); 3, forelimb proximal to the elbow joint; 4, forelimb distal to the elbow joint; 5, 6, and 7, trunk divided into 3 dorsoventral regions (cranial, middle, and caudal) of equal width; 8, hind limb proximal to the stifle joint; and 9, hind limb distal to the stifle joint. The circular areas of skin that were excised were 2, 4, or 6 cm in diameter by use of templates; for regions 4 and 9, the circular regions were contoured to the curvature of the dorsal aspect of the limbs. The excisional depth included subcutaneous fat and 1 deep fascial plane for all samples. Fifty-four samples were collected from each dog (total number of samples, 216); there were 72 samples of each original diameter. After excision and after formalin fixation, diameter measurements were made in 4 orientations for each canine skin sample. The direction of tension lines was marked on each skin sample and diameter measurements were made as follows: 1, diameter parallel to skin tension lines; 2, diameter perpendicular to skin tension lines; 3, diameter in dorsoventral direction; and 4, diameter in craniocaudal (or rostrocaudal) direction. Because the diameter measurements did not differ, a mean value for each sized sample at each time point was calculated from which an overall mean was determined.

Table 2—Overall change in diameter of 2-, 4-, and 6-cm-diameter skin samples removed from both sides of 4 canine cadavers by body region.

Variable	Diameter change	Body region 1	2	3	4	5	6	7	8	9
Mean total shrinkage (difference between pre-excision and postfixation diameters)	Mean ± SE (mm)	4.3 ± 0.44	4.1 ± 0.65	7.3 ± 0.74	6.6 ± 0.47	7.2 ± 0.53	7.3 ± 0.44	6.4 ± 0.56	5.9 ± 0.46	7.1 ± 0.56
	%	10.75	10.25	18.25	16.5	18.0	18.25	16.0	14.75	17.75
Mean excision-related shrinkage (difference between pre-excision and postexcision diameters)	Mean ± SE (mm)	3.8 ± 0.39	3.6 ± 0.60	6.4 ± 0.65	5.6 ± 0.44	6.1 ± 0.47	6.5 ± 0.36	5.6 ± 0.56	5.1 ± 0.44	6.5 ± 0.53
	%	9.5	8.5	16.0	14.0	15.5	16.0	14.0	12.75	16.0
Mean formalin fixation-induced shrinkage (difference between postexcision and postfixation diameters)	Mean ± SE (mm)	0.5 ± 0.30	0.6 ± 0.50	0.9 ± 0.43	1.1 ± 0.31	1.1 ± 0.32	0.9 ± 0.27	0.8 ± 0.34	0.8 ± 0.37	0.6 ± 0.34
	%	1.25	1.75	2.25	2.75	2.5	2.25	2.00	2.0	1.75

See Table 1 for key.

between percentage of skin shrinkage and surgeon, body side, body region, or initial diameter of the skin sample assessed. For all regions, the diameters of all samples decreased immediately after excision. The mean percentage of excision-related skin shrinkage for each region was significantly greater than the percentage decrease in the samples' diameters attributed to formalin fixation (**Table 2**). No significant differences in percentage of skin shrinkage were found between skin samples that were excised and measured in the first hour after euthanasia versus those that were excised and measured in the fourth hour after euthanasia. The mean total skin shrinkage (difference between sample diameter before excision and that after excision followed by formalin fixation for 24 hours) ranged from 10.20% for samples from the neck region to 18.45% for samples from the middle trunk region; for all regions combined, mean overall total shrinkage was 15.6% (mean ± SE overall change in diameter from pre-excision value, 6.2 ± 0.7 mm; $P < 0.001$). For all body regions, the difference between sample diameter before excision and that after excision (mean change in diameter, 5.5 ± 0.7 mm) was significant ($P < 0.001$). Sample diameters immediately after excision and after formalin fixation for 24 hours did not differ significantly. The mean percentage of skin shrinkage that occurred in specimens immediately after excision was 13.67% (range, –25% to 33%), and the mean percentage of skin shrinkage that occurred in specimens after formalin fixation was 1.69% (range, –30% to 30%).

Discussion

The effect of postexcisional handling of histologic specimens has been described in the human and veterinary medicine literature. Shrinkage in specimens of tissues including cervical tissue, breast cancers, lungs, brain, and muscles was attributed to fixation in formaldehyde, dehydration due to processing or freezing, or inherent properties of the tissue itself.[7–13]

Shrinkage of cutaneous specimens presents a unique challenge because of several intrinsic properties of skin. The superficial layer of the dermis (stratum papillare) contains a fine network of reticular and elastic fibers that give skin an elastic nature. However, purely elastic forces are mitigated by an underlying dense collagen layer (stratum reticulare).[6] When a stress is applied to skin, the collagen fibers immediately begin to reorient and expand along the lines of tension in a phenomenon known as relaxation. Because of the dynamic nature of the collagen fibers, a constant stress applied to skin will lead to an initial strain, followed by a gradual increase in strain that has been called mechanical creep. Furthermore, the reorienting and lengthening of the collagen fibers is not completely reversible, such that under cyclic loading, skin will retain an increasing amount of tension after each load cycle applied to it, a phenomenon termed hysteresis.[14] In humans, it has been shown that cutaneous hysteresis curves are not altered by age, but can be altered by states that alter the collagen or elastin quantity or quality, including Ehlers-Danlos syndrome, scleroderma, and osteogenesis imperfecta.[15]

In vivo, skin is placed under tension. Lines of tension were first described in humans by Langer in 1862[16] and in dogs by Irwin in 1966.[5] Because of this constant tension, a degree of relaxation can be expected to occur following excision of an area of skin, but the degree of relaxation cannot be predicted by standard stress-strain curves because of hysteresis.[13] The effect of excision of cutaneous masses on the amount of skin shrinkage in humans has been studied.[2–4,17–20] These studies attempted to quantify both the effect of inherent skin properties as well as fixation methods on the shrinkage of excised specimens. Owing to the prospective, clinical nature of most of these studies, no controls were assessed with regard to the lesion types, margins removed, or orientation along tension lines in many cases. Overall shrinkage of skin area varied from 14%[3] to 31%.[1] When assessing possible contributions to this phenomenon, most researchers found no correlation between location of tumor site and skin shrinkage,[1,3,19] although Dauendorffer et al[2] determined that excised lesions of a limb had a greater degree of skin shrinkage than did excised lesions of the head or neck. Most tissue shrinkage occurred after excision but before formalin fixation (80.4% of total shrinkage in 1 human study)[19]; in most studies,[1–3,21] the contribution of formalin fixation to overall shrinkage was 0% to 29%. Gregory et al,[17] however, detected equal amounts of shrinkage in human skin samples during the postexcisional period as well as after formalin fixation. The reason postulated for the contribution of formalin fixation to skin shrinkage involves dehydration of the tissues during the fixation process.

To the authors' knowledge, only 1 study[22] of postexcisional skin shrinkage has been conducted in live anesthetized dogs. In that study,[22] 2 specimens each were obtained from the neck, thorax, and crus of each dog. Two lateral dimensions (length and width) were measured, and from those data, a surface area was calculated as well as specimen depth, which was not measured in the present study. Results of that study[22] indicated that the degree of shrinkage of the lateral margins of skin specimens obtained distal to the stifle joint was greater than that for specimens obtained from the neck or thorax, a finding that differs from that of the present study. Possible reasons for this discrepancy may include the fact that in the previous study,[22] skin samples were retrieved from anesthetized dogs instead of from recently euthanized dogs, were elliptical rather than round, and were obtained in smaller numbers (36 skin specimens in the previous study, compared with 216 samples in the present study). In the previous study,[22] no effect was noted regarding inclusion of fascia or muscle at the deep margin. No conclusions were made regarding during what step of processing most skin shrinkage occurred, and no attempt was made to assess the effect of tension lines on shrinkage of skin specimens.

In the present study, we assessed the effects of sample site, size of excised area, and tension lines on shrinkage of skin samples. No significant correlation was found between any of these variables and degree of skin shrinkage; this was in contrast to the study by Reimer et al,[22] in which a significant association between degree of skin sample shrinkage and site of origin was identified. The reason for this difference is unknown but may be attributable to a difference in the location of the excised skin, difference in shape of the

tissue excised, differences between tissues from living animals versus cadavers, or differences in breed characteristics.

Results of the present study indicated that most skin sample shrinkage occurred in the immediate postexcisional period, with formalin fixation contributing minimally to skin shrinkage. These findings echo the observations made previously in human medicine[1–3,21] and demonstrate that the intrinsic properties of skin are more important to skin shrinkage than is formalin fixation.

The present study had several limitations. A small number of dogs were used, and although significant differences were able to be identified, the uniformity of age and size of the dogs used may not accurately reflect true population differences. The degree of shrinkage in skin samples collected from toy breed dogs or dogs with different amounts of skin tension (Shar Pei vs Greyhound) may differ from the findings of the present study.

The dogs in the present study were all apparently free of gross cutaneous abnormalities and were all young healthy dogs unlikely to be affected with diseases that may affect skin tension, such as hyperadrenocorticism or hyperthyroidism. However, no histologic analysis was performed on the skin samples collected in the study, and, as such, microscopic pathological changes may have been present that affected the results. The duration of formalin immersion in the present study (24 hours) reflects the amount of time that specimens remain in formalin in our clinic. We cannot rule out the possibility that decreasing or increasing the duration of fixation may change the amount of skin sample shrinkage that occurs. Furthermore, the method of measurement of sample diameters (ie, by means of a straight ruler) could be subject to user error. Variation was minimized by having a single investigator measure all specimens at all time points. The use of more advanced measuring techniques (digital calipers or imaging software) may help decrease user error further.

In the present study, a circular excision was made in the skin. Conventional skin incisions are performed in an ellipse in an attempt to improve cosmesis of wound closure by minimizing redundant skin at the edge of the incision (so-called dog ear formation). For a classically taught elliptical incision, the length is 3 to 4 times the width and forms angles of 30° along its long axis.[23] However, clinical specimens rarely conform to those exact measurements. In the previous study[22] in veterinary patients, the length of the specimen was twice the width, and the specimens were true ellipses with no angles along the long axis. To attempt to determine what the underlying determinants of shrinkage in excised skin samples were, we elected to remove circular samples (to assess the effect of skin tension lines) and to vary specimens by size (to assess the effect of amount of tissue). It is possible, however, that there is a more complex interaction between specimen shape and skin shrinkage, which should be taken into account when different shapes are used for skin sample excision.

Canine cadavers were used in the present study. Although attempts were made to excise the skin samples within a short time after euthanasia, it is possible that changes in skin elasticity, innervation, or myocutaneous cell contractility occur rapidly after death and may alter the extent of skin sample shrinkage. To the authors' knowledge, no study has been conducted to compare the changes in skin contractility from skin samples obtained from living and nonliving specimens. In the present study, no changes in shrinkage were detected between the samples collected in the first hour after euthanasia and the samples collected in the fourth hour after euthanasia, indicating that within the 4-hour period of the sample collection, no significant changes in contractility of the skin occurred. Although comparison of these data to the results of the study by Reimer et al[22] are difficult because we reported changes in skin sample diameter whereas Reimer et al[22] reported changes in surface area, the overall change in diameter (15.8%) was within the range (14% to 31%) previously identified in human studies.[1,3] Nevertheless, any conclusions from the present study must be tempered against the fact that cadaver tissues were used.

The results of the present study have suggested that cutaneous samples undergo characteristic shrinkage after excision and that formalin fixation contributes minimally to overall skin sample shrinkage. Furthermore, no correlation between the skin samples' anatomic site of origin, tension lines, or overall diameter and degree of skin sample shrinkage was observed. The information obtained in this study is only the first step in understanding the effect of pathological skin conditions on skin sample shrinkage. It appears that different cutaneous tumor types cause variable alterations in skin morphology in humans[1] and likely have different effects on skin sample shrinkage, and these observations may also be true in dogs. The amount of shrinkage that occurs within a sample from an area affected by a pathological process may differ from the amount of shrinkage that occurs within the healthy skin surrounding the sample. Further studies are necessary to elucidate these differences.

When surgical margins are determined on the basis of recommendations from histologic studies, it is important that tissue shrinkage is considered. Further studies should be conducted to determine the effect of neoplasia on the degree of skin sample shrinkage before histologic determination of tissue margins can be used to accurately determine surgical margins.

a. SAS, version 9.3, SAS Institute Inc, Cary, NC.

References

1. Hudson-Peacock MJ, Matthews JS, Lawrence CM. Relation between size of skin excision, wound and specimen. *J Am Acad Dermatol* 1995;32:1010–1015.
2. Dauendorffer JN, Bastuj-Garin S, Guero S, et al. Shrinkage of skin excision specimens. *Br J Dermatol* 2009;160:810–814.
3. Blasdale C, Charlton FG, Weatherhead SC, et al. Effect of tissue shrinkage on histological tumour-free margin after excision of basal cell carcinoma. *Br J Dermatol* 2010;162:607–610.
4. Kerns MJ, Darst MA, Olsen TG, et al. Shrinkage of cutaneous specimen: formalin or other factors involved? *J Cutan Pathol* 2008;35:1093–1096.
5. Irwin DH. Tension lines in the skin of the dog. *J Small Anim Pract* 1966;7:593–598.
6. Pavletic MM. Tension lines. In: Paveletic MM, ed. *Atlas of small animal wound management and reconstructive surgery*. 3rd ed. Ames, Iowa: Wiley-Blackwell, 2010;248–249.

7. Baraibar MA, Schoning P. Effects of freezing and frozen storage on histological characteristics of canine tissues. *J Forensic Sci* 1985;30:439–447.
8. Boonstra H, Oosterhuis JW, Oosterhuis AM, et al. Cervical tissue shrinkage by formaldehyde fixation, paraffin wax embedding, section cutting and mounting. *Virchows Arch A Pathol Anat Histopathol* 1983;402:195–201.
9. Ladekarl M. The influence of tissue processing on quantitative histopathology in breast cancer. *J Microsc* 1994;174:93–100.
10. Lum H, Mitzner W. Effects of 10% formalin fixation on fixed lung volume and lung tissue shrinkage. A comparison of eleven laboratory species. *Am Rev Respir Dis* 1985;132:1078–1083.
11. Mouritzen Dam A. Shrinkage of the brain during histological procedures with fixation in formaldehyde solutions of different concentrations. *J Hirnforsch* 1979;20:115–119.
12. Schned AR, Wheeler KJ, Hodorowski CA, et al. Tissue shrinkage correction factor in the calculation of prostate cancer volume. *Am J Surg Pathol* 1996;20:1501–1506.
13. Stickland NC. A detailed analysis of the effects of various fixatives on animal tissue with particular reference to muscle tissue. *Stain Technol* 1975;50:255–264.
14. Edwards C, Marks R. Evaluation of biomechanical properties of human skin. *Clin Dermatol* 1995;13:375–380.
15. Pedersen L, Hansen B, Jemec GE. Mechanical properties of the skin: a comparison between two suction cup methods. *Skin Res Technol* 2003;9:111–115.
16. Langer K. On the anatomy and physiology of the skin, III (1862). Translated by Gibson T. *Br J Plast Surg* 1978;31:185–199.
17. Gregory N, Mulvaney M, Pattinson T, et al. Shrinkage of skin excision specimens and downcoding. *Arch Dermatol* 2003;139:542–543.
18. Silverman MK, Golomb FM, Kopf AW, et al. Verification of a formula for determination of pre-excision surgical margins from fixed-tissue melanoma specimens. *J Am Acad Dermatol* 1992;27:214–219.
19. Davis M, Monk B. Surgical vs. pathological excision margins—an excision too far. *Eur J Plast Surg* 2010;33:117–120.
20. Salmhofer W, Rieger E, Soyer HP, et al. Influence of skin tension and formalin fixation on sonographic measurement of tumor thickness. *J Am Acad Dermatol* 1996;34:34–39.
21. Golomb FM, Doyle JP, Grin GM, et al. Determination of preexcision surgical margins of melanomas from fixed tissue specimens. *Plast Reconstr Surg* 1991;88:804–809.
22. Reimer SB, Seguin B, DeCock HE, et al. Evaluation of the effect of routine histologic processing on the size of skin samples obtained from dogs. *Am J Vet Res* 2005;66:500–505.
23. Weisberg NK, Nehal KS, Zide BM. Dog-ears: a review. *Dermatol Surg* 2000;26:363–370.