Histological changes in skeletal muscle during death by drowning. An experimental study

Abstract

A diagnosis of drowning is a challenge in legal medicine as there is generally a lack of pathognomonic findings indicative of drowning. This paper investigates whether the skeletal muscle undergoes structural changes during death by drowning. Eighteen Wistar rats were divided into three equal groups according to the cause of death: drowning, exsanguination and cervical dislocation. Immediately after death, samples of the masseter, sternohyoid, diaphragm, anterior tibial, soleus, and extensor digitorum longus muscles were obtained and examined by light and electron microscopy.

In the drowning group, all muscles except the masseter displayed scattered evidence of fiber degeneration, and modified Gomori trichrome staining revealed structural changes in the form of abnormal clumps of red material and ragged red fibers. Under the electron microscope, there was myofibrillar disruption and large masses of abnormal mitochondria. In the exsanguination group, modified Gomori trichrome staining disclosed structural changes and mitochondrial abnormalities were apparent under light microscopy; however, there was no evidence of degeneration. No alterations were observed in the cervical dislocation group.

As far as we know, this is the first time that these histological findings are described in death by drowning and are consistent with rhabdomyolysis and intense anoxia of skeletal muscle.

Introduction

Histological examination of skeletal muscle in legal and forensic medicine tends to focus on four major objectives: to establish the sequence of postmortem changes; [1-3] to identify artifactual postmortem changes; [4] to recognize certain histological changes which enable the myotoxic effects of drugs and toxins to be established and characterized; [5] and to identify microscopic features enabling the cause of death to be established. [6-8]

Within the latter objective, determination of the cause and manner of death for a body recovered from the water is hampered by a lack of autopsy findings specific for drowning. [9] Our study was prompted by the unexpected finding of muscle fiber abnormalities during earlier research into the association of diatoms with drowning in peripheral rat tissue. [10] In muscle pathology, intense red staining using modified Gomori trichrome staining is widely regarded as indicative of a range of structural abnormalities, including mitochondrial clumping, nemaline bodies, tubular aggregates, and cytoplasmic bodies. [11]

We seek to investigate structural abnormalities in various muscles that might be associated with death by drowning. For comparative purposes, two other causes of death were included: exsanguination and cervical dislocation. Our results confirm earlier muscle changes observations in death by drowning, suggesting that these changes may be linked to intense anoxia and muscle strain. However these changes can not be considered as specific of drowning deaths.

Material and Methods

Animals and groups

Eighteen 12-week-old male Wistar rats (380-425g) were divided equally into three groups according to the cause of death: Group 1: death by drowning; Group 2: exsanguination; Group 3: cervical dislocation.

Rats in Group 1 were placed in a cage which was then immersed into tap water at room temperature for 30 minutes. Rats in Groups 2 and 3 were previously anesthetized with an intraperitoneal injection of 75mg/kg ketamine (Imalgene® 100mg/ml, Merial Laboratorios, Lyon, France). For exsanguination (Group 2), anesthetized rats were placed in the supine position for transthoracic cardiac puncture; blood was then quickly aspirated into a 10-ml syringe.

Samples of masseter, sternohyoid, anterior tibial, soleus, and extensor digitorum longus muscles (right and left) were taken from all rats; the diaphragm was split in two and each half was examined separately. These muscles were chosen because they are widely used in myology and experimental myopathology, and their histological and histochemical characteristics are therefore well documented. [12-16] Moreover, hemorrhagic changes have been reported in the human sternohyoid during drowning. [9]

Ethical approval

All procedures were carried out in accordance with Directive 2010/63/EU of the European Parliament and of the Council, of 22 September 2010, on the protection of animals used for scientific purposes, [17] and the study was approved by the University of Córdoba Bioethics Committee.

Light and electron microscopy

All muscles were examined grossly immediately after extraction. For histological and histochemical analysis, specimens were embedded in OCT, flash-frozen in isopentane cooled with liquid nitrogen (-160ºC) and transversely sectioned at 8 μm using a cryostat at -20°C. Sections were stained with haematoxylin-eosin (H-E), modified Gomori trichrome (MGT), Masson's

trichrome, nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-tr), succinate dehydrogenase (SDH), alkaline phosphatase (AKP), and acid phosphatase (AP) using standard staining procedures. [18]

Small fragments from each specimen were fixed in 2.5% glutaraldehyde in 0.1M phosphate buffer at pH 7.4, postfixed in 1% osmium tetroxide for 2 hours, dehydrated through an acetone series, and then embedded in Epon. Ultrathin sections were obtained with an LKB 8800 ultramicrotome, and sections were placed on copper grids and stained with uranyl acetate and lead citrate. They were examined with a JEOL JEM 201 high-resolution transmission electron microscope (SCAI, University of Córdoba).

Quantitative and statistical analysis

Whole transverse histologic sections were examined at x 200 magnification. The frequency of changes was determined by counting lesions over a minimum of twelve 0.24 mm^2 fields per muscle using a Sony Exwaved HAD digital camera mounted on a Nikon Eclipse E1000 microscope (Nikon, Tokyo, Japan) and connected to a personal computer. The extent of lesions was scored using an *ad hoc* semiquantitative classification by two different observers (see note to Table 1). For classification purposes the maximum lesion score for each muscle was taken as valid, and the mode for maximum values for each lesion in each group was taken as the representative value for that group.

Results

Gross examination of muscle yielded no relevant findings in any of the groups. Histological changes observed, together with the results of semiquantitative analysis for the three groups, are shown in Table 1. No structural abnormalities were noted in any of the muscles in the cervical dislocation group, but changes were observed in the other two groups. The nature and frequency of these changes differed between the exsanguination and drowning groups.

Drowning group (Group 1)

At histological examination, rounded, swollen fibers that stained more intensely were identified as hypercontracted fibers (Fig. 1). Larger fibers observed in some muscles, often exhibiting fissures and internal nuclei, were classed as whorled fibers (Fig. 2); these were sometimes found adjacent to fibers displaying pale staining zones, which were identified as ghost fibers (Fig. 3), and others with a granular appearance (Figs. 3 and 4). Some specimens contained occasional areas of edema and degenerative phenomena including ghost fibers as well as others staining intensely red to Masson's trichrome (Fig. 5).

MGT staining revealed fibers containing bright red masses which displayed various morphological patterns: peripheral balls or balloons on fiber surfaces; half-moon or crest shapes, or hood-like patterns covering most of the fiber, while the rest of the sarcoplasm continued to appear normal (Figs. 6-8). In other instances, the reddish staining pattern was clearly consistent with that of a *ragged red* fiber; peripheral clumping was observed around the whole fiber, together with an intrasarcoplasmic granular pattern (Fig. 9). Apparently-isolated, reddish-staining masses also were visible between muscle fibers, their patterns varying even within a single transverse section; these masses stained positively for SDH (Fig.10) and NADH-tr (Fig.11) —confirming their mitochondrial nature— but negative for other stains tested (AKP and AP). Histochemical staining for AP and AKP yielded no significant findings.

Electron microscopy disclosed major ultrastructural changes involving both mitochondria and myofibrils. Interestingly, these degenerative alterations were more marked in the diaphragm than in the other muscles. Myofibrillar changes were less extensive than mitochondrial changes, which were apparent in the majority of instances. Many fibers contained large clumps of abnormal subsarcolemmal and intermyofibrillar mitochondria (Fig.12). Most were swollen with broken crests; in some instances, plate-like inclusions were visible, while others displayed numerous vesicles (cystic degeneration) and vacuolization (Figs. 12-15).

These clumps of abnormal mitochondria were observed both in fibers with virtually-unchanged myofibrils and in fibers displaying extensive myofibrillar damage. There was evidence of hypercontraction, focal Z-disc streaming, and myofibrillar disruption (Figs. 13-15); these changes were particularly apparent in the diaphragm, where they were accompanied by myofibrillar rupture and myofilament loss.

There was no sign of intramuscular bleeding, capillary injury or plasma extravasation.

Exsanguination group (Group 2)

MGT staining revealed peripheral balls or balloons on fiber surfaces containing red material with MGT (Fig. 16) and *ragged red* fibers, although these were less numerous than in the drowning group. None of the specimens displayed evidence of fiber degeneration of the kind observed in the previous group, except for occasional, isolated hypercontracted fibers. Under electron microscopy (Fig. 17), a number of fibers exhibited mitochondrial changes similar to those detected in the drowning group. No myofibrillar alterations were observed.

Cervical dislocation group (Group 3)

Histological findings with both H-E and MGT staining in the cervical dislocation group were normal for all muscles (Fig. 18). Histochemical staining revealed no cytoarchitectural or fiber-type changes (Fig. 19). No ultrastructural changes —either mitochondrial or myofibrillar— were observed.

Discussion

These findings confirmed that death by drowning prompts changes in skeletal muscle structure consistent with intense anoxia and rhabdomyolysis; however, the frequency of these changes varied depending on the muscle concerned. Interestingly, no such changes were observed in the cervical dislocation group, while exsanguinated rats displayed only changes consistent with anoxia.

Histological evidence of anoxia included fibers containing abnormal deposits of red material and other fibers identified by MGT staining as *ragged red*. Histochemistry and electron microscopy detected mitochondrial masses, most displaying significant degenerative changes consistent with anoxia.[19] *Ragged red* fibers, a well-documented finding in muscle pathology, are widely regarded as a non-specific change linked to mitochondrial disorders; however, some authors[20] view them as a special kind of pathological reaction which may involve abnormal accumulation of mitochondria. Since their detection in the rats was not due to underlying mitochondrial pathology, they may be linked to the death. It is unlikely to be a post-mortem change as there are no report describing this in the literature, and post-mortem degenerative changes in skeletal muscle occur at a later stage. [2] Moreover, these changes were not observed in the cervical dislocation group, after the same post-mortem interval. Here, *ragged red* fibers, or fibers closely resembling them, occurred over a very short duration of time, since death was very fast (less than 2 minutes in the drowning and exsanguination groups). These mitochondrial changes thus took place very quickly; the speed with which they appeared suggests that they were formed equally fast.

Although MGT-detected structural abnormalities were much more marked in the drowning group, they also were observed—albeit less frequently—in the exsanguination group, suggesting a common or similar mechanism in both types of death. This may be linked to the marked hypoxemia undergone by muscle fibers in both situations: in the drowning group due to asphyxia caused by liquid entering the airways, and in the exsanguination group to sudden hypovolemic shock. It seems that the intense "hypoxia" underwent by the exsanguination and drowning rats gives time for the mitochondrial changes to occur whilst the group of cervical dislocation does not

have enough time. In any case, these muscle changes suggestive of intense anoxia, might be seen in other kind of asphyxial deaths and can not be considered as specific of drowning deaths.

Although the precise mechanism leading to the formation of *ragged red* fiber and abnormal clumps of red material in the muscles studied here cannot yet be identified beyond doubt, it may well be that in response to the lack of oxygen prompted both by drowning and by hypovolemic shock, muscle mitochondria accumulate rapidly in the vicinity of muscle capillaries in order to enhance the efficacy of their oxidative metabolism, or simply that they undergo an immediate pathological reaction. In normal muscle fibers, subsarcolemmal mitochondria are known to accumulate close to capillaries in order to optimize the supply of oxygen and substrates. [21, 22] Modifications in the capillary network and in muscle fibers -particularly changes in mitochondrial volume density- are known to take place in response to changing functional demands due to hypoxia linked both to extreme altitude [23] and to prolonged submersion. [24] Adaptation of this kind, however, takes place progressively; here, rather than a gradual adaptation, modifications were part of a very fast fiber response to severe hypoxia. This might lead to the rapid formation of large mitochondrial clumps and thus to the appearance of ragged red fibers staining abnormally to MGT. This would seem to be borne out by the mitochondrial response to hypoxia. Muscle mitochondria display an early reaction to ischemia; 2-24 h after application of a tourniquet, rat skeletal muscle mitochondria undergo alterations leading to the appearance of ragged red fibers; [25] at the same time, there is evidence of mitochondrial crest loss or condensation. [26] Interestingly, Isozaki *et al* [27] reported abundant ragged red fibers at autopsy in the diaphragm of patients with chronic obstructive pulmonary disease, suggesting that they were formed under the relative hypoxic state in the overworking diaphragm.

However, whilst MGT staining revealed structural abnormalities in both the drowning and the exsanguination groups, one striking phenomenon was observed only in the drowning group: clear evidence of fiber degeneration, in the form of hypercontracted fibers, ghost fibers, whorled fibers and coil fibers, suggesting additional damage involving myofibrillar rupture later confirmed by electron microscopy. In our opinion these degenerative changes would appear to provide structural evidence of rhabdomyolysis caused by drowning. One cause of acquired rhabdomyolysis is linked to stress and metabolic disorders prompted by a near-drowning situation. [28, 29] Changes observed in the present study may be attributable to muscle damage as a result of sudden contractions caused by the muscular exertion involved in trying to get out of the water, and by forced breathing. However, we can not rule out completely the possible role of agonal convulsions as a cause of these degenerative changes, which could also take place in other asphyxial deaths as a consequence of intense muscle spasms. The high levels of myoglobin in urine reported in some causes of death, including drowning, are regarded as an indicator of muscle hyperactivity. [30]

Concentric muscle actions -including swimming and diaphragmatic breathing- appear to reproduce the patterns seen in traumatic injuries such as crush or over-strain. [31] Strenuous high-intensity exercise is known to lead to muscle damage characterized by rupture or disruption of contractile material; [32, 33] forced lengthening contractions have been found to induce the formation of swollen, rounded fibers due to disruption of the plasma membrane, apparent in irregular dystrophin staining.[34] It has been suggested that severe damage to the plasma membrane due to mechanical trauma may cause a massive influx of Ca^{++} and thence to hypercontraction. [35] Ghost-like fibers are non-specific degenerative fibers appearing in a whole range of myopathic disorders, including rhabdomyolysis; the main morphological feature of these early degenerative fibers is their pallor on histological staining and loss of activity on histochemical examination. [35] These varying degrees of myofibrillar lesion may therefore be regarded as an expression of muscle over-strain, linked either to an overworking diaphragm due to forced breathing, or to more intense contractions of anterior tibial, soleus, and EDL muscles as the subject tries to get out of the water. The changes are similar to those reported following strenuous exercise or in severe stress-induced rhabdomyolysis. No intramuscular bleeding was observed here, although it has been reported as the only significant finding in human neck, trunk, and arm muscles in cases of drowning. These haemorraghes have been attributed to agonal convulsions, hypercontraction and overexertion of the affected muscle groups. [8]

One limitation of this work is that we only studied three different causes of death: drowning, exsanguination and cervical dislocation. The reasons behind this election are restrictions about our experimental study with animals. [17] It would have been better not to use anesthesia in any of the groups, as well as to add some groups of other mechanical asphyxia -manual or ligature strangulation, hanging, smothering…- but ethical reasons prevent us from doing so.

In conclusion, our experimental findings indicate that there are histologically-evident changes in skeletal muscle after death by drowning. Microscopic features of these changes suggest two possible mechanisms: intense anoxia, a mechanism common to death by drowning and by exsanguination; and mechanical trauma -found only in death by drowning- linked to intense muscle contractions due to the over-strain involved in forced breathing and in trying to get out of the water. Nevertheless, future research on human skeletal muscle in drowning and other asphyxial deaths is needed to confirm these findings and its possible usefulness in the diagnosis of this type of death, taken in conjunction with other forensic data.

Acknowledgments

We are grateful to Mr. Antonio Agüera for all technical support and assistance.

References

1. Kobayashi M, Takatori T, Nakajima M, Saka K, Iwase H, Nagao M, Niijima H, Matsuda Y. Does the sequence of onset of rigor mortis depend on the proportion of muscle fibre types and on intra-muscular glycogen content? *Int J Legal Med*. 1999;112:167–171.
2. Tomita Y, Nihira M, Ohno Y, Sato S. Ultrastructural changes during in situ early postmortem autolysis in kidney, pancreas, liver, heart and skeletal muscle of rats. *Legal Medicine*. 2004;6:25–31.
3. Tavichakorntrakool R, Prasongwattana V, Sriboonlue P, Puapairoj A,Pongskul J, Khuntikeo N, Hanpanich W, Yenchitsomanus P, Wongkham C, Thongboonkerd V. Serial analyses of postmortem changes in human skeletal muscle: A case study of alterations in proteome profile, histology, electrolyte contents, water composition, and enzyme activity. *Proteomics Clin Appl.* 2008;2:1255–1264.
4. Henssge C, Wang H, Hoppe B. Light microscopical investigations on structural changes of skeletal muscle as artifacts after postmortem stimulation. *For Sci Int.* 2002;125:163–171.
5. Sieb JP. Myopathies due to drugs, toxins, and nutritional deficiency. In: *Myology. Basic and Clinical.* (Eds. AG Engel, C Franzini-Armstrong). Vol 2. Third edition, McGraw-Hill, Medical Publishing Division. New York; 2004:1693-1712.
6. Sigrist T, Germann U. Homicide by mechanical suffocation - yes or no? The value of histology of skeletal muscles. *Z Rechtsmed.* 1989;102:549-557.
7. Tabata N. Morphological changes in traumatized skeletal muscle: The appearance of 'opaque fibers' of cervical muscles as evidence of compression to the neck. *For Sci Int.* 1998;96:197–214.
8. Puschel K, Schulz F, Darrmann I, Tsokos M. Macromorphology and histology of intramuscular hemorrhages in cases of drowning. *Int J Legal Med.* 1999;112:101-106.
9. Alexander RT, Jentzen, JM. Neck and scleral hemorrhage in drowning. *J Forensic Sci.* 2011;56:522-525.
10. Badu IK, Girela E, Beltrán CM, Ruz-Caracuel I, Jimena I. Diatoms in forensic analysis. A practical approach in rats. *Med Sci Law.* 2015;55:228–235.
11. Sewry CA, Goebel HH. General pathology of muscle disease. In: *Muscle disease: pathology and genetics.* (Eds., HH Goebel, CA Sewry, RO Weller). International Society of Neuropathology. John Wiley & Sons, Ltd; 2013:19-38.
12. Davies AS, Gunn HM. Histochemical fibre types in the mammalian diaphragm. *J Anat.* 1972;112:41-60.
13. Yellin H. Differences in histochemical attributes between diaphragm and hindleg muscles of the rat. *Anat Rec.* 1972;173:333-340.

14. Pullen AH. The distribution and relative sizes of three histochemical fibre types in the rat tibialis anterior muscle. *J Anat.* 1977;123:1-19.
15. Pullen AH. The distribution and relative sizes of fibre types in the extensor digitorum longus and soleus muscles of the adult rat. *J Anat.* 1977;123:467-486.
16. Dulhunty AF, Dlutowski M. Fiber types in red and white segments of rat sternomastoid muscle. *Am J Anat.* 1979;156:51-62.
17. Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. Official Journal of the European Union. 20/10/2010;276:33-79.
18. Dubowitz V, Sewry CA. *Muscle Biopsy: A Practical Approach*, 3rd edn, Saunders Elsevier, London; 2007.
19. Engel AG, Banker BQ. Ultrastructural changes in diseased muscle. In: *Myology. Basic and Clinical* (AG Engel, C Franzini-Armstrong, eds). Third edition. McGraw Hill, New York; 2004:749-887.
20. Swash M, Scwartz MS, Sargeant MK. The significance of ragged-red fibres in neuromuscular disease. *J Neurol Sci.* 1978;38:347-355.
21. Baba K, Kawamura T, Shibata M, Sohirad M, Kamiya A. Capillary-tissue arrangement in the skeletal muscle optimized for oxygen transport in all mammals. *Microvascular Research.* 1995;49:163-179.
22. Hoppeler H, Weibel ER. Structural and functional limits for oxygen supply to muscle. *Acta Physiol Scand.* 2000;168:445-456.
23. Howald H, Hoppeler H. Performing at extreme altitude: muscle cellular and subcellular adaptations. *Eur J Appl Physiol.* 2003;90:360-364.
24. Bae KA, An NY, Kwon YW, et al. Muscle fibre size and capillarity in Korean diving women. *Acta Physiol Scand.* 2003;179:167-172.
25. Heffner RR, Barron SA. The early effects of ischemia upon skeletal muscle mitochondria. *J Neurol Sci.*1978;38:295-315.
26. Carmo-Araújo EM, Dal-Pai-Silva M, Daal-Pai V, Cecchini R, Anjos-Ferreira AL. Ischaemia and reperfusion effects on skeletal muscle tissue: morphological and histochemical studies. *Int J Exp Path.* 2007;88:147-154.
27. Isozaki E, Miyamoto K, Tanabe H, Oda M. Morphological changes in human diaphragm-ragged red fiber, core/targetoid fiber, cytoplasmic body, and ring fiber. *Rinsho Shinkeigaku.* 1989;29:726-733.
28. Warren JD, Blumbergs PC, Thompson PD. Rhabdomyolysis: a review. *Muscle Nerve.* 2002;25:332-347.
29. Seon EY, Rhee H, Lee N, Lee SJ, Song SH, Lee DW, Lee SB, Sol MY, Kwak IS. A Case of Severe Acute Kidney Injury by Near-Drowning. *J Korean Med Sci.* 2012;27:218-220.

30. Zhu BL, Ishida K, Quan L, Taniguchi M, Oritani S, Kamikodai Y, Fujita MQ, Maeda H. Post-mortem urinary myoglobin levels with reference to the causes of death. *For Sci Int.* 2001;115:183-188.
31. Stauber WT, Smith CA. Cellular responses in exertion-induced skeletal muscle injury. *Mol Cell Biochem.* 1998;179:189-196.
32. Salminen A, Vihko V. Susceptibility of mouse skeletal muscles to exercise injuries. *Muscle Nerve.* 1983;8:596-601.
33. Magaudda L, Di Mauro D, Trimarchi F, Anastasi G. Effects of physical exercise on skeletal muscle fiber: ultrastructural and molecular aspects. *Basic Appl Myol.* 2004;14:17-21.
34. Komulainen J, Takala TES, Kuipers H, Hesselink MKC. The disruption of myofibre structures in rat skeletal muscle after forced lengthening contractions. Pflügers Arch - *Eur J Physiol.* 1998;436:735-741.
35. Carpenter S, Karpati G. *Pathology of skeletal muscle.* 2nd edition. Oxford University Press. New York; 2001.

Legends to Figures

Fig 1 *Drowning group. Soleus muscle*. Hypercontracted fibers (arrows) showed great size, high staining and rounded profiles. Masson trichrome, 20x (original magnification, *om)*

Fig 2 *Drowning group. Anterior tibial muscle*. Many very large fibers showing distortions of their architecture (whorled fibers) (arrows). H&E, 10x *(om)*

Fig 3 *Drowning group. Anterior tibial muscle.* Several degenerative muscle fibers showing different patterns: whorled fibers with acidophilic sarcoplasm, fisures, and internal nuclei (thin arrows), granular fibers (arrowheads) and ghost fibers with pale sarcoplasm (thick arrow). H&E, 40x *(om)*

Fig 4 *Drowning group. Anterior tibial muscle.* Three muscle fibers showed basophilic granular stain (head arrows) which corresponds with the *ragged red fiber.* H&E, 40x *(om)*

Fig 5 *Drowning group. Sternohyoid muscle.* Muscle fibers showing degenerative changes include ghosts or pale fibers (arrows) along with others containing reddish mass inside. There is also interstitial edema. Masson trichrome, 40x *(om)*

Fig 6 *Drowning group. Anterior tibial muscle.* Presence of red-staining material with different morphologies in several muscle fibers are prominent: peripheral balloons (arrowhead), crescent or ridge (thick arrow) and interstitial red material (thin arrows). MGT, 40x *(om)*

Fig 7 *Drowning group. Anterior tibial muscle.* Muscle fibers with reddish clusters as a hood. MGT, 40x *(om)*

Fig 8 *Drowning group. Anterior tibial muscle.* Muscle fiber similar to the above. MGT, 40x *(om)*

Fig 9 *Drowning group. Anterior tibial muscle.* A typical ragged red fiber. MGT, 40x *(om)*

Fig 10 *Drowning group. Anterior tibial* muscle. The arrows indicate small muscle fibers, some of them are fully occupied by material of high oxidative activity.SDH, 40x *(om)*

Fig 11 *Drowning group. Diaphragm muscle.* Several muscle fibers containing intense oxidative enzyme activity in peripheral and internal regions. NADH-tr, 20x *(om)*

Fig 12 *Drowning group. EDL muscle*. Low-magnification of part of three fibers with large subsarcolemmal aggregates of normal and abnormal mitochondria (asterisks). Many of the intermyofibrillar mitochondria also have degenerative changes. Transmission Electron Microscopy (TEM).

Fig 13 *Drowning group. Diaphragm muscle.* Large masses of degenerative mitochondria between hypercontracted myofibrils. Mitochondria vary in size and shape, and many of them are swollen with breaks in their cristae. TEM

Fig 14 *Drowning group. Diaphragm muscle.* Massive accumulation of degenerative mitochondria showing vacuolization and swelling. TEM

Fig 15 *Drowning group. Diaphragm muscle.* The myofibrils are broken and shows streaming and disintegration of the Z disks (arrows), mitochondria are swollen and the sarcotubular profiles are dilated. TEM

Fig 16 *Exsanguination group. Diaphragm muscle.* Several muscle fibers showing red peripheral balloons. MGT, 20x *(om)*

Fig 17 *Exsanguination group. Anterior tibial* muscle. The fiber shows no evidence of myofibrillar degeneration but many vacuolated mitochondria with disorganized or absent cristae can be seen in the subsarcolemmal and intermyofibrillar locations. Normal mitochondria are also observed (asterisk). TEM

Fig 18 *Cervical dislocation group. Diaphragm muscle.* Muscle fibers do not show abnormalities. MGT, 40x *(om)*

Fig 19 *Cervical dislocation group. Masseter muscle.* Normal checkerboard distribution of histochemical fiber types; note the absence of abnormalities in muscle fibers. NADH-tr, 20x *(om)*