Histological Changes in Bovine Skin Exposed to Natural Environmental Conditions

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Background: Animal mutilations have been a recurring enigma since the first highly publicized cases in the late 1960s (1). In the intervening thirty years in spite of hundreds, possibly thousands of reports of these cases, no perpetrator has been caught or charged. Thus, animal mutilations remain a source of continuing financial hardship to the ranching community nationwide, but particularly in the West and Southwest of the United States and in Western Canada.

There has been substantial controversy in the animal mutilation field over the interpretation of the wounds that have been observed on cattle that have died from unknown causes[1]. Many veterinarians have concluded that animal mutilations are in fact no more than cattle that have died of natural causes or predation and that have subsequently been attacked by a variety of scavengers (coyotes, foxes, wild dogs, etc.). Further, because of lying exposed to the elements sometimes for several days after death, the edges of the wounds are even more difficult to interpret because of ultra-violet damage, oxidation, decomposition and a variety of other complicating factors. Yet ranchers and many investigators of animal mutilations continue to insist that the sharp "surgical" cuts displayed on hundreds of animals are not consistent with predation or scavenger activity.

The National Institute for Discovery Science began researching the animal mutilation field in 1997. It quickly became apparent that there is a lack of objective data in the field with many different investigations being conducted without standardized protocols or standard criteria for defining animal mutilations. In an effort to address this problem, the following paper attempts to publish a photographic database of the histological changes that accompany cattle hide that is cut with a sharp instrument or torn by pliers (to simulate teeth marks) and left for several days exposed to the elements. The aim is to provide, and to continually upgrade, a reference database for those researching histological evidence in animal mutilation cases.

Purpose: in order to have a set of reference images on gradual effects of outside environmental conditions on the bovine skin, which could help in evaluating morphologic changes in spontaneous animal mutilation cases, we set up an experiment that would enable comparing macroscopic and microscopic changes in tissues. In addition, we needed to establish the types of physical changes induced by cutting with sharp instruments and tearing. This kind of research was not done before, to our knowledge.

Material and Method

- One ear and two pieces of 30/10-cm bovine skin, along with the about 4 cm thick subjacent tissues (to avoid a too rapid dehydration), were collected from two cows that had been recently slaughtered in the usual manner
- One long edge has been cut by a sharp instrument (knife)
- The other edge was cut with a regular plier intending to imitate the tooth cut

[1] See *Animal Mutilations: What We Don't Know* and *Animal Mutilations: What We Know* by G. Onet, www.accessnv.com/nids

- The skin was nailed down on a piece of wood (see Fig. 1) and exposed to outside conditions for the whole period of time of 144 hours, day (not under direct sun shine) and night. The weather was throughout the 7-day period clear, with no precipitation.
- Pieces of 0.6 cm thick were cut from the skin and put in buffered 10% formaldehyde solution at the following intervals of time:

1. fresh
2. 2 hours
3. 4 hours
4. 8 hours
5. 20 hours
6. 24 hours (1 day)
7. 48 hours (2 days)
8. 72 hours (3 days)
9. 96 hours (4 days)
10. 120 hours (5 days)
11. 144 hours (6 days)

All these tissue samples were submitted for histology preparation at the Purdue University Animal Disease Diagnostic Laboratory and Utah State University Veterinary Diagnostic Laboratory. Sections were obtained after paraffin embedding. Bichromic (hematoxylin-eosin) and trichromic staining (hematoxilin-eosin-brilliant green) were used for differentiation of various tissue components (2). The microscopic examination and interpretation was done by NIDS' veterinary pathologist, by using a Nikon microscope, with an attached Olympus Dp-10 digital camera for microphotography.

The whole experiment was meant to help properly evaluate tissue samples from spontaneous animal mutilation cases in establishing the nature of lesions found.

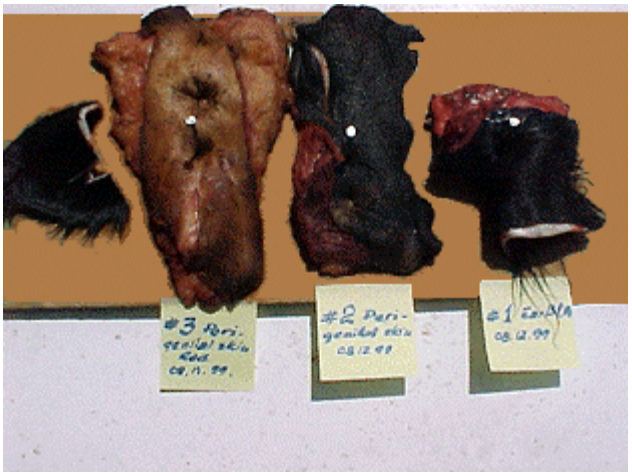

Fig. 1. Ear and external female genital organs collected from slaughter house and exposed to outside environmental conditions. At left — the tip of the ear, followed by two anuses and vulvas, with the subjacent tissues and the bottom of the ear.

Figure 2 shows the morphology of the fresh bovine skin at the time of collection, prior to being exposed to outside conditions.

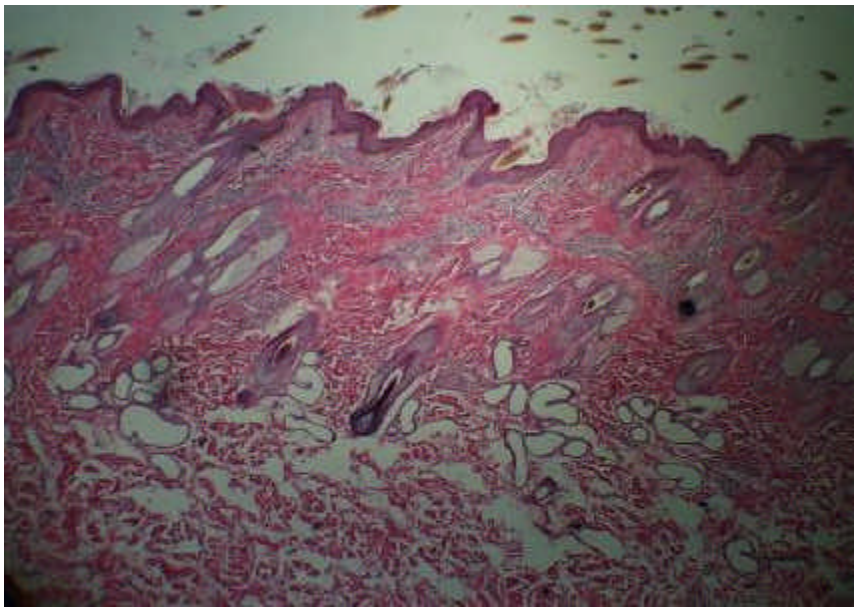

Fig. 2. Histological section through the fresh skin tissue (x40). Bichromic staining.

The normal structure of the skin layers consists of epidermis, dermis and hypodermis. The epidermis has a basal layer, comprising of distinctive cuboidal cells, with hyperchromatic nuclei. Above, the stratum spinosum is composed of polyhedral cells, which assume a more flattened morphology as they progress to the surface. Progressing to the surface is the stratum granulosum, a granular layer. The nuclei appear pale or shrunken, and the cytoplasm is filled with dark basophilic granules. The next layer — stratum corneum — consists predominantly of the skeletons of dead keratinocytes, that is, keratin filaments. The epidermis also is populated with scattered cells of multiple functions, like melanocytes (dendritic cells that make melanin, the pigment of the skin) and Merkel cells, in intimate association with myelinated nerve fibers.

Beneath the epidermis, separated by the basement membrane, lies the dermis that provides the tensile strength of the skin due to the abundance of the collagen. Elastic fibers and extracellular matrix consisting of fibronectin, glycoso-aminoglycans and filamentous glycoproteins are found in the interstices of collagen fibers and provides resilience and substrates important to cell migration and attachment. The dermis harbors between rete ridges of the dermal papillae hair follicles, sebaceous and sweat glands, vessels (arterioles and venules, lymph vessels) and nerves. It is also endowed with a number of different cell types, including fibroblasts, macrophages, endothelial and mast cells, lymphocytes and other migratory cells.

In the hypodermis or subcutis the predominant component is the adipous tissue, accompanied by a smaller number of capillaries (3,4).

Figure 3 depicts, at a higher magnification (x400), a skin section with the basal membrane, stratum spinosum, stratum granulosum in which a hair follicle is shown at the right.

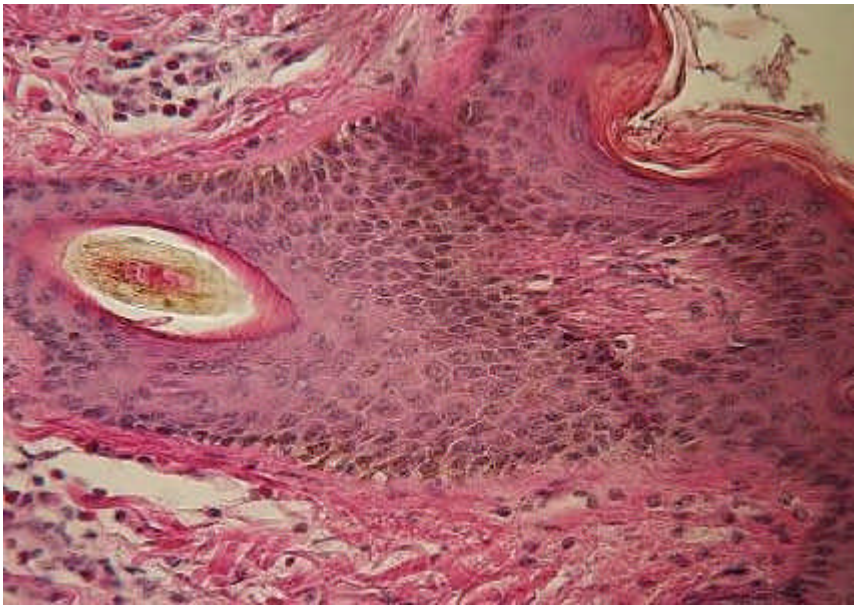

Fig. 3. Fresh skin section (x400). Stratum spinosum and granulosum harboring a hair follicle (at right).

In the case of ear samples, it needs to be mentioned that they have two skin layers, one outside and the other inside of the ear pavilion. The outside skin has the same basic structures as skin from other areas, but the adipous layer of the hypodermis is less represented. The inner skin has more sebaceous glands and is lacking the hair follicles. Between the two skin cushions, there is a cartilage layer, with cartilage cells (chondrocytes), arranged in an orderly manner and with a regular polarity in a cartilaginous matrix (see Figure 4).

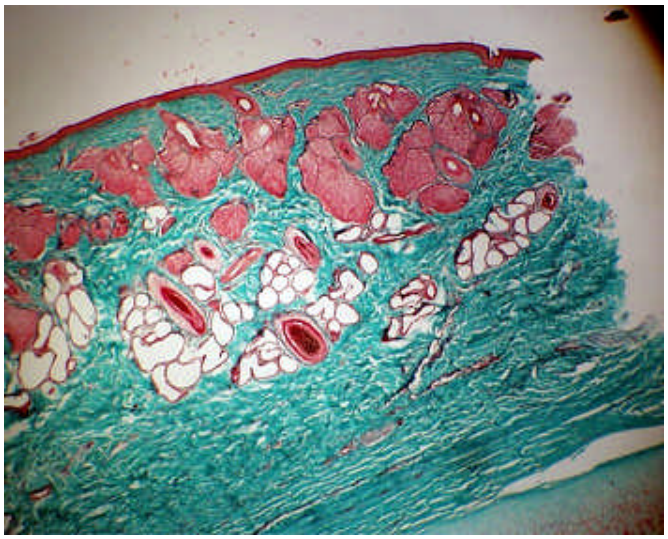

Fig. 4. Fresh ear section (x40). In the lower right corner — a portion of the ear cartilage.

Results

The first major change in the tissues was the progressive dehydration. Although a rather thick (two inches) layer of subjacent fatty and connective tissues were taken along with the superficial skin, the loss of water seemed to have been quicker than in natural cases, when the skin remains attached to the carcass and the moisture from inside hinders, at least partly, a rapid drying of superficial layers. Dehydration is expressed by loss of the characteristic structure of exposed tissues with an evident eosinophylic staining (red). This gradual eosinophily of the components of different skin layers decreases with the deepness in the lower layers. Dehydration makes tissues progressively brittle and, during the histological preparation, parts of those layers tend to break and separate from the rest of the section, which made the interpretation more difficult. However, the most significant changes were registered and allowed to describe and illustrate the process.

Compared to these normal structures, the skin samples collected showed significant changes, in direct correlation with the time elapsed.

Ear samples

After two hours of exposure the only moderate change was induced by dehydration of the superficial epidermal layer, translated in an increased eosinophily and a slight distortion of the exposed fiber component (Fig. 5).

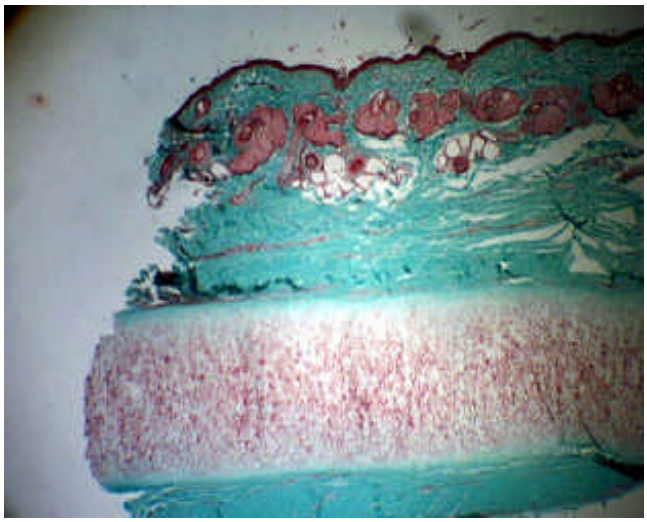

Fig. 5. Ear after 2 hours (x40)

After 4 hours, the eosinophilic tendency expands deeper in the skin layers up to the cartilage edges, while spaces between fibers become more evident (Fig. 6).

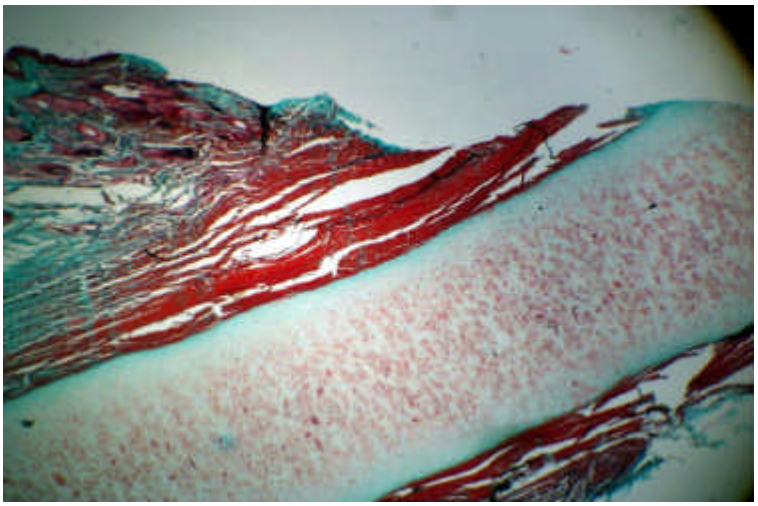

Fig. 6. Ear after 4 hours (x40)

The process of dehydration, with increased eosinophily of the exposed tissues, will accentuate gradually and is accompanied by a less than expected shrinkage of the ear cartilage (Figs. 7, 8, 9 and 10).

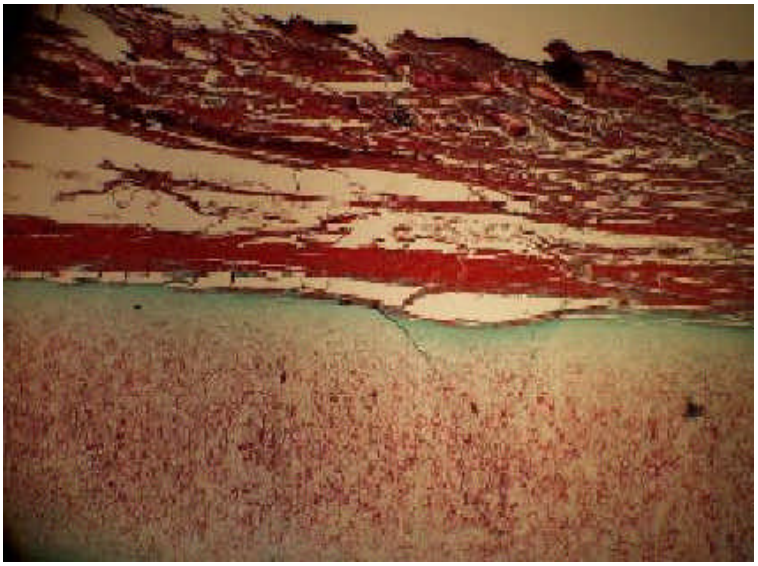

Fig. 7. Ear after 24 hours (x40)

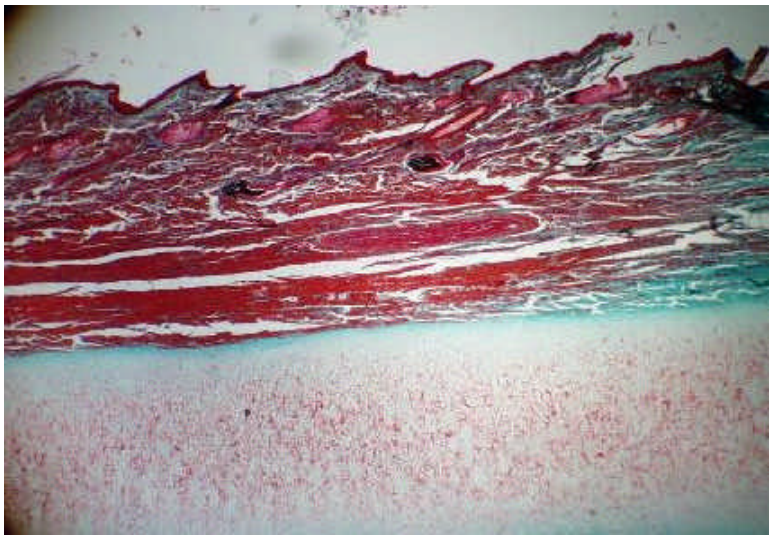

Fig. 8. Ear after 48 hours (x40)

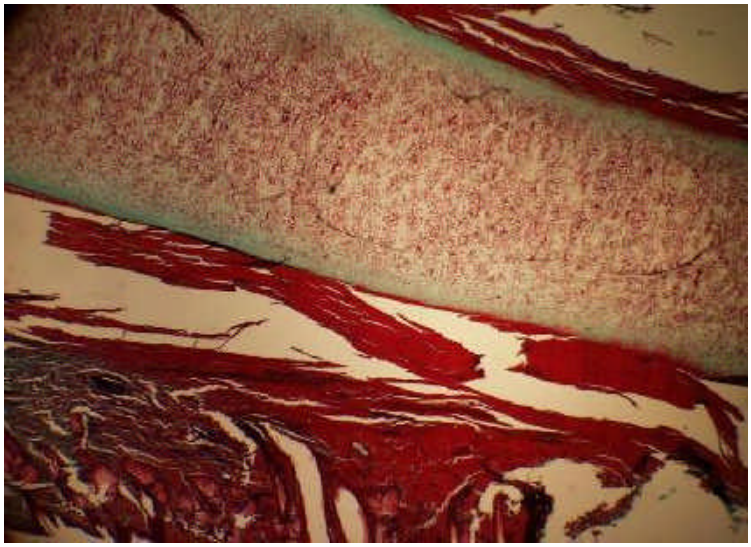

Fig. 9. Ear after 96 hours (x40)

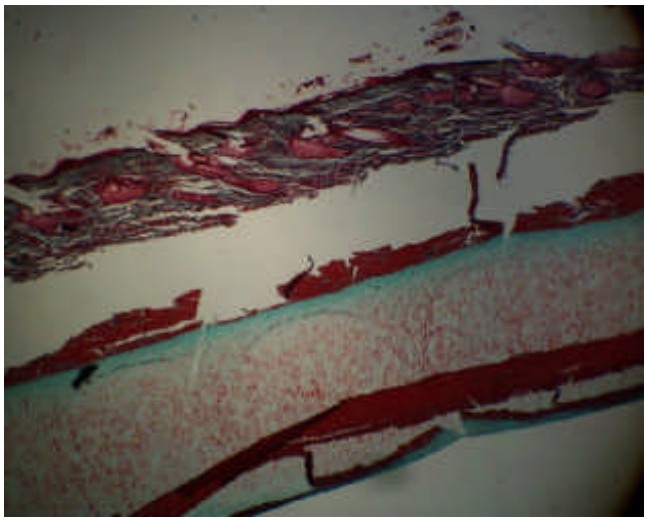

Fig. 10. Ear after 144 hours (x40)

At a higher magnification different stages of nucleic alterations, from picnosis to cariolysis in chondrocytes can be seen. The cell delimitations become more condensed (Fig. 11)

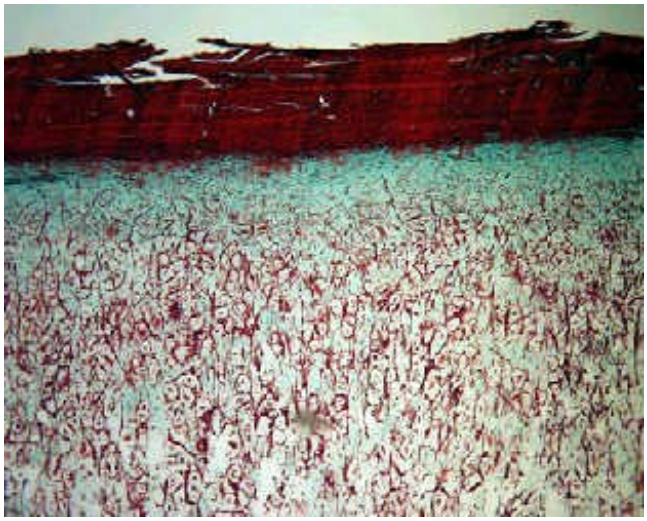

Fig. 11. Ear after 144 hours (x200)

Skin samples

After two hours, only a slight eosinophily in the superficial epithelium can be seen (Fig. 12) while the rest of the subjacent layer keeps a relatively normal stain affinity. The dehydrated parts tend to fragment.

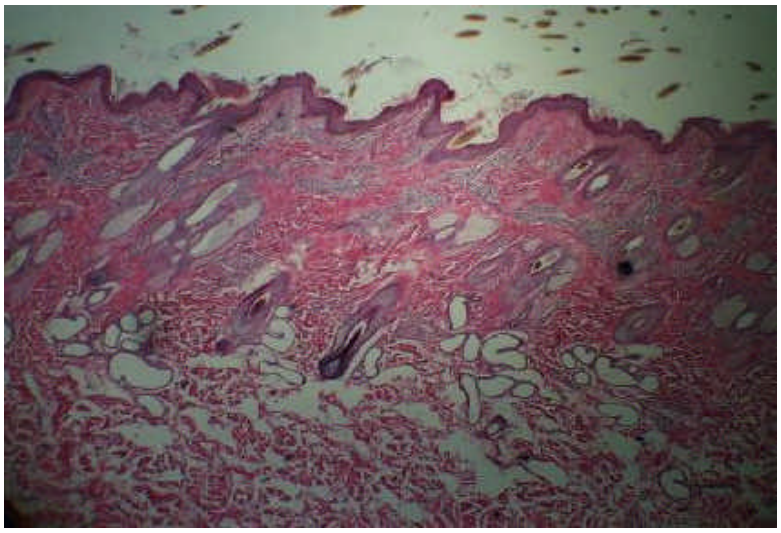

Fig. 12. Skin after 2 hours (x40

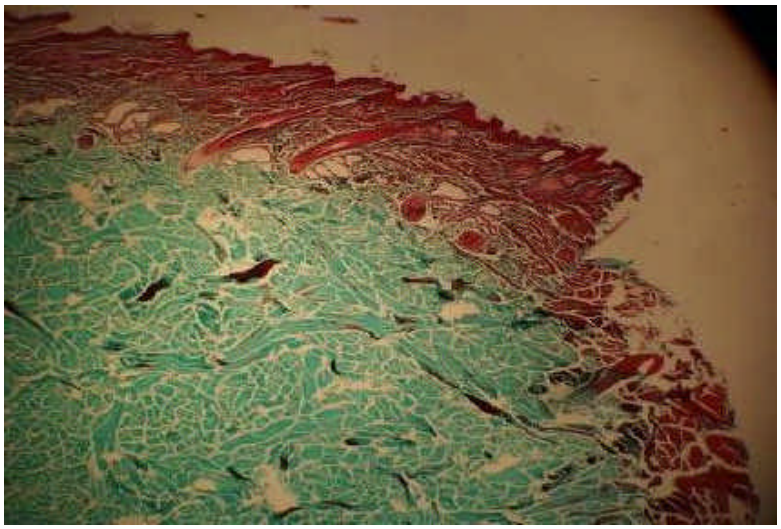

Fig. 13. Skin after 4 hours (x40)

After four hours, the dehydration progresses deeper and, consequently, the eosinophily and the fragmentation increases (Fig. 13). At higher magnification, nuclear and cytoplasmic changes are noticed in the hair follicle and gland cells.

The eosinophily and shrinkage encompasses larger areas and the cell damages are more and more evident with the time elapsed (Figs. 14 and 15).

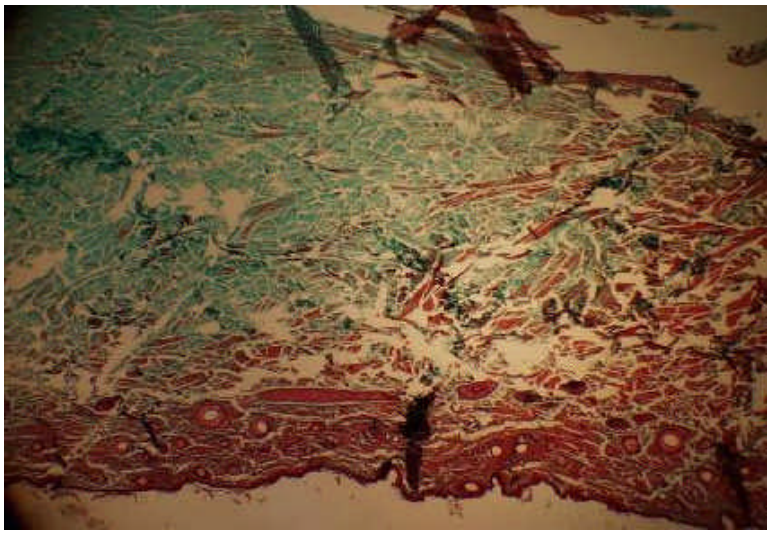

Fig. 14. Skin after 20 hours (x40)

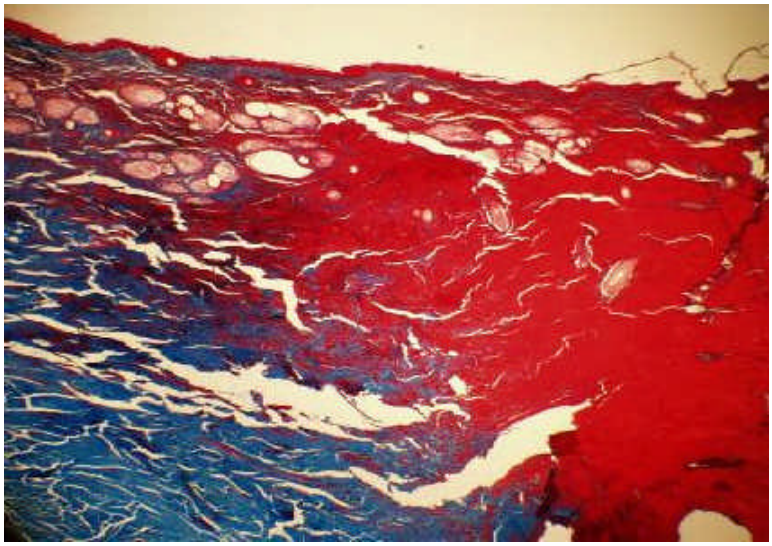

Fig. 15. Skin after 144 hours (x40)

Collagen become fractured and separated from the adjacent dermis with clefts (drying artifact). This histological aspect might of particular interest, in light of some data in published literature referring to so called “cooked collagen”. Due to physical and biochemical changes induced by dehydration, changes suffered by collagen could be interpreted microscopically as the result of heat application. The lack of similar alterations of neighboring tissues and the morphological aspect of the structures underneath the superficial-exposed tissues raises the question of possible misinterpretation.

After 20 and 24 hours the hypereosinophily is more evident, with fracture of collagen bundles. At a higher magnification, the hair follicle structures become less well defined, while the surrounding tissues look like a compact eosinophilic mass, with cleavages easy to detect (Fig. 16).

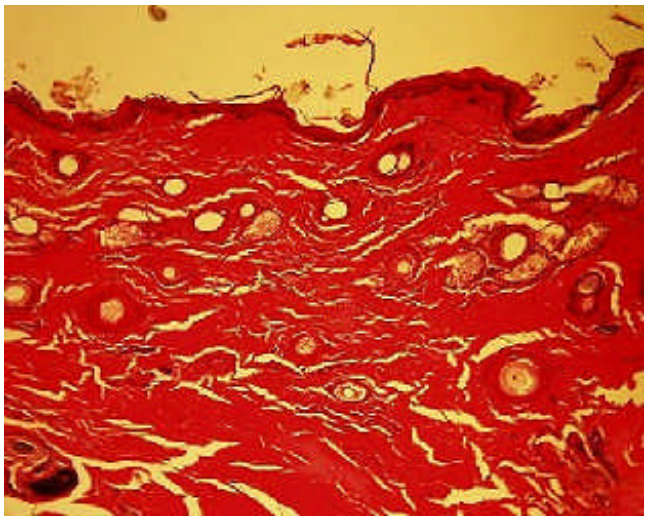

Fig. 16. Skin after 144 hours (x200)

After 144 hours the epidermis and dermis are transformed in a completely red and fragmented mass, in which detailed cell structures a hard to define (Fig. 17). Sebaceous gland cells suffer visible nucleic alterations, such as karyopycnosis, karyorrhexis and karyolysis, depending on the length of time elapsed (Fig. 18).

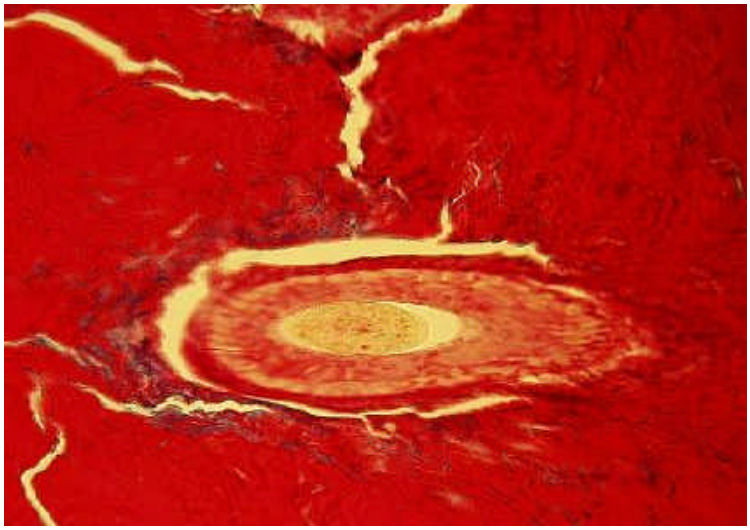

Fig. 17. Skin after 144 hours (x400)

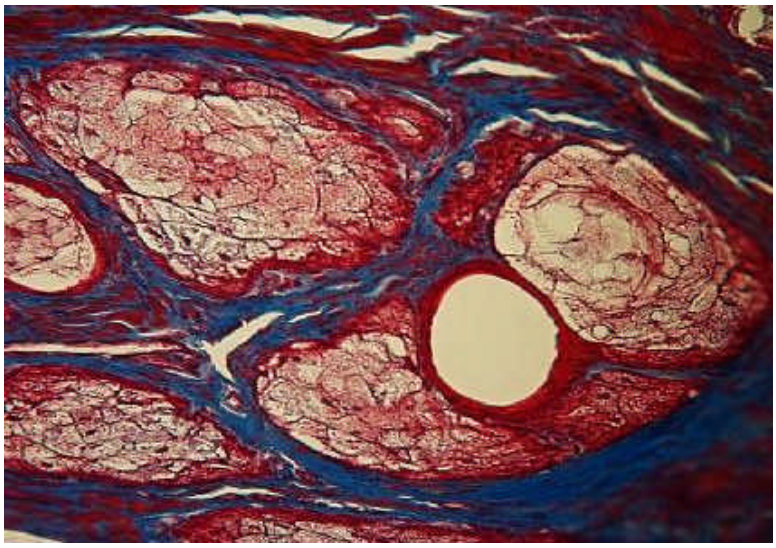

Fig. 18. Skin after 144 hours (x400). Sebaceous gland cells with nucleus alterations (karyopycnosis, karyorrhexis and karyolysis (x400)

Another objective of this experiment was to establish morphological differences between sharp cutting and teeth tearing which would allow histologically to differentiate a sharp cut from a more blunt separation of tissues. Trying to imitate the tearing by teeth as it happens in spontaneous cases, when predators work on carcasses, we used an ordinary plier with which we cut the skin and the ear. Figs. 19 and 20 show suggestive differences between the ear cut with a scalpel and with a plier. While the sharply cut tissues neatly preserve the structure of different layers and show a rather regular edge, tissues cut by plier show the results of the compression, with deformation of the epithelium and fragmentation of the cartilage.

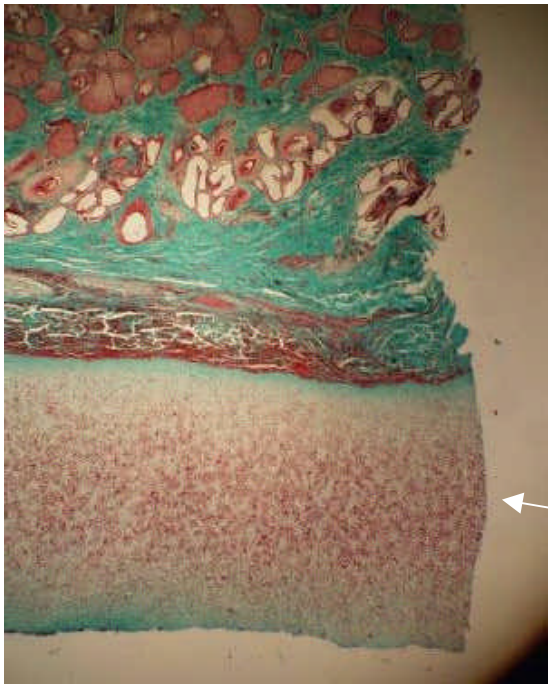

Fig. 19. Ear — sharp cut. Noticeable regular edge on the right, especially of the cartilage (x40)

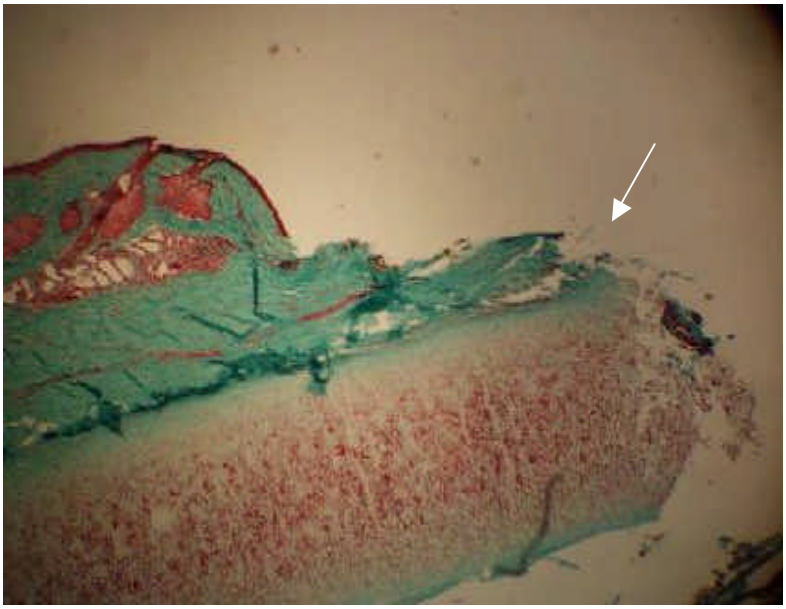

Fig. 20. Ear — plier cut. Irregular edges of the skin and cartilage, with evident tissue disruption (x40)

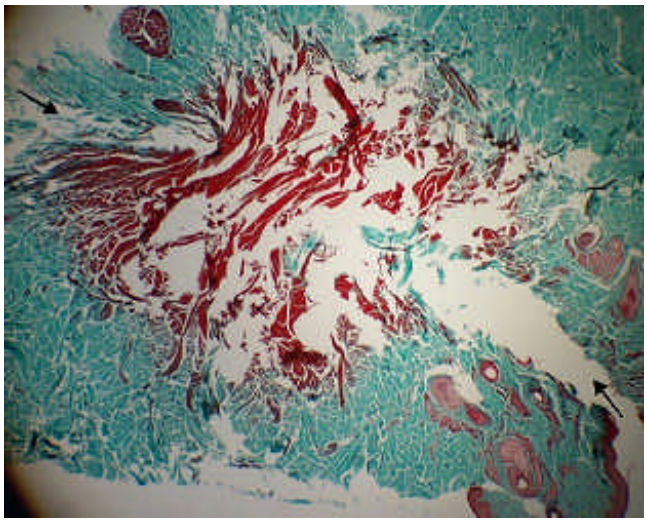

Fig. 21. Skin — plier cut. Arrows indicate the penetration path, with collagen fiber dislocation and eosinophilic staining (x40)

In the case of the skin, tissues cut by plier were not completely separated. Arrows show the trajectory of the two plier teeth advancing deep into the skin, deforming and separating the collagen fibers somehow irregularly (Figs. 21 and 22). Compared to these images the sharply cut skin shows a much more regular edge, with no laceration and tissue destruction (Fig. 23).

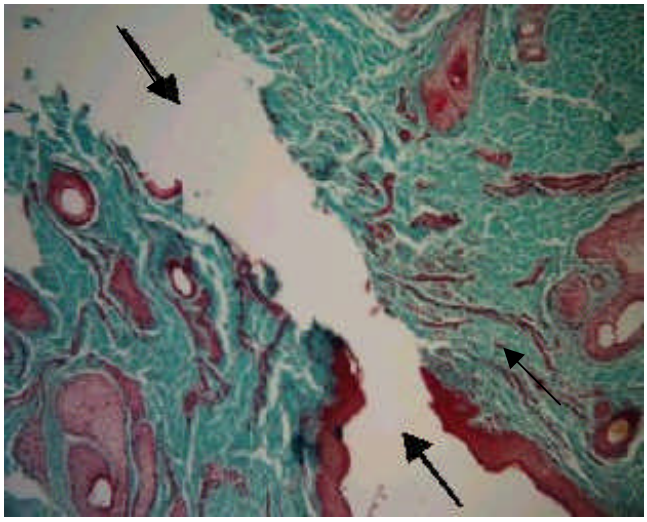

Fig. 22. Skin — plier cut (x200)

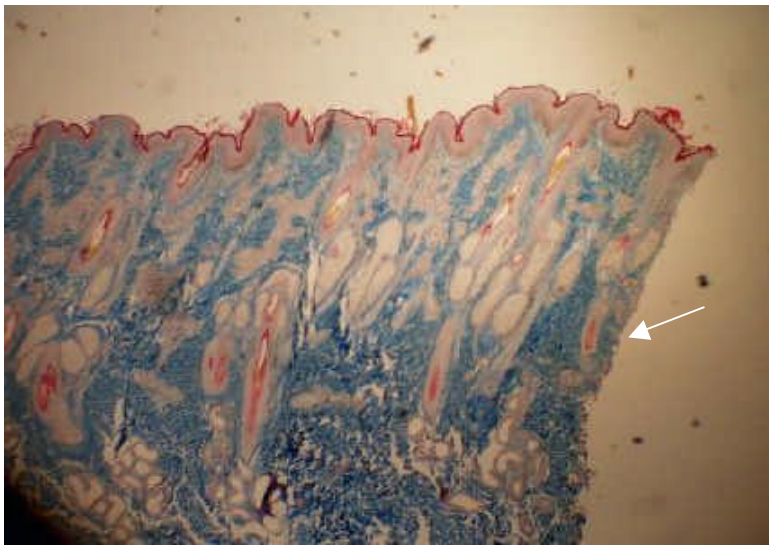

Fig. 23. Skin — sharp instrument cut. Noticeable regular edge on the right (x40)

Discussion

The focus of this paper is twofold: to provide information on histological differences between cuts from a sharp instrument and those caused by tearing on cow hide, and secondly to show the often dramatic changes that occur to cattle hide over time during exposure to the elements. The reason for scrutinizing the differences between sharp instruments and torn cattle hide at the level of histology is that

there is an ongoing, thirty years controversy about the origins of the cuts found in mutilated animals. The ranching community and animal mutilation investigators have interpreted some of the cuts as originating from sharp instruments (knives, scalpels, lasers etc.), whereas the veterinary community and some others have ascribed the cuts to predator or scavenger attacking the carcass. This impasse can only be resolved with the availability of more objective data. The data in this paper provide a beginning towards this goal.

Figures 5 and 6 (ear sample) and Figures 12 and 13 (skin sample) show that even within four hours of exposure, noticeable dehydration manifesting as increased eosinophilia is evident in the region nearest where the hide is cut. The ear was cut in these experiments because in animal mutilation cases, the ear is one of the most common body parts missing. We recognize that the rate of this dehydration will be substantially influenced by both humidity and altitude in different areas. The experiment was done in an area of moderate humidity (27%) with daily temperature highs and lows being 78^{o}F and 29^{o}F, respectively.

One consequence of dehydration and oxidation is that the collagen bundles become fractured and separate from the dermis within 24 hours (see Figure 14). This process accelerates progressively over the following days. Therefore, only in very fresh tissue samples is it possible to interpret collagen changes.

In the second part of the experiment, we attempted to assess the differences between the use of sharp instruments to cut the hide, versus the use of a set of pliers to mimic the tearing of the hide by a scavenger or predator. The comparison was conducted for both the ear and the hide. Figures 19 and 20 show that the ear cartilage indicates a strong difference between the use of a sharp instrument and the pliers. The smooth cut is markedly apparent (Fig. 19) in the cartilage of the ear and is very different from that seen with the tearing action of pliers (Fig. 20). Thus, in the cases where the ear is missing in an animal mutilation case, samples that include ear cartilage should always be removed for analysis. Comparing Figures 21, 22 and 23 show that the differences between a sharp cut (Fig. 23) and pliers (Figs. 21 and 22) are also apparent with the pliers leaving a telltale track through the tissue.

It is the position of NIDS that careful and detailed histological analysis, as well as low powered microscopy, is required to definitively delineate the differences between a sharp cut and tearing of hide in cases of suspected animal mutilation.

Finally, the sometimes dramatic changes that are seen even within 24 hours of exposure to weather, underlines the need for investigators of animal mutilation to sample the tissues as quickly as possible after death, and for the ranching community to notify NIDS immediately after their animal is found dead of unknown causes. As can be seen, even a delay of 24 hours in making a telephone call can introduce extra complications into the interpretation of these already complex cases.

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