Histological Characterization of Hemorrhages in Muscles of Broiler Chickens

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ABSTRACT Hemorrhages in meat of broiler chickens are major quality defects. The objective of our study was to characterize the various types of hemorrhages in thigh and breast muscles with respect to their morphological appearance, location, and origin. Chickens were stunned using a water-bath stunner and were either exsanguinated and fixed or perfused with fixative. The morphological appearance of the hemorrhages was determined by the type of tissue in which they were found and by the amount of extravasating blood. Origins of hemorrhages were found only at sites of rupture of venous structures, such as postcapillary venules and small collecting veins. The absence of significant leukocyte infiltration strongly indicated that muscle tissue damage and hemorrhage occurred within the 24 h preceding stunning and slaughter. The locations and types of hemorrhages indicate different underlying mechanisms.

(*Key words*: muscle, hemorrhages, broiler, histology, electrical stunning)

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INTRODUCTION

Hemorrhages in meat are considered as major quality defects (Warrington, 1974; Grandin, 1980; Bilgili, 1992; Veerkamp, 1992; Kan, 1993). They occur in all species of animals slaughtered and are located in the muscle as well as in intermuscular fat and connective tissue (Leet *et al.*, 1977; Lambooy and Sybesma, 1988; Cochram and Lee, 1991). All red discolorations caused by the presence of extravascular hemoglobin are considered as hemorrhages. The discolorations include antemortem as well as postmortem hemoglobin effluxes from the vascular system.

Hemoglobin can leave the vascular system *via* intact erythrocytes or *via* blood plasma, after cell lysis or permeation has occurred. Antemortem and perimortem hemoglobin extravasation are primarily due to rupture of the vascular wall, i.e., hemorrhage *per rhexin*. Postmortem effusion of hemoglobin occurs during processing of carcasses, especially upon dissection.

Broiler chickens that have reached slaughter weight must be (manually) caught, crated, and transported to the processing plant. The involved strain, stress, exposure to extreme environmental conditions, and trauma can cause lesions and fractures or can predispose the animal to (muscle) tissue damage caused by subsequent restraining, stunning, and slaughter. Electrical stunning prior to slaughter induces muscular and vascular spasms that may cause muscular and vascular damage (Sokolova *et al.*, 1988; Poels and Gabreëls, 1993; Block *et al.*, 1995). Once the vascular wall has been focally damaged or ruptured, the amount of blood extravasating will depend on vasomotion, hemodynamics, hemorheology, and blood coagulation (hemostasis).

Postmortem hemoglobin extravasation from dissected carcasses will depend on the amount and distribution of the residual blood present in the bled carcass, the extent of hemolysis, and the degree of coagulation of intravascular blood. It can cause sanguineous smears on the meat surface, especially when the blood has not been fully coagulated.

In the present report we describe histological observations in muscles containing various types of hemorrhages frequently observed in electrically stunned broiler chickens. Our objective was to characterize these hemorrhages with respect to morphological appearance, origin, and location.

MATERIALS AND METHODS

Ten 6-wk-old broiler chickens were obtained from a local slaughter house. The birds were suspended by the legs in iron shackles. They were stunned electrically in a water bath for 4 s at 120 V, 50 Hz AC. The current flowing through the animals was measured using a multimeter.[2] The average current measured was 108 ± 15 mA, sufficient

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[2]Fluke 8024B multimeter, Eindhoven, The Netherlands.

to induce heart fibrillation. Four animals were exsanguinated by cutting the jugular vein unilaterally, 15 s after stunning. Six birds were used for perfusion fixation. Their abdomens were opened and their hearts exposed immediately after stunning. Perfusion was performed *via* the apex of the heart, using a 0.55-mm steel needle. Each bird was perfused with 250 mL heparinized phosphate buffered saline (pH 7.4) and, subsequently, with 250 mL phosphate-buffered formaldehyde (4%).

Following exsanguination perfusion, the skin was removed, and photographs were taken of hemorrhages on the medial surface of the thighs, with Kodak 5124 GPF films.[3] Subsequently, the pectoral muscles were excised bilaterally. Photographs were taken of hemorrhages on the medial (attachment) side of the pectoral muscle pairs.

Muscle samples containing hemorrhages were collected from the medial part of the thighs and breasts. They were stretched with pins on cork squares. Muscle samples from the four nonperfused birds were fixed in a phosphate-buffered solution containing 2% glutaraldehyde and 4% paraformaldehyde, pH 7.4, whereas those from the six perfused carcasses were fixed for 5 h in Carnoy fixative (glacial acetic acid, chloroform, and ethanol at a ratio of 1:3:6) (Romeis, 1968). All samples were dehydrated and embedded in paraffin.

Serial sections, 5 μm thick, were cut and stained with hematoxylin and eosin (Romeis, 1968). The morphology of perfusion and submersion fixed muscles was essentially the same. Fixation by submersion is therefore adequate to study hemorrhages histologically. Micrographs were taken with Kodak Ektachrome 64 T 5 EPY 135-36 color reversal films.[3] Hemorrhages were classified according to their morphology, location, and origin. Morphology was based on size and appearance. The nomenclature is derived from literature concerning human pathology and hemorrhages in muscles of slaughter animals (Mandrup, 1964; Leet *et al.*, 1977; Gilbert and Devine, 1982; Hillebrand, 1993) and is as follows:

1. Petechiae: small pin-point like blood spots. Petechiae in the intermuscular fat and *perimysium* are called speckles (Gilbert and Devine, 1982).
2. Striae: hemorrhages with an oblong appearance, also termed striated hemorrhages. In muscle tissue they follow the direction of the muscle fibers.
3. Ecchymosis: hemorrhages with a surface area of several square millimeters. Ecchymosis of muscle tissue from lambs is called blood splash (Leet *et al.*, 1977).
4. Sugillation: clear-cut hemorrhage with a diameter of several centimeters.

Blood vessels were typified according to their morphology, i.e., size, presence of typical vessel wall structures (*tunicas*), and lumen diameter relative to blood vessel wall thickness (Hodges, 1974; Jerusalem, 1986).

[3]Eastman Kodak, Rochester, NY 14650.

RESULTS

Hemorrhages on a Macroscopic Level

Photographs of dissected parts (breast muscles and thighs) are presented in Figures 1 to 3. In Figure 1a, a survey of the medial side of a breast muscle pair with different types of hemorrhages is shown. A detail showing the suggillation at the site where the breast muscle pair is attached to the apex of the sternum is presented in Figure 1b. The sugillation extends over both superficial breast muscles (*Pectoralis superficialis*) on either side of the connective tissue connection (*raphe*). Figure 1c shows striae near the tendon of the left *Pectoralis profundus* muscle. The blood follows the direction of the muscle fibers. A clear-cut ecchymotic hemorrhage is presented in Figure 1d. It is located on the distal side of the right *P. profundus* muscle.

Figure 2 shows the medial part of the thigh of a left leg near the knee joint. Numerous petechial hemorrhages are present in the fat tissue surrounding the distal part of the *Sartorius*, near the knee. More petechial hemorrhages can be seen in the *Sartorius* muscle itself, as well as in the connective or fat tissue between the medial part of the *Quadriceps femoris* and the internal *Adductor*. An example of a frequently observed reddish, sanguineous *Adductor* muscle is presented in Figure 3.

Hemorrhages on a Microscopic Level

In several muscles, ruptured venules and veins were found in the inter- and intramuscular adipose tissue (Figures 4a,b, and 5a,b). We were not able to find any ruptured vessel that could be typified as arterial in any of about 1,500 sections examined. In perfused as well as in submersion-fixed muscles, venous structures were often completely filled with erythrocytes. This phenomenon occurred in all types of veins, muscular as well as nonmuscular. In the vicinity of such veins, hemorrhages were often observed. Filled veins were, however, not always associated with hemorrhages, as shown in Figure 6. Neither were hemorrhages limited to veins filled with erythrocytes.

Sometimes, hemorrhages were present at locations of general or local disruption of the muscle fiber structure. At these sites, muscle tissue was characterized by hypercontraction of myofibers (Figure 7). Other intramuscular hemorrhages, in contrast, occurred at normal appearing sites, showing no signs of disruption or hypercontraction (Figure 8). In some sections, hypercontracted areas were found without hemorrhages. Degenerating muscle fibers with infiltrations of inflammatory cells were seldom seen.

In Figure 9, a sugillation of the pectoral muscle at the site of the sternum apex is shown. Hemorrhages were located in the muscular part as well as in the *raphe*, consisting of dense connective tissue with numerous fibroblasts and fibrocytes and irregular collagenous fibers, typical for tendons. We were not able to locate the origin of these types of blood extravasations. Damage of the *raphe* occurred at sites where the direction of longitudinal oriented muscle fibers was more or less perpendicular to

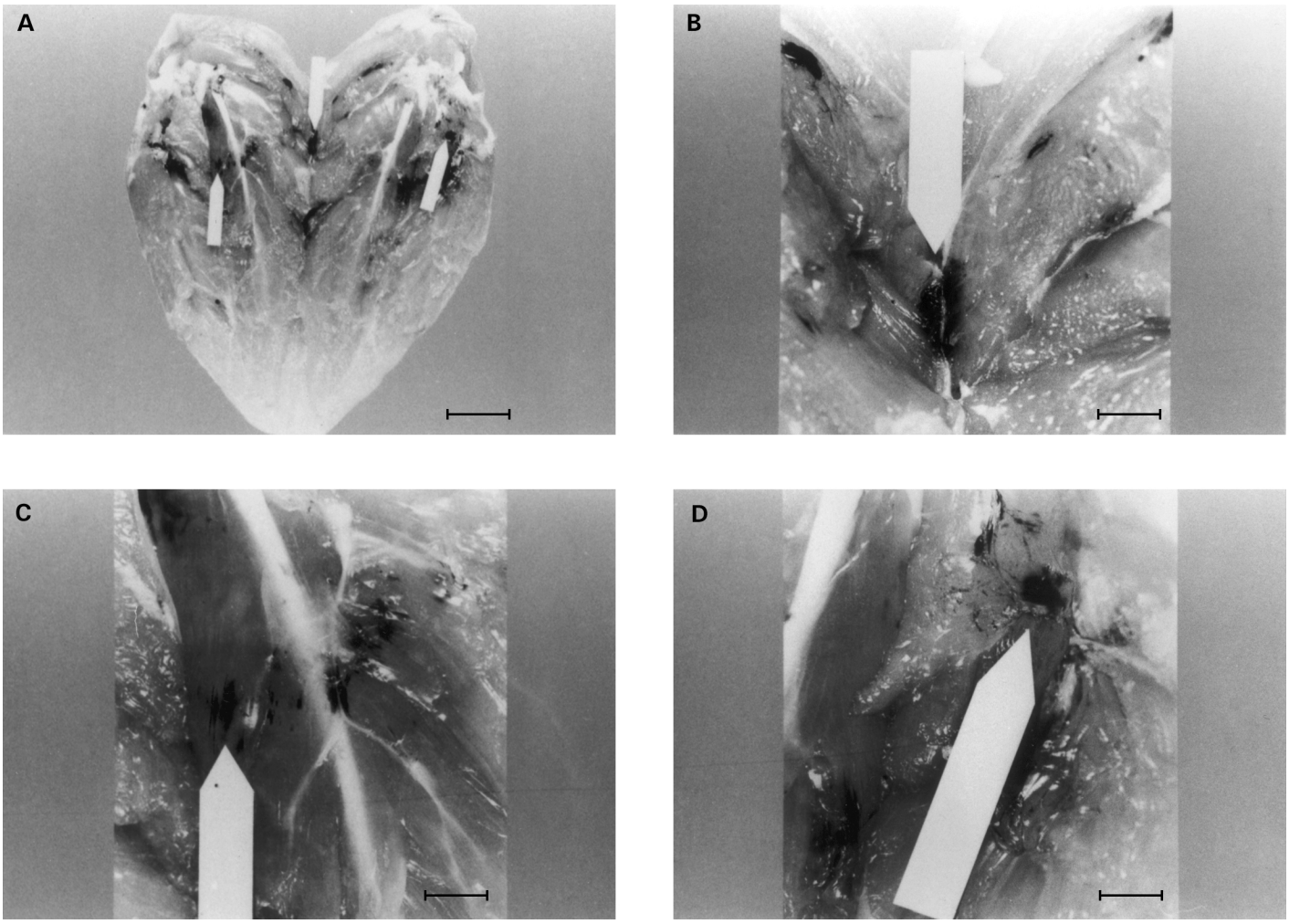

FIGURE 1. Survey of the medial (attachment) side of a pectoral muscle pair with different types of hemorrhages. Indicated are hemorrhages presented in detail in Figure 1 b to d. Bar: 2 cm (a). Sugillation at the attachment site of the superficial pectoral muscle pair to the apex of the sternum. Bar: 0.5 cm (b). Striae near the tendon of the left *Pectoralis profundus* muscle. Bar: 0.5 cm (c). Ecchymosis on the distal side of the right *Pectoralis profundus* muscle. Bar: 0.5 cm (d).

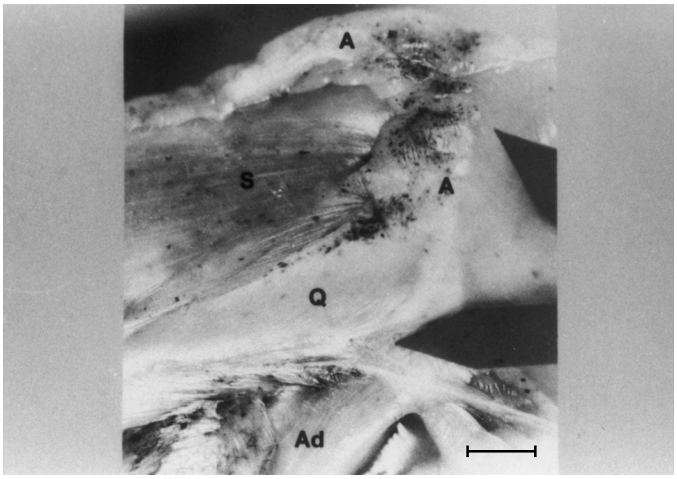

FIGURE 2. Medial part of the left thigh. Petechial hemorrhages in muscle tissue, fat surrounding the knee joint, and intermuscular fat or connective tissue. A = Adipose tissue; S = *Sartorius*; Q = *Quadriceps femoris*; and Ad = *Adductor*. Bar: 0.5 cm.

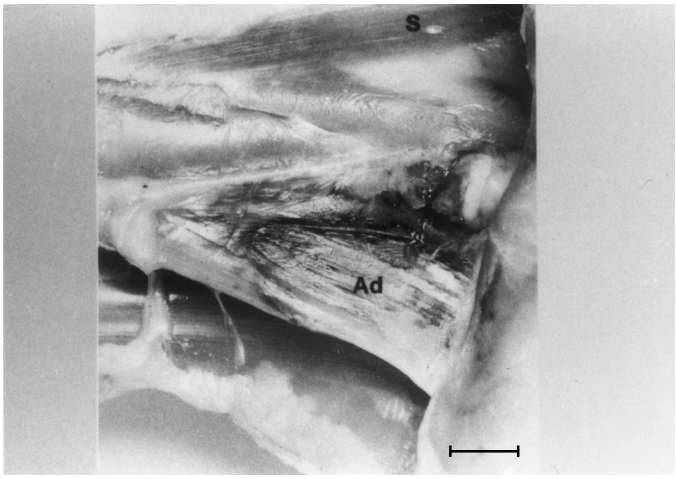

FIGURE 3. Medial part of the right thigh. *Adductor* muscle (Ad) with a reddish sanguineous appearance. S = *Sartorius*. Bar: 0.5 cm.

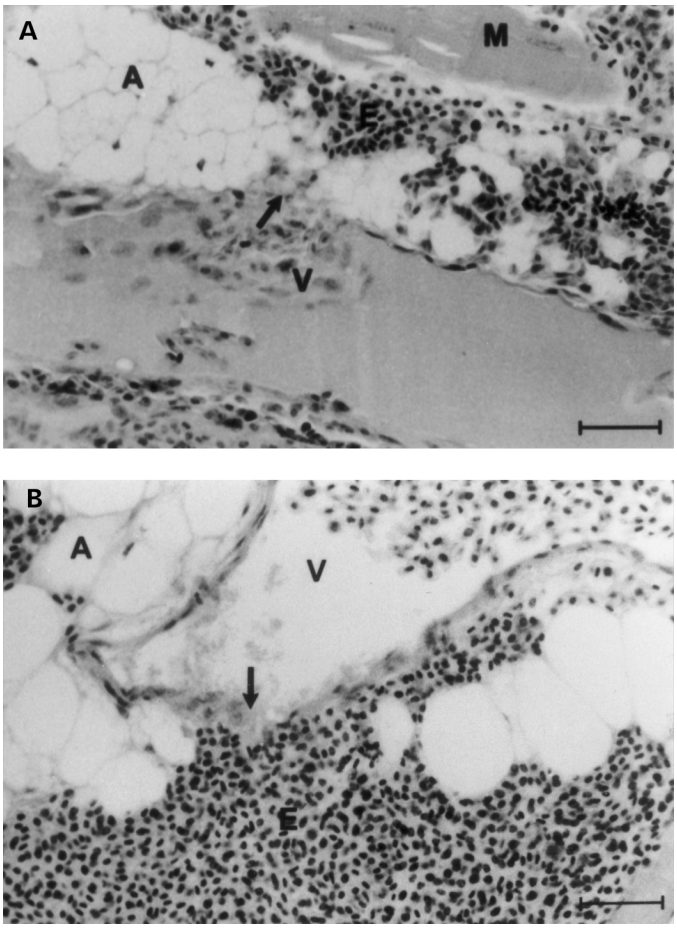

FIGURE 4. *Pectoralis profundus*. Small collecting vein (V) within intramuscular adipose tissue (A) with rhexis (arrow). M = Muscle fiber; E = extravascular erythrocytes. Bar: 25 μm (panel a). *Adductor*. Small collecting vein within intramuscular adipose tissue. Site of rhexis is indicated with an arrow. Bar: 25 μm (panel b).

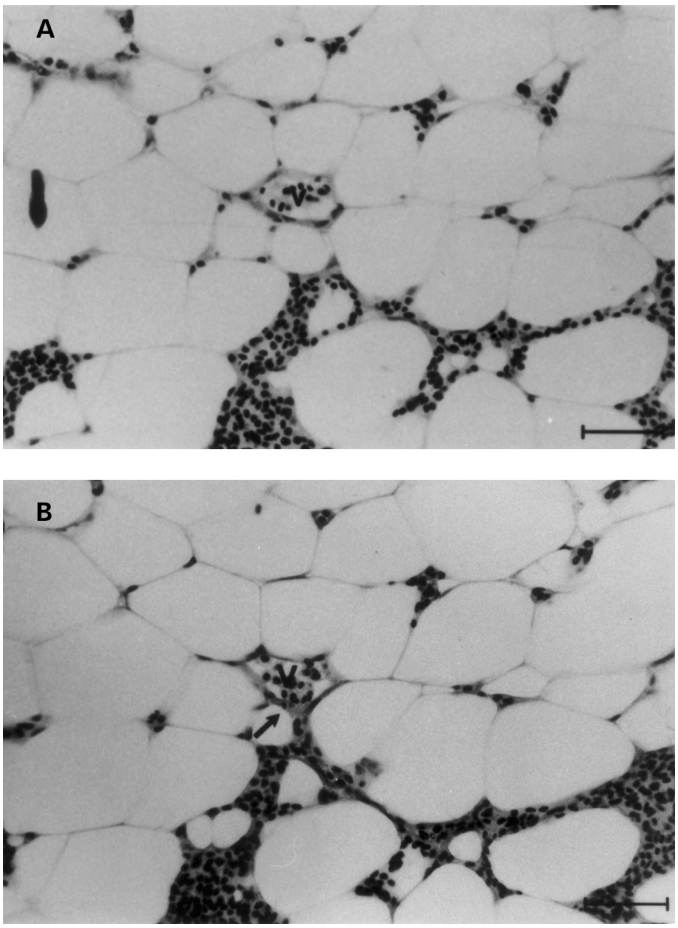

FIGURE 5. Petechial hemorrhage within intermuscular fat. First of two serial sections showing an intact postcapillary venule (V). Bar: 25 μm (a). Second of two serial sections showing the postcapillary venule (V) from Panel a with rhexis (arrow). Bar: 25 μm (b).

the connective tissue. Junctions between *raphe* and muscle fibers were mostly unaffected.

Hemorrhages were found between muscle fibers, within the intra- and intermuscular fat, and adjacent to or within the dense connective tissue enveloping fascicles (*perimysium*) and muscles (*epimysium*), as well as in the more loosely connective tissue. Typical petechial hemorrhages as occurring in intra- as well as intermuscular fat are presented in Figure 10. Some of them were observed to originate from small, thin-walled vessels, identified as postcapillary venules (Figures 5a and b). The origin of the majority of these blood spots, however, could not be revealed. An example of a striated hemorrhage, located in the pectoral muscle, is shown in Figure 11. The erythrocytes were arranged in an oblong stripe or branched form, following the direction of the muscle fibers. No unambiguous origin was found. Figure 12 shows an example of a diffuse blood extravasation within the loosely connective tissue adjacent to the *perimysium* consisting of dense connective tissue. In some cases, venous rupture could be observed in the *perimysium* (not shown).

Numerous hemorrhages with variable size, located between muscle fibers as well as in the intramuscular fat, were typical for *Adductor* muscles with a sanguinous appearance. Although many fibers had a hypercontracted appearance, degenerating fibers, characterized by infiltrations of heterophilic granulocytes and mononuclear cells, were absent. Sometimes adhering blood was found on the

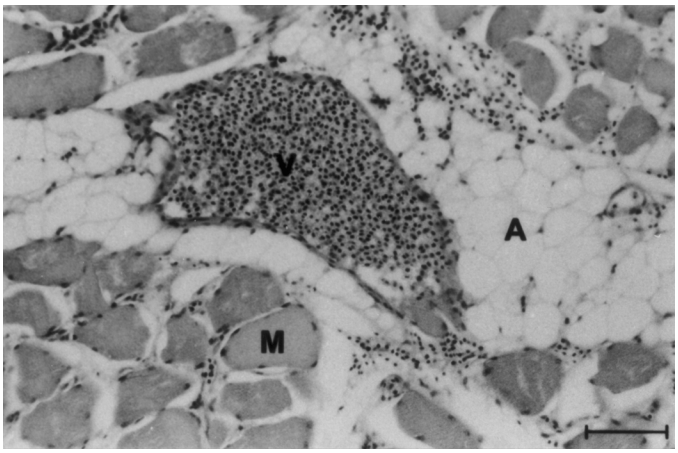

FIGURE 6. *Adductor*. Small collecting vein (V) filled with erythrocytes. M = Muscle fiber; A = adipose tissue. Bar: 50 μm.

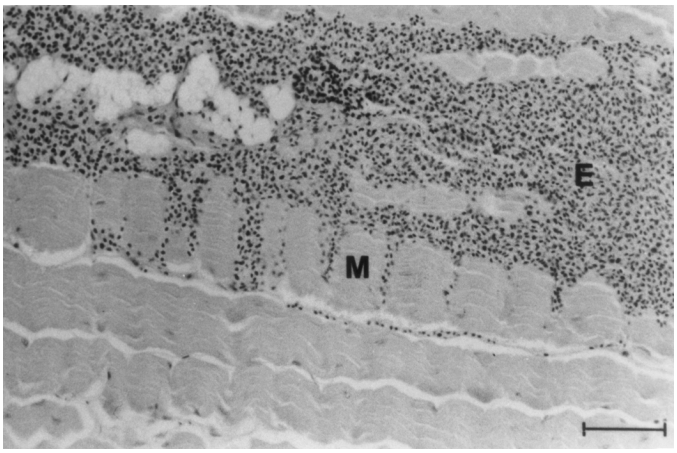

FIGURE 7. *Pectoralis profundus*. Intramuscular hemorrhage (E) associated with hypercontracted muscle fibers (M). Bar: 50 μm.

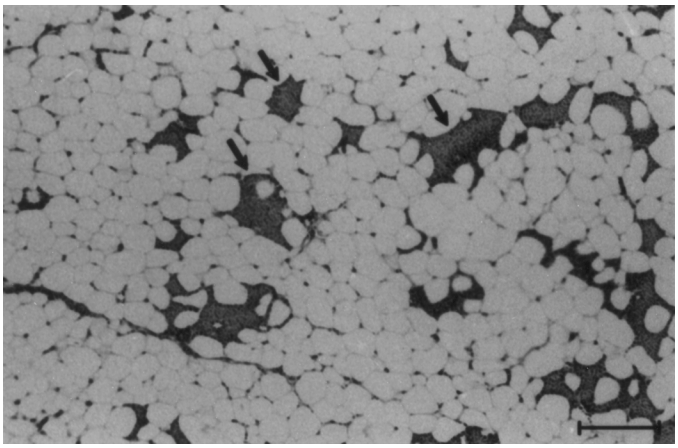

FIGURE 10. Petechial hemorrhages (arrows) within intermuscular fat. Bar: 100 μm.

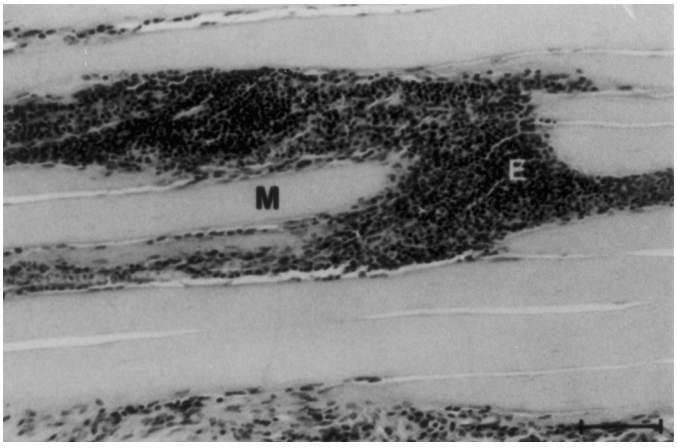

FIGURE 8. *Pectoralis superficialis*. Intramuscular hemorrhage (E) associated with noncontracted muscle fibers (M). Bar: 50 μm.

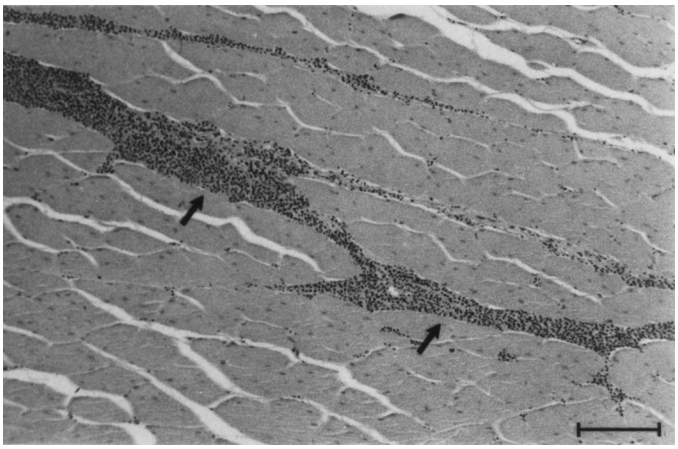

FIGURE 11. *Pectoralis profundus*. Striated hemorrhage (arrows). Bar: 100 μm.

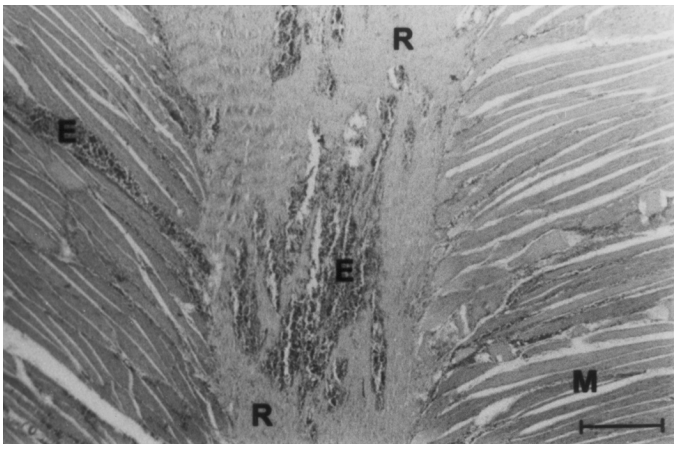

FIGURE 9. Hemorrhage at the attachment site of the superficial pectoral muscle pair to the sternum apex. R = *Raphe;* E = extravascular erythrocytes; and M = muscle fibers. Bar: 250 μm.

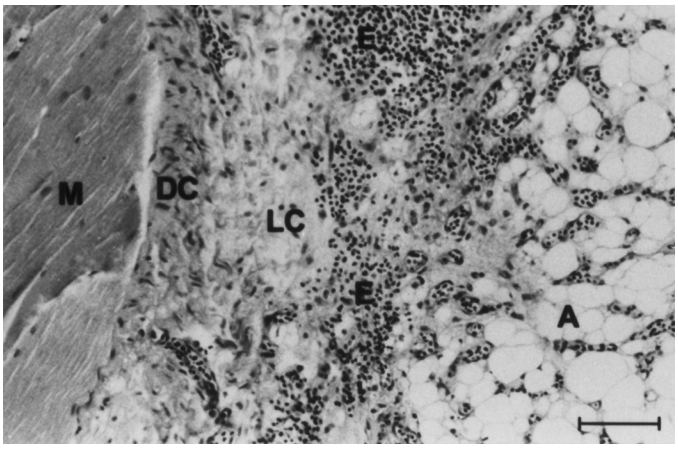

FIGURE 12. Diffuse hemorrhages (E) in loose connective tissue (LC) between the dense connective tissue (DC) of the epimysium and adipose tissue (A). M = Muscle fibers. Bar: 50 μm.

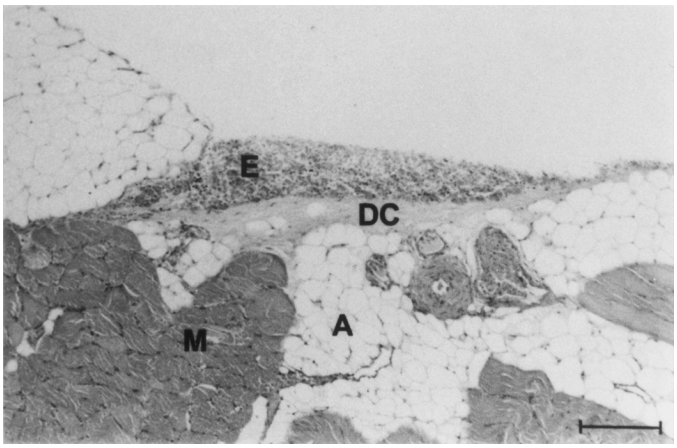

FIGURE 13. *Adductor*. Erythrocytes (E) adhering to the muscle surface. M = Muscle tissue; A = adipose tissue; and DC = dense connective tissue. Bar: 100 μm.

surface of such muscles (Figure 13). A survey of the different types of hemorrhages with their location and origin is presented in Table 1.

DISCUSSION

The morphology of the hemorrhages investigated depended on the tissue in which they occurred. In the pectoral muscles, extravasating blood was often found to follow the direction of the muscle fibers. It gave these hemorrhages their typical oblong, striated, or branched appearance. In fat tissue, the form of the hemorrhages was determined by the mesh structure of the adipocytes. The majority of the hemorrhages had a petechial appearance. More diffuse hemorrhages were found in loose connective tissue. Erythrocytes were scattered throughout the loosely arranged extracellular matrix and fibrocytes. The structure of the tissue at the site of hemorrhage determines whether the extravasation of erythrocytes is diffuse or defined. The amount of blood extravasating determines the size.

The sugillation near the sternum apex extended over the connection (*raphe*) of the two superficial pectoral muscles. This connection, consisting of dense connective tissue with fibroblasts and fibrocytes typical for tendons, was frequently disrupted. Especially at sites where the fibers were more or less perpendicularly directed to the connection, damage and hemorrhage occurred. The force generated upon simultaneous contraction by both pectoral muscles, as occurring during water-bath stunning of chickens, probably disrupted the connective tissue. The fact that the connective tissue was damaged, whereas the junctions between muscle fibers and connective tissue (myo-tendinous junctions) remained intact, indicates that connective tissue development has not kept up with muscle development, as was suggested for turkeys by Swatland (1990). Collagen and connective tissue content relative to total muscle protein are age-dependent (Khalili and Zarkadas, 1988). In broiler chickens slaughtered at young age, muscle force generated by the stunning procedure may therefore exceed tension resistance of the muscular connective tissue.

In many muscles investigated, signs of hypercontraction of muscle fibers were observed. The muscles demonstrated disruptions of myofibers similar to those described for electrically stimulated rat muscles (Block *et al.*, 1995). Although hemorrhages occurred in such hypercontracted areas, they were also found in noncontracted muscle parts. It is likely that mechanisms other than physical strain, tension, or force generated by severe muscle contractions can cause hemorrhages in muscle tissue as well. Several hypercontracted areas were found to be free from hemorrhages. Therefore, hypercontraction leading to disruption of the muscular structure may or may not cause rupture of blood vessels.

At the moment of stunning, all muscle fibers will be excited simultaneously. The intramuscular pressure will rise considerably. The increased pressure is believed to compress the arteries and veins passing through the muscle but not the capillaries and precapillary resistance vessels (Gray *et al.*, 1967; Laughlin, 1987). Under normal conditions, upon muscle contraction blood is pressed out of the veins toward the heart. As a result of whole-body stunning, however, vasoconstriction of the larger muscular veins, similar to that observed in electro-stimulated muscles of rats, is likely to occur (Hussman *et al.*, 1995). This vasoconstriction might even reverse the blood flow. Consequently, blood will accumulate, and pressure will rise in the small, thin-walled venules and collecting veins. Venules or small veins that are not able to resist the increased pressure will rupture. We frequently found venules and small collecting

TABLE 1. Hemorrhages: type, location and origin

Type of hemorrhage	Location	Origin
Petechiae	• Inter- and intramuscular adipose tissue	(Postcapillary) venules and small collecting veins ?
Striae (also branched)	• Muscle fascicles	?
Sugillations at sternum apex	• *Raphe* • Muscle adjacent to *raphe*	? ?
Diffuse hemorrhage	• Loose connective tissue between *perimysium* and intermuscular adipose tissue • Loose connective tissue between *epimysium* and intramuscular adipose tissue	(Postcapillary) venules and small collecting veins ? (Postcapillary) venules and small collecting veins ?

? = Hemorrhages for which no origin could be found.

veins to be packed with erythrocytes. They were often associated with extensive hemorrhages. In cases in which the origins of the hemorrhages were found, they were always venous. Some of the petechiae in fat originated from postcapillary venules. More extensive hemorrhages originated from larger venules or small veins. Not one site of arterial rupture was found. These results strongly indicate that a (local) rise of blood pressure because of accumulation of blood causes venous rupture. Connective tissue and fat surrounding ruptured veins frequently had a normal appearance, which indicated that these hemorrhages were not due to violent disruption of the entire tissue structure, supporting the mechanism described above.

The electrical field itself may affect blood vessel wall integrity. The vascular wall can be damaged by mechanisms like Joule heating and cell permeation (Tsong, 1991; Reilly, 1994). Most severe damage is likely to occur at sites of high current density, like the joint of the knee. Local Joule heating therefore may have damaged the vasculature in the fat tissue of the thighs.

Adductor muscles with a sanguineous appearance have numerous fibers with a hypercontracted and disrupted appearance. There are, however, no signs of massive infiltration of leukocytes. Leukocytes start to infiltrate hypercontracted muscle fibers of rats and rabbits about 24 h after damage has occurred (Fisher *et al.*, 1990; Nikolaou *et al.*, 1987). Thus, the damage inflicted on the *Adductor* muscle occurred within the 24 h preceding slaughter and cannot be the result of dystrophia or damage resulting from rearing.

The histological study of hemorrhages in different types of muscles showed that the morphological appearance of the blood extravasation is determined by the structure of the tissue as well as by the amount of blood leaving the circulation. The diversity in hemorrhage type and location indicates that hemorrhages are caused by several different mechanisms.

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