

Microscopic Changes In Bovine Tissues Exposed To Microwave Radiation, High Heat And Cauterization

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Introduction

The animal mutilation enigma has been ongoing for over thirty years. During this history, there have been persistent anecdotal reports of extremely sharp cuts on cattle from which body parts and organs have been removed (see, for example <http://www.nidsci.org/articles/articles2.html> and references therein). There have also been reports that “laser surgery” has been conducted on these animals by unknown perpetrators. Further, a small number of pathology reports include descriptions of high heat, cooked collagen and cautery as possible methodologies for producing some of the wounds in animal mutilations (1, 4). These reports are controversial and are regarded with skepticism by the majority of veterinarians who have encountered animal mutilations. Biophysical research on grass and vegetation samples found near and around the mutilated carcasses have suggested that the grass, and by implication the animals, have been subjected to some form of radiation during the mutilation. The favored candidate historically has been microwave radiation for accomplishing these changes in the grass and vegetation (3). Thus the majority of the evidence underpinning the proposal that microwave radiation is involved in animal mutilations has come from analysis of the vegetation surrounding the dead animal, rather from the tissues of the animal itself. However, the few studies by pathologists of the tissue itself have also tended to invoke microwave radiation as being involved in the mutilation process.

The purpose of the experiments reported in this paper is (a) to simulate microwave radiation, high heat and cautery on cattle skin and tissue and (b) to conduct detailed histology and microscopic photography on the tissues that have been treated in this way for different time periods. Thus, a database of the incremental changes that occur at the cellular level in tissues subjected to microwave radiation, high heat and cautery can be used as a comparison in future studies of tissues and skin from mutilated animals. These histological changes can be used to compare reports already in the literature regarding “laser surgery”, cautery and the use of heat in animal mutilations. They also will help future investigations by having proper reference data and will enable morphologic evaluation of changes in tissues collected from spontaneous mutilation cases.

Materials and methods

- A set of different bovine tissue samples were collected from a slaughterhouse (skin from the periorbital and genital areas and liver) and transported on ice to NIDS facility in Utah for further processing.

- Three sets of pieces of 20/10/6 mm were cut in order to be exposed to microwave radiation at a known energy emitted by a 850 Watt microwave oven. The adjusted microwave power on the tissue would be 1.4 Watts (J/sec); the sample volume was calculated as 1.4×10^{-6}

cubic meters. Therefore, a 5 second pulse would equal a microwave intensity of $6.43 \times 10^6 \text{ J/m}^3$. These figures are approximate, since the manufacturer did not provide the exact figures on the grounds that the information was proprietary. The exposure of the samples to microwave radiation was for variable intervals of time, as follows:

- 10 seconds
- 30 seconds
- 1 minute
- 2 minutes
- 5 minutes
- Immediately after being exposed, the tissue samples were placed in 10% buffered formaldehyde solution that had been precooled to 4°C for fixation and kept at 4°C . This was to stop the heating effects on tissues as rapidly and uniformly as possible.
- A second set of pieces of similar dimensions were exposed to the same amounts of radiation, but the half of each piece were covered with aluminum foil to prevent direct radiation effects. Covered surfaces were marked with a black ink marker for later identification during the histologic processing.
- A third set of samples were exposed to regular 400° temperature kitchen oven for different intervals of time, such as:
 - 1minute
 - 2 minutes
 - 4 minutes
 - 5 minute
 - 8 minutes
- A set of samples was cut by using a regular electric solder.
- Fixed tissues were trimmed after 24 hours of fixation
- Tissue samples were submitted for histologic preparation consisting of paraffin embedding and Masson's trichromic staining, which selectively stains cell components and fibers (2, 5). We preferred the use of this staining procedure because we wanted to have the fibers and collagen details better revealed.

The evaluation of changes in the examined tissues was done by using a Nikon Apophot universal research microscope at different enlargement degrees and the photographs were taken with an Olympus DP-10 digital camera.

Results

A. Changes induced by microwave radiation

1. Skin samples

Skin tissues exposed to microwave radiation suffer severe structural alterations, which are easy to identify microscopically. Changes in the skin samples from genital and periorbital areas were generally similar, because of their histologic similarity.

After 5 seconds the exposed half of the section displays a visible loss of blue staining of collagen, due to its denaturation, with replacement by pink-red staining, which indicates, along with biochemical changes, a shifting in stainability. The epidermis is stained lighter in red color than normal. The dermis and subdermis collagen is also more lightly stained than normal. Deep in the dermal tissue there is a zone expressing severe degenerative change in the collagen. In the genital area skin, a severe circumscribed destruction of the normal structure can be detected deep in the dermal tissue (see arrow in Photo 1). Around an empty area, reddish stained collagen constitutes an easy to distinguish surrounding. The epithelium does not seem to suffer important changes (Photos 1 and 2).

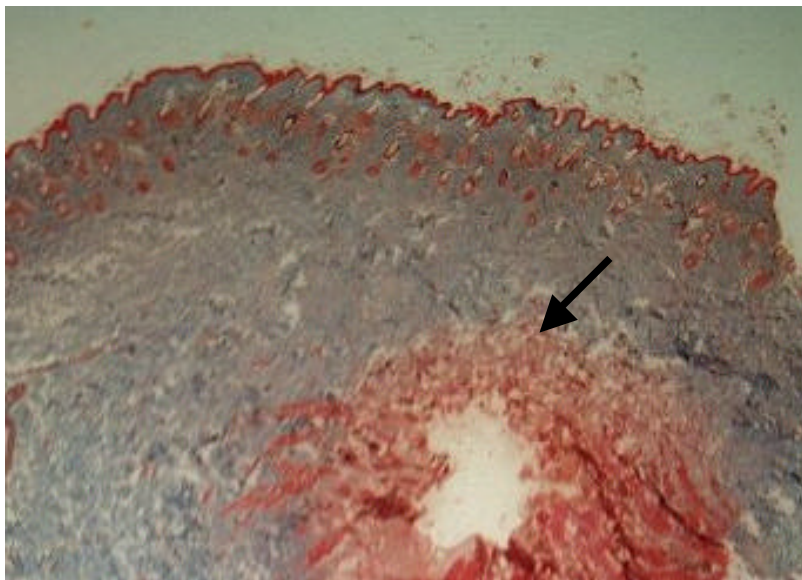


Photo 1. Genital area skin, microwave exposure 5 seconds, x 15

After 10 seconds, irregular empty spaces can be seen under the epithelium, while the collagen in the exposed area loses the blue stain (Photo 2). The deeper subcutis does

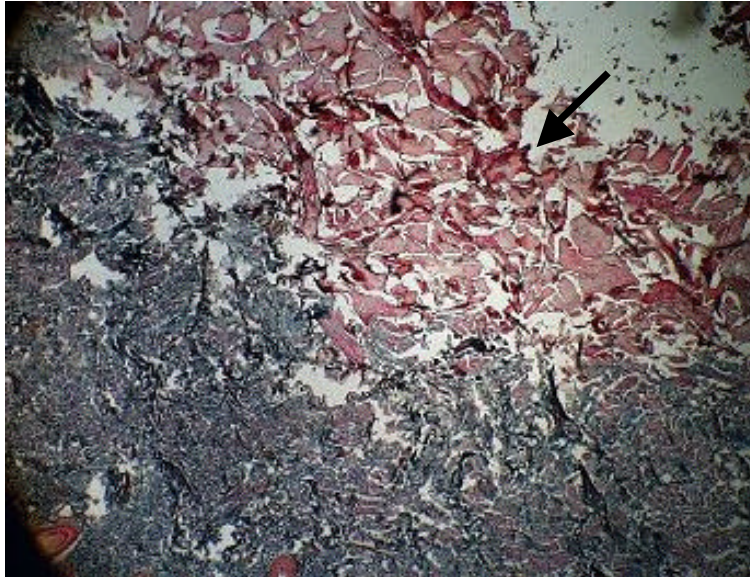


Photo 2. Genital area skin, microwave exposure 5 seconds, x 22

have more loss of staining qualities and this becomes extensive in the half not protected by aluminum foil (see arrows in Photo 2 and 3). The cellular structure is also damaged in the dermis around the hair follicles and deep in the sub dermis (Photos 3 and 4).

In the section of the skin samples exposed for 15 seconds to microwave radiation appear lighter stained. Scattered collagen fibers in the dermis and sub dermis have a pink reddish color. They express clear differences in staining of the two areas: the protected by

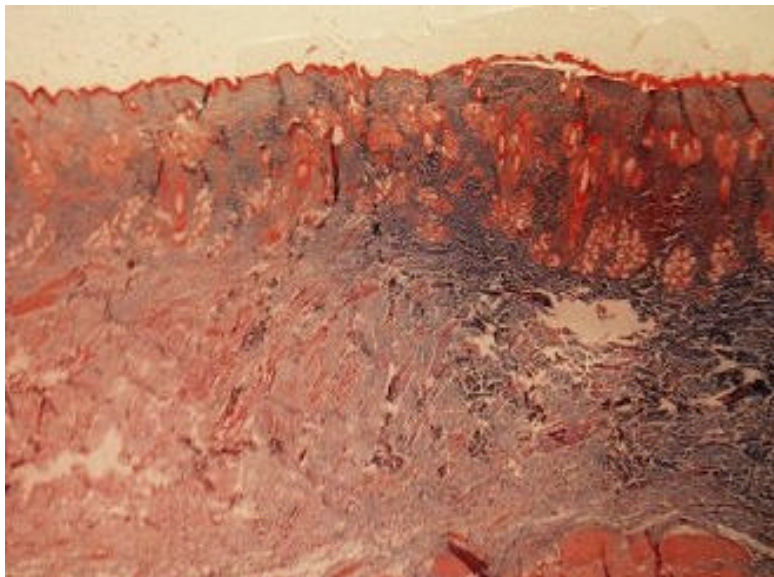


Photo 3. Periorbital area skin, microwave exposure 10 seconds, x 15

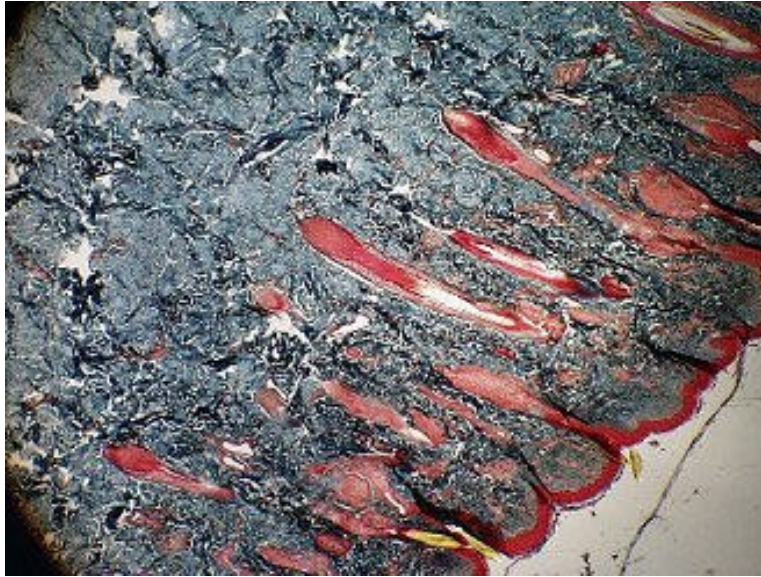


Photo 4. Genital area skin, microwave exposure 10 seconds, x 22

aluminum foil and non-protected. The exposed area displays a complete loss of cellular detail and stains an obvious pink color. This area is about five millimeters wide (Photo 5). There are thin areas of superficial dermis that have some blue collagen staining. The epidermis overlying these areas appears somewhat abnormal, with some vacuolation (Photo 6).



Photo 5. Periorbital area skin, microwave exposure 15 seconds, x 22



Photo 6. Genital area skin, microwave exposure 15 seconds, x 22

After 20 seconds of microwave exposure while the protected zone keeps some of the normal structure and bluish staining, the exposed area shows severe structural damage and a pronounced pink staining (see arrow in Photo 7). The skin completely loses the cellular detail and the collagen staining has a generalized eosinophilic appearance. The epidermis on much of the section appears vacuolated and detached from the dermis. The destruction encompasses tissues from the epithelium to deep sub dermis. The subepidermic tissues look fragmented, with red stained irregular compact masses leaving empty spaces (see arrow in Photo 8).

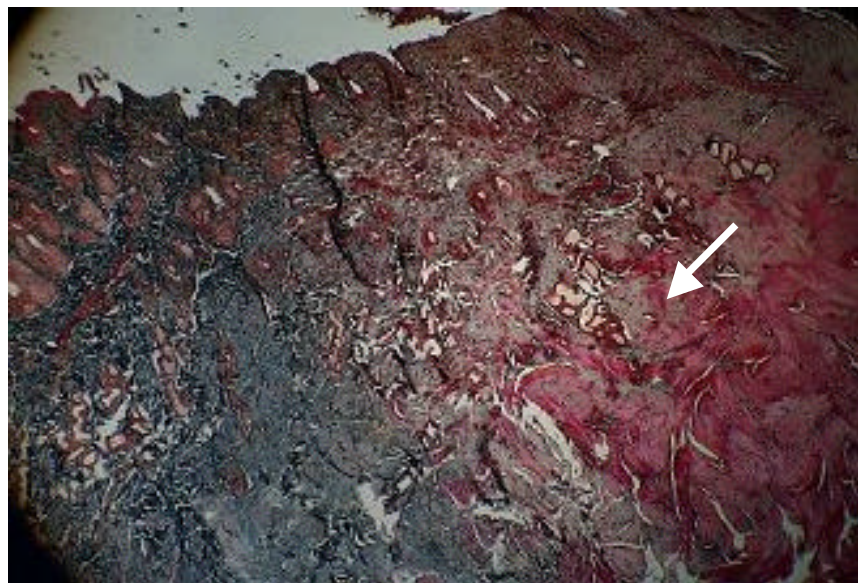


Photo 7. Genital area skin, microwave exposure 20 seconds, x 22

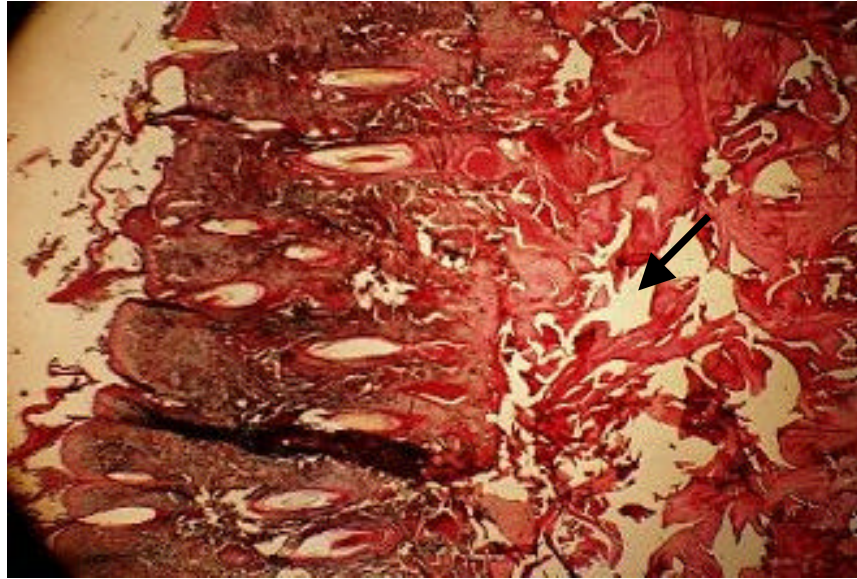


Photo 8. Periorbital area skin, microwave exposure 20 seconds, x 22

Thirty seconds of exposure results in complete destruction of the normal tissue architecture of the skin, from epithelium to the subdermic layers. The intense red coloring of the collagen makes it appear as amorphous red stained conglomerates. (Photos 9 and 10).



Photo 9. Genital area skin, microwave exposure 30 seconds, x 22

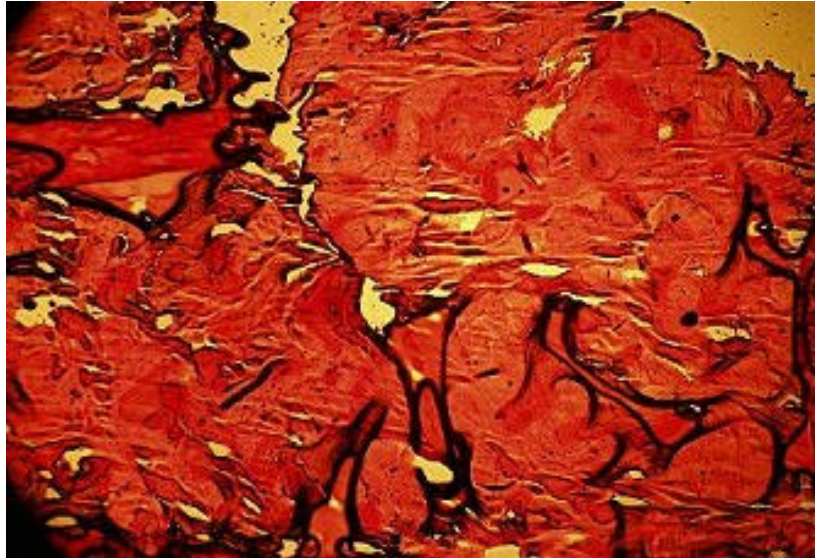


Photo 10. Periorbital area skin, microwave exposure 30 seconds, x 44

b. Liver samples

The liver tissue exposed to microwave radiation suffers important morphologic changes, which go from slight, uneven degeneration of the hepatic lobules to creation of numerous empty spaces close to the central vein (see arrow in Photo 11), or randomly disposed bubble-like formations, which dislocate the cords and disrupt the normal hepatocyte hexahedral arrangement (Photo 12). The hepatic cords become hard to distinguish and the sinusoids lose their normal anatomic disposition. Some of them display a pale staining. The exposed half of the sample shows a darker red staining, with loss of cellular detail and fragmentation of the parenchyma resulting in the appearance of numerous irregular vacuoles. The capsule over this portion is also stained red, with loss of the blue staining of collagen.



Photo 11. Liver, microwave exposure 20 seconds, x 55

B. Oven heat exposure

a. Skin samples

The high temperature exposure results in gradual changes in the structure and appearance of the periorbital and perigenital area skin samples.

After 1 minute the epidermis and dermis appear not too much damaged and shows a red staining of the epithelial elements and blue staining of the collagen. The epithelium seems to have been dislocated and leaves some epithelial empty spaces (see arrow in Photo 13). The deep portion of the dermis extending into the subdermis has a non-stained appearance, with poor cell definition (Photo 14).

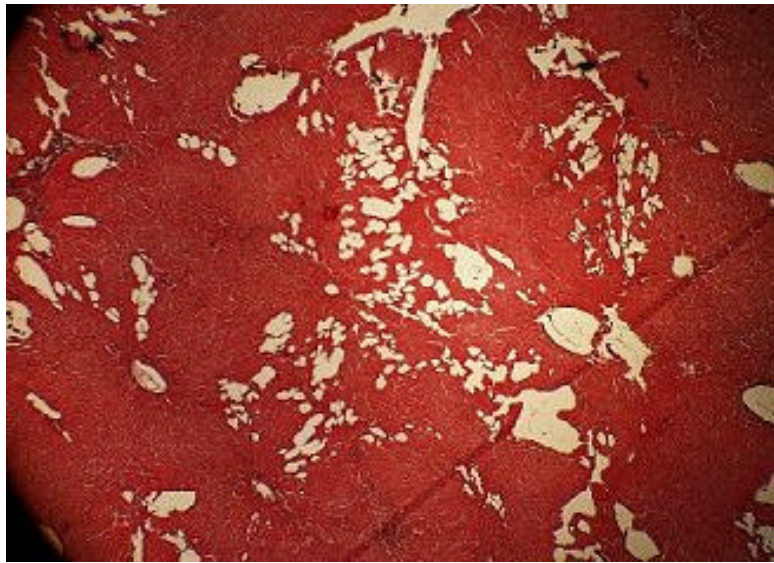


Photo 12. Liver, microwave exposure 30 seconds, x 55



Photo 13. Genital area skin, oven heat exposure 1 minute, x 15

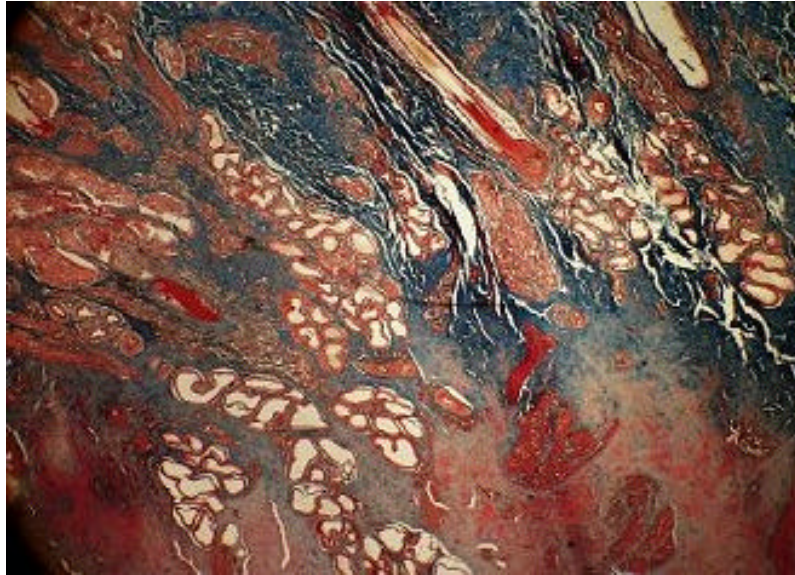


Photo 14. Genital area skin, oven heat exposure 1 minute, x 22

After 2 minutes, around the hair follicles and subepithelial glands tissue degeneration becomes obvious and is expressed by the loss of cellular details and discoloration (Photo 15).

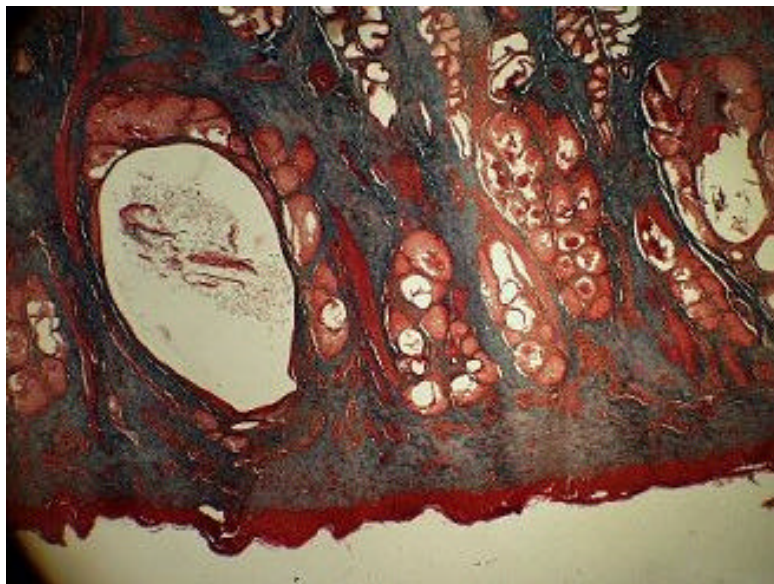


Photo 15. Genital area skin, oven heat exposure 2 minutes, x 22

Three minutes of oven heat exposure result in reduced blue staining, loss of subepithelial normal histological structure, with increased eosinophily and creation of irregular empty spaces due to fractured collagen (see arrow in Photo 16).

These destructive consequences are even more relevant after four and five minutes of exposure. Severe collagen coagulation, with numerous cracks in the deep dermal and subdermal tissue are easy to detect (Photo 17).

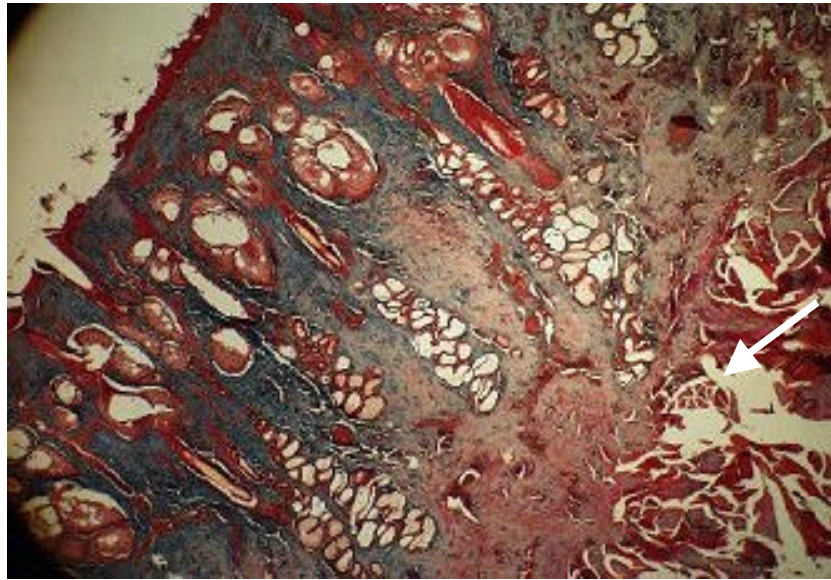


Photo 16. Genital area skin, oven heat exposure 3 minutes, x 22

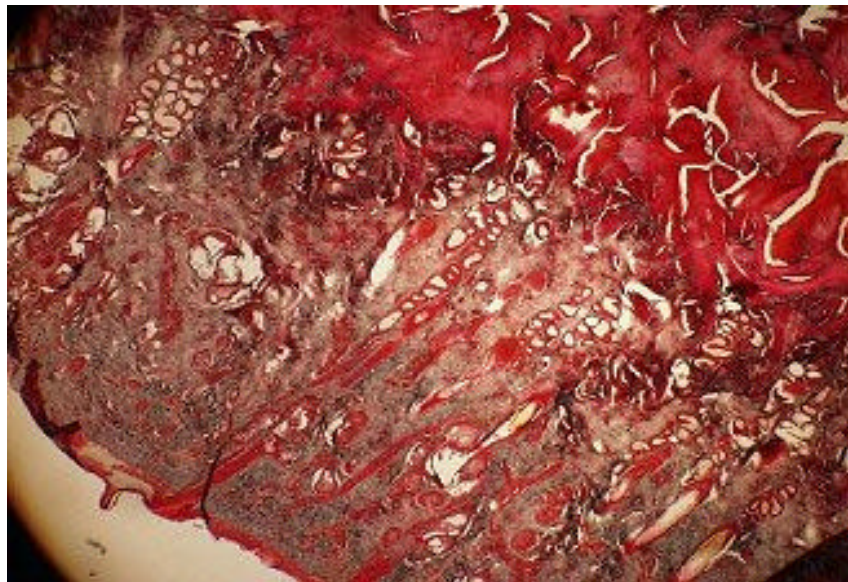


Photo 17. Genital area skin, oven heat exposure 5 minutes, x 22

b. Liver samples

After 1 minute, the normal hepato-cellular architecture seems not to be significantly altered. The capsule also appears normal, except for the very superficial half, which stains lightly blue as opposed to deeper blue color observed in the deep half of the capsule.

After 2 minutes, the liver parenchyma appears relatively normal. The only notable changes seen in the capsule, which has very little blue staining, suggesting some type of alteration of the structure of the collagen.

After 3 minutes of heat, exposure hepatocytes are more lightly stained and have no blue staining. This suggests that the collagen in the capsule as well as in the trabecular tissue extending into the parenchyma have lost their normal staining characteristics (Photo 18).

Four minutes of exposure to heat result in advanced loss of cellular detail and loss of typical blue collagen stain. In addition, empty irregular and uneven spaces can be seen in the deep subcapsular hepatic tissue (Photo 19).



Photo 18. Liver, oven heat exposure 3 minutes, x 22

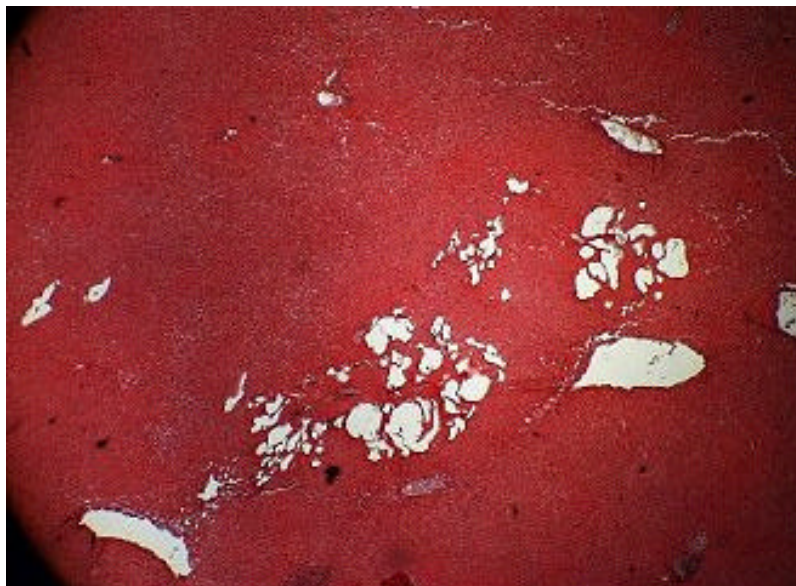


Photo 19. Liver, oven heat exposure 4 minutes, x 22

After 5 minutes, severe changes can be found in the liver capsule and the subjacent tissue (see arrow in Photo 20)

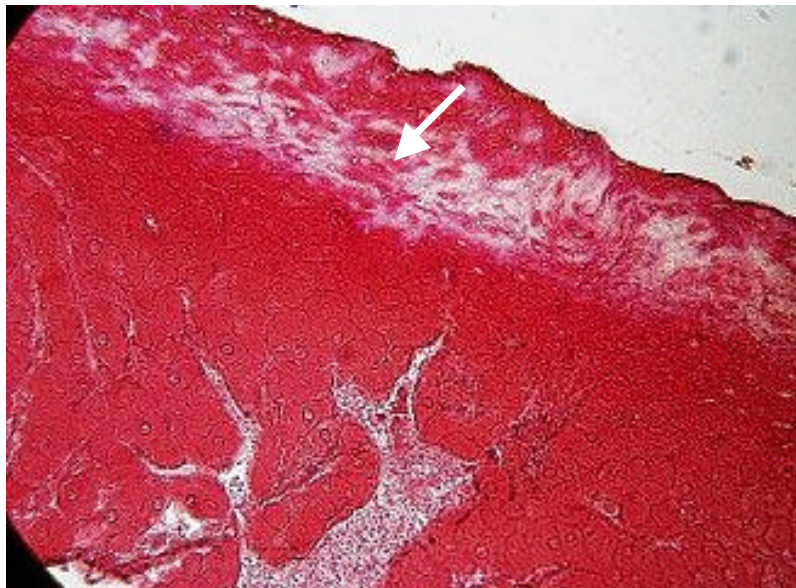


Photo 20. Liver, oven heat exposure 5 minutes, x 22

C. Cauterization

a. Skin samples

Both tissue samples, from periorbital and perigenital areas cut with an electric solder showed similar morphological changes. The epidermis and an important part of the subdermal structures, measuring about 1 mm, appear severely altered, showing a pronounced red-pinkish staining, due to collagen coagulation. Cellular detail is in good part lost. Ballooning degeneration of the epidermis is visible as well (Photos 21, 22, 23).

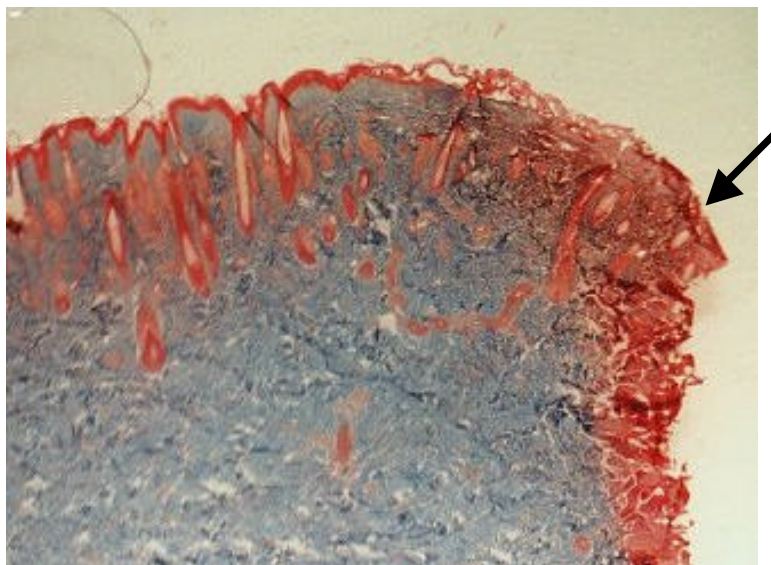


Photo 21. Periorbital skin, cauterized, x 15

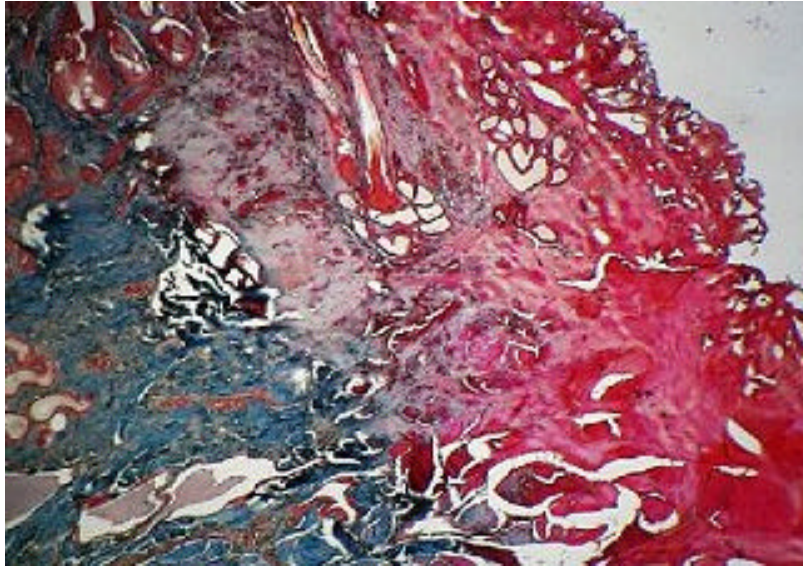


Photo 22. Genital skin, cauterized, x 22

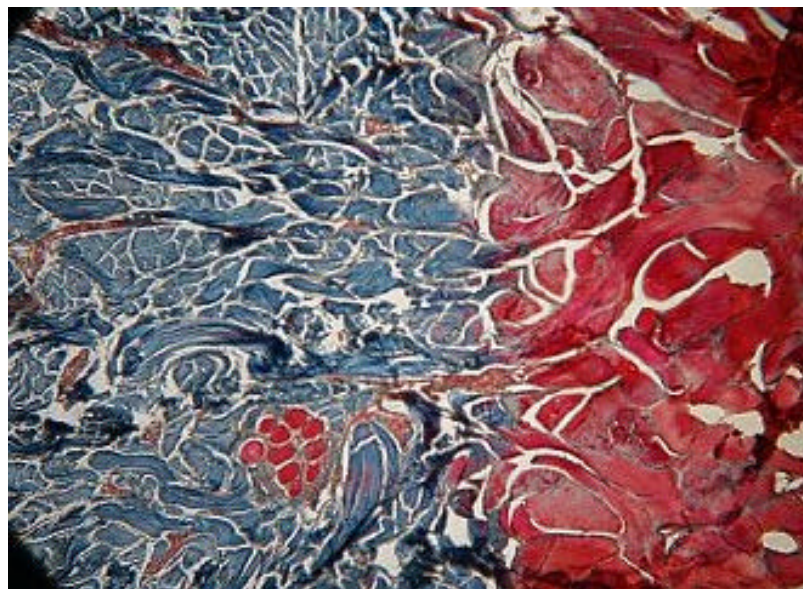


Photo 23. Periorbital skin, cauterized, x 44

b. Liver samples

The liver has an evident cooked appearance about one millimeter deep. The capsule seems normal, except for the very superficial portion, which is lightly blue in staining. The cauterization edge shows severe morphological damage, with loss of cellular detail and rearrangement of the tissue to form cords and large vacuoles (see arrow in Photo 24).

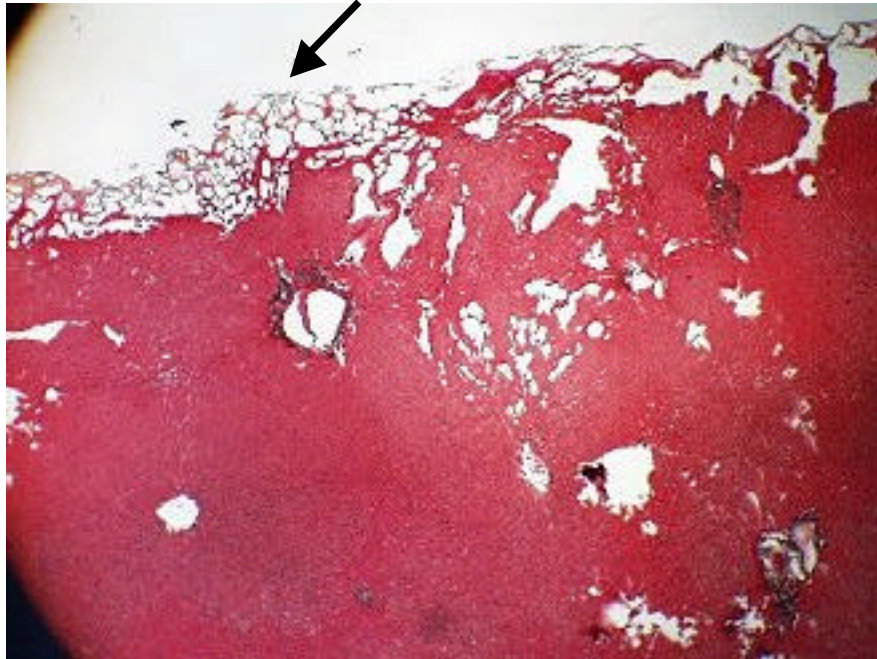


Photo 24. Liver, cauterized, x 22

Discussion

The purpose of these experiments was to mimic some of the alleged cellular changes seen in animal mutilations. It is intended that by examining the histological changes involved to provide a reference database for future comparative work with mutilated tissues. These effects involve: microwave radiation, non-specific high heat and cauterization. During the slide preparation Masson trichromic stain was used so that even slight changes to collagen architecture as a result of microwaves, heat or cauterization could be documented. It can be seen that even after 5 seconds exposure to microwaves (4250 Joules), noticeable changes occur in the subdermal collagen (Photos 1 and 2) in both periorbital and genital skin as well as pronounced changes to the epidermal layer itself. These changes intensify as the exposure increases from 5 seconds to 30 seconds (compare Photos 1 through 10). The conformation of the collagen bundles changes even after 5 seconds and this is reflected in a shift in the color of the stain from blue to pink. This possibly also reflects a lowering of the pH around the collagen bundles that is caused by cell lysis. Therefore, we suggest that future histology studies on tissue from mutilated animals should use trichromic stain and should closely examine and document changes to the architecture of the collagen.

Further, the liver samples exposed to microwave radiation can also be compared with similar tissues from mutilated animals to compare possible electromagnetic radiation effects on internal organs, as has been claimed previously.

The changes induced by the soldering in these experiments were also noteworthy. Photos 21, 22 and 23 show extensive damage as evidenced by the strong pink-red staining. Most of the internal reddish color can again be ascribed to collagen denaturation. Photos 21, 22 and 23 also

show that the epidermis itself is denatured. It is of interest that others have described “collagen cooking” as a result of “laser surgery” in tissues from mutilated animals (see Photos 3 and 4 in pp. 101 and 102 of reference #1). It will be of interest to examine future tissues from mutilated animals with the procedure outlined here, trichromic staining and high magnification microscope photography.

Selected References

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