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ACUTE RHABDOMYOLYSIS ("TYING-UP") IN STANDARDBRED HORSES

A MORPHOLOGICAL AND BIOCHEMICAL STUDY*

By

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LINDHOLM, A., H.-E. JOHANSSON & P. KJÆRSGAARD: *Acute rhabdomyolysis ("tying-up") in standardbred horses. A morphological and biochemical study.* Acta vet. scand. 1974, *15*, 325—339. — Morphological, biochemical and histochemical changes were studied in muscle needle biopsy specimens (gluteus medius) from 59 standardbred trotters with acute clinical symptoms of the "tying-up" disease. All horses had increased levels of serum enzymes SGOT and SCPK. The biopsy specimens were taken at various intervals after onset of clinical symptoms (1—4 hrs., 18—24 hrs. and 2—20 days). By light microscopy it was shown that the muscular alterations had a focal distribution and were of the hyalin degeneration type with insignificant inflammatory reaction and slight calcification. The ultrastructural changes apparently commenced with myofibrillar waving, mitochondrial and sarcotubular alterations and terminated with myofibrillar degeneration and necrosis with invasion of inflammatory cells. The inflammatory cells were ultrastructurally similar to monocytes and macrophages. The degenerative changes mainly comprised fast twitch (FT and FTH) fibres as histochemically evidenced by myofibrillar ATPase and alkaline phosphatase staining.

Biopsies from diseased muscle 1—4 hrs. after the onset of "tying-up" contained a low muscle concentration of glycogen, ATP and CP and a high concentration of lactate and glucose. Hence it is suggested that the described muscular alterations may be caused by a deranged carbohydrate metabolism caused by a local hypoxia.

It was found that the "tying-up" disease resembled idiopathic rhabdomyolysis in man and was thus designated "equine rhabdomyolysis".

histochemistry; horse; rhabdomyolysis; skeletal muscle; "tying-up"; ultrastructure.

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"Tying-up" or "equine paralytic myoglobinuria" is a well-known myopathy in race-horses and is found both in standardbred trotters and in thoroughbreds (e. g. *Lindholm* 1972, *McLean* 1973). The disease is seen throughout the year (*Meginnis* 1957). Clinical symptoms are rather uniform, but the intensity of symptoms may vary greatly.

The initial signs, consisting of profuse sweating and gait disturbance, may appear after only 10—20 min. of light exercise (slow trotting at 5 m $\times$ sec.$^{-1}$). In severe cases, horses are unable to move their hindlegs, and swelling and rigidity in croup muscles soon develop. Myoglobinuria is often seen. This disease is haematologically characterized by elevated serum enzyme levels for SGOT (glutamic-oxalacetic-transaminase) and SCPK (creatine-phosphokinase), the levels of which are considered to reflect the severity of the disease, (e. g. *Cardinet et al.* 1967 and *Lindholm* 1972). The light microscopic (LM) morphology is well-known and is characterized by hyalin degeneration, fragmentation of the fibres and insignificant inflammatory reaction (*Jubb & Kennedy* 1963, *Smith & Jones* 1966). However, the ultrastructural morphology has not been investigated.

The purpose of the present investigation, using a muscle biopsy technique, was to elucidate morphological electron microscopic (EM) and light microscopic muscular alterations at different times after the onset of acute "tying-up" symptoms in standardbred race-horses. In addition, a biochemical and histochemical study was carried out in an attempt to gain a better understanding of the disease.

MATERIAL AND METHODS

The investigation comprised 59 standardbred trotters, 28 females and 31 males (16 stallions and 15 geldings), 2—8 years old, with clinical symptoms of "tying-up" and elevated serum enzyme levels for SGOT and SCPK. Six horses (1 mare, 2 stallions and 3 geldings), 4—8 years of age, with no clinical signs of myopathy and with normal SGOT and SCPK blood enzyme values (*Lindholm* 1972), served as controls. All examined horses were in regular training by professional trainers at Solvalla Race Track, Stockholm, and were regularly raced.

Biopsy specimens were taken for light microscopic, electron microscopic, biochemical and histochemical investigations. The light microscopic study was performed on 43 biopsies from 35

horses whereas the electron microscopic investigation comprised 30 biopsy specimens from 17 horses. Biochemical analyses comprised 33 biopsy specimens from 25 horses and the histochemical investigation 6 biopsies from 6 horses. The biopsy specimens were taken from the gluteus medius muscle as described by *Lindholm & Piehl* (1974) at various times after the onset of the disease (1—4 hrs., 18—24 hrs. and 2—20 days).

Muscle biopsy samples for light microscopic investigation were fixed in 10 % neutral formalin after a 10—15 min. exposure to open air. Paraffin sections were stained with haematoxylin and eosin, Gomori's trichrome and van Kossa methods. Muscle specimens for electron microscopic investigation were immediately fixed in cold 3 % glutaraldehyde, 0.067 M cacodylate buffer (pH 7.4). Post-fixation was performed in 2.67 % OsO_4 and 0.067 M s-collidine buffer. The specimens were embedded in Epon and sectioned on a LKB Ultrotome. Ultrathin sections were stained with uranyl acetate or lead citrate and examined in a Philips RA 201 electron microscope or a Siemens Elmiscope 101.

The biopsy specimens for biochemical analyses were immediately frozen in liquid nitrogen, and muscle concentrations of glycogen, glucose, glucose-6-phosphate (G-6-P), adenosinetriphosphate (ATP), creatine phosphate (CP) and lactate were determined with the Lowry methods as described by *Karlsson et al.* (1970). In histochemical analyses, the identification of muscle fibres was based on the intensity of staining for myofibrillar adenosine triphosphatase (ATPase) (*Padykula & Herman* 1955) and reduced nicotinamide adenine dinucleotide diaphorase (NADH-diaphorase) (*Novikoff et al.* 1961). The various fibres were identified according to *Lindholm & Piehl*. In addition, 1 section was stained for alkaline phosphatase using the method described by *Gomori* (1939).

SGOT was determined according to *Reitman & Frankel* (1957) and SCPK according to *Kuby et al.* (1954), as modified by *Okinaka et al.* (1961), with Sigma reagent no. 661.

Statistical methods

Student's t-test was used for statistical comparison of mean group values. The following degrees of probability were used for significance: highly significant $P < 0.001^{***}$, significant $0.001 < P < 0.01^{**}$ and almost significant $0.01 < P < 0.05^{*}$.

RESULTS

Light microscopy, controls

No morphological alterations were seen in the muscle biopsy specimens.

Electron microscopy, controls (Figs. 1, 2 and 3)

Two major fibre types were identified by electron microscopic means (Fig. 1). One type was rich in mitochondria (the mitochondria having a subsarcolemmic and interfibrillar position) and lipid vacuoles having a juxtamitochondrial position (Fig. 2). The other fibre type had a smaller number of mitochondria and lipid vacuoles (Fig. 3), but was rich in glycogen, especially at subsarcolemmic sites. On the basis of histochemical staining, 3 major fibre types have been identified in horse skeletal muscle (*Lindholm & Piehl* 1974). In the present investigation, however, no attempts at ultrastructural classification of the 3 different fibres were made.

Light microscopy, 1—4 hrs. after the onset of acute symptoms (Figs. 4, 5 and 6)

Moderate diffuse interstitial oedema was present in most biopsy specimens. Degenerative fibre alterations were common. Degenerative changes had a typically focal character, comprising either small parts of single fibres or larger areas in several fibres, as evidenced in serial sectioning. Degenerative changes occurred in the form of fibre homogenization with a loss of cross-striation. The involved fibre parts were stained a pale, uniform red by haematoxylin and eosin. With Gomori's trichrome stain the same areas were either bright red or consisted of alternating bright red and pale red to light blue segments (Fig. 4). The nuclei of degenerated fibres were pyknotic. The altered fibres frequently displayed segmental derangement. This derangement usually comprised the sarcoplasm, leaving an empty sarcolemma (Figs. 5 and 6). Derangement was in some cases associated with an accumulation of mononuclear cells within the endomysium (Fig. 6). In addition to homogenized and unaltered fibres, wavy fibres were frequently seen. These fibres were void of distinct cross-striation.

Electron microscopy, 1—4 hrs. after the onset of acute symptoms (Figs. 7, 8 and 9)

Ultrastructural changes at this time were dominated by a broadening of the subsarcolemmic space in single fibres or groups of muscle fibres in all examined horses. This alteration was frequently associated with a broadening of the intermyofibrillar space (Fig. 7). These spaces were rich in glycogen. Uncommon fibres with pronounced broadening of the subsarcolemmic and intermyofibrillar spaces revealed slightly wavy myofibrils. The myofibrillar architecture was generally well-preserved. Slight focal dissociation of the myofilaments was occasionally seen in myofibres with wavy fibrils (Fig. 8). In general, the mitochondria were greatly enlarged with a dilated matrix of low electron density. Membranes and cristae were well-preserved. However, some enlarged mitochondria occasionally displayed lysis of the cristae. The sarcoplasmic reticulum was greatly dilated (Fig. 9).

Light microscopy, 18—24 hrs. after the onset of acute symptoms (Fig. 10)

Extensive interstitial oedema was present in most of the examined muscle biopsy specimens. This oedema was more pronounced around degenerated fibres. Degenerative fibre changes were similar to those described in the 1—4-hr. period. The inflammatory cells consisted exclusively of mononuclear cells. With van Kossa stain, numerous subsarcolemmic deposits of calcium were demonstrated in homogenized fibres (Fig. 10).

Electron microscopy, 18—24 hrs. after the onset of acute symptoms (Figs. 11—17)

During this period, both intact and altered fibres were seen, but altered fibres were more frequent than in the preceding period. Myofibrils frequently displayed dissociation and disruption of the myofilaments (myofibrillar degeneration). This disruption comprised single or multiple sarcomeres and was associated with both waving and dissociation of the Z-lines (streaming of the Z-line, *Mair & Tomé* 1972). Intact sarcomeres were shorter and a narrow I-band was prevalent, whereas disintegrated sarcomeres were considerably longer (Figs. 11 and 12). In addition to enlarged mitochondria similar to those seen in the preceding period, frequent small mitochondria with a condensed

appearance were seen. Partially disintegrated mitochondria were noted in large conglomerates. The presence of frequent large, electron-dense deposits was typical of all mitochondria. These changes were most frequently seen in fibres with few mitochondria (Fig. 13). The fibres relatively richer in mitochondria and lipid vacuoles frequently displayed increased numbers of juxtamitochondrial lipid vacuoles.

The presence of fibres completely void of cross-striation and glycogen was a noticable difference, as compared to the preceding period. These degenerated fibres contained a granular material in which filaments and mitochondria were seen. The mitochondria generally appeared condensed and contained numerous electron-dense deposits (Fig. 14). Conglomerates of bizarre-shaped mitochondria were seen on the periphery of these fibres, these mitochondria also containing numerous dense deposits (Fig. 15). Occasionally invasion of inflammatory cells were seen in the degenerated fibres. The cells were mainly of monocyte type and cells with macrophagic properties (Figs. 16 and 17).

Biochemical investigation

The gluteus medius muscle was found to contain more glycogen in the acute stage (1—4 hrs.) of "tying-up" than in the 2—20-day period following the onset of the disease ($0.01 < P < 0.05$) (Table 1). On the whole, glucose, G-6-P, ATP, CP and lactate concentrations were quite similar to values stated for normal standardbred trotters (*Lindholm & Piehl*).

In the early stage (1—4 hrs.), however, 4 horses exhibited values which deviated from those found in the other 8 horses in this group (Table 2), displaying low muscle ATP and CP concentrations (2.6 and 9.9 mmol $\times$ kg^{-1} wet muscle, respectively). However, they had high muscle lactate and glucose concentrations (5.3 and 3.1 mmol $\times$ kg^{-1}, respectively). Glycogen content was normal (106 mmol of glucose units $\times$ kg^{-1}). The remaining 8 horses displayed a high mean glycogen concentration (156 mmol of glucose units $\times$ kg^{-1}), whereas their lactate, ATP, CP, glucose and G-6-P levels were within normal limits. The maximal values observed for serum enzyme SGOT and SCPK were also higher in the 4 horses, with mean maximal values of 4465 and 2602 units, respectively. Corresponding values for the 8 remaining horses were 2877 and 894 units, respectively.

Table 1. Concentrations of muscle metabolites in the gluteus medius muscle of standardbred horses at different intervals after onset of the "tying-up" disease.

		1—4 hrs.	18—24 hrs.	2—20 days
		mmol × kg^{-1} wet muscle		
Glycogen (glucose units)	m.	139	131	118
	s	29.5	40.1	20.3
	range	91—179	56—168	89—164
	n	12	6	15
Lactate	m.	3.6	2.7	3.7
	s	1.69	0.55	1.62
	range	1.5—7.5	2.4—3.5	1.6—7.5
	n	12	6	15
ATP	m.	4.1	3.7	4.1
	s	1.43	1.34	1.12
	range	1.7—6.7	1.8—5.4	2.6—5.9
	n	12	6	15
CP	m.	15.1	16.3	17.9
	s	5.18	5.45	3.93
	range	6.3—21.7	7.3—21.4	9.2—25.1
	n	12	6	15
G-6-P	m.	0.7	1.0	0.8
	s	0.34	0.29	0.39
	range	0.3—1.3	0.8—1.3	0.4—1.3
	n	12	6	15
Glucose	m.	1.5	0.7	0.8
	s	1.45	0.40	0.23
	range	0.3—4.5	0.3—1.1	0.3—1.6
	n	12	6	15

In the second stage (18—24 hrs.), 1 horse was similar to the 4 horses of the first stage (1—4 hrs.) and displayed low muscle glycogen, ATP and CP concentrations; its lactate concentration was apparently normal. The remaining 7 horses exhibited a mean glycogen value as high as 146 mmol of glucose units × kg^{-1}, whereas their lactate, ATP, CP, G-6-P and glucose concentrations were normal. The maximal mean serum enzyme levels for SGOT and SCPK in this group amounted to 6366 and 1033 units, respectively.

In the third stage (2—20 days) the horses exhibited values similar to those reported in healthy standardbred horses (*Lindholm & Piehl*), but there were also a few minor exceptions in

Table 2. Concentrations of muscle metabolites in the gluteus medius muscle of standardbred horses 1—4 hrs. after onset of the disease. The horses are divided into 2 groups (A = 4 horses with less than 120 mmol × kg^{-1} w.m. and B = 8 horses with more than 120 mmol × kg^{-1} w.m.) according to the muscle glycogen concentration. The t-value denotes the significance of the difference between the mean values of the 2 groups.

		Group A		Group B
		mmol × kg^{-1} wet muscle		
Glycogen (glucose units)	m.	106		156
	s	10.0		18.6
	range	91—114		127—179
	t		6.13***	
Lactate	m.	5.3		2.7
	s	1.93		0.68
	range	3.0—7.5		1.5—3.4
	t		2.67*	
ATP	m.	2.6		5.0
	s	0.65		0.89
	range	1.7—3.2		4.1—6.7
	t		4.39**	
CP	m.	9.9		18.0
	s	4.51		2.73
	range	6.5—15.9		14.0—21.7
	t		3.30**	
G-6-P	m.	0.8		0.7
	s	0.38		0.35
	range	0.5—1.2		0.3—1.3
Glucose	m.	3.1		0.7
	s	1.51		0.37
	range	1.5—4.5		0.3—1.3
	t		2.73*	

this stage, although not as striking as in the first and second stages. The maximal mean values noted for SGOT and SCPK in this group were 7281 and 1315 units, respectively.

In order to classify the damaged fibres, a histochemical investigation was performed on 5 biopsy specimens obtained 1—4 hrs. after the onset of the disease. Twenty-four % of the fibres were ST, 50 % FTH and 26 % FT, a distribution similar to that reported for healthy standardbred horses (*Lindholm & Piehl*). Abnormal and extensive oedema with a broadening of the space between myofibres was seen in 3 horses.

An additional staining with alkaline phosphatase as described by *Gomori* (1939) was performed in the case of 1 horse biopsied 2 days after the onset of acute symptoms. According to *Engel & Cunningham* (1970) degenerated muscle fibres are darkly stained by this stain, and in this particular horse several darkly stained fibres were recognized in the muscle. These darkly stained, degenerated fibres were found to correspond to mainly fast twitch fibres on the basis of the myofibrillar ATPase staining. However, it was not possible to distinguish between degenerated FT and FTH fibres on the basis of NADH-diaphorase staining (Fig. 18).

DISCUSSION

The light microscopic investigation confirmed earlier observed muscular alterations in horses with "tying-up", i.e. a hyalin degeneration and necrosis of muscle fibres with an insignificant calcification and a slight inflammatory reaction (*Jubb & Kennedy* 1963, *Smith & Jones* 1966). Degenerative changes also proved to have a typical focal distribution.

The ultrastructural muscular alterations apparently take place in fast twitch fibres (i.e. FT and/or FTH fibres), which was supported by the finding of initial changes mainly in these fibres. The ultrastructural changes seen in the present investigation can be summarized as follows: myofibrillar waving, mitochondrial and sarcotubular alterations, myofibrillar degeneration and necrosis with the infiltration of inflammatory cells. The latter changes were present in a small number of fibres in contrast to the former more delicate initial changes which were seen in a great number of fibres.

It is suggested that the broadening of the subsarcolemmic and interfibrillar spaces at least in part is caused by an increased glycogen deposition. This assumption is supported by the increased values of glycogen biochemically determined in simultaneous biopsies.

Myofibrillar degeneration was seen in fibres displaying the aforementioned alterations, i.e. fibrillar waving and mitochondrial and sarcotubular swelling. Myofibrillar degeneration was seen as Z-line streaming and myofilamental dissolution which initially comprised single sarcomeres but involved whole fibres in later stages. It was observed that disintegrated sarcomeres were longer than adjacent intact sarcomeres, which were contracted.

The glycogen content of fibres declined as the spread of the myofibrillar degeneration increased, and completely destroyed fibres lacked glycogen. The end stage of the degenerative events occurred as a necrosis. At this stage scavenger cells of mononuclear type occurred within the necrotic sarcoplasm. These cells displayed ultrastructural similarities to monocytes and macrophages. The invasion of inflammatory cells within necrotic fibres was seen in a few cases already in the 1—4-hr. period. This surprisingly early occurrence of inflammatory cells can be explained by an eventual initial subclinic phase of the "tying-up" myopathy.

Biochemically most of the horses investigated displayed muscle glycogen and metabolite concentrations similar to normal healthy standardbred trotters. However, 4 horses in the 1—4-hr. stage and 1 horse in the 18—24-hr. stage displayed diverging values of glycogen and metabolites as well as serum enzymes SCPK and SGOT. These horses also displayed widespread electron microscopic alterations. It is suggested that these biopsies were obtained from muscle tissue with advanced degenerative changes. Since none of the examined horses had carried out strenuous exercise imposing lactacid anaerobic demands, an enhanced glycogen breakdown in the diseased muscles of these horses must have been due to some other cause. The fact that 1 horse in the 18—24-hr. group displayed glycogen, ATP and CP values similar to values in the 4 horses in the 1—4-hr. group indicates that the myopathy may be progressive and of long duration. Clinical observations support this surmise, as some horses could remain "tied-up" 24 hrs. after the outbreak of the disease.

The degenerative cellular events described above closely resemble those found in idiopathic rhabdomyolysis in man. *Scarpelli et al.* (1963), *Schutta et al.* (1969) and *Savage et al.* (1971) have shown that the mitochondrion in muscle fibres of man is the target organelle of the hitherto unknown insult or insults imposed on the muscle cell. *Schutta et al.* frequently noted excessive deposits of glycogen and suggested a possible abnormal carbohydrate metabolism. The aetiologic factors in idiopathic rhabdomyolysis in man remain unknown, although strenuous exercise (*Sparpelli et al.*) is one of the factors which have been noted in conjunction with the onset of acute symptoms of myopathy. *Kontos et al.* (1963) provided evidence that a defect in skeletal muscle metabolism, such as an uncoupling of oxidative

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Abbreviations in the figures

A	A-band	Mi	Mitochondrion
BC	Blood capillary	N	Nucleus
BM	Basement membrane	PM	Plasma membrane
E	Endothelial cell	RBC	Red blood corpuscle
FM	Filamentous material	Sa	Sarcomere
G	Glycogen	SR	Sarcoplasmic reticulum
H	H-zone	T	T-system
I	I-band	Tr	Triad
LB	Lipid body	Z	Z-line
M	M-line		

Figure 1. Control horse. A longitudinal section of 2 adjacent muscle fibres demonstrating differences in glycogen content and number of mitochondria. The upper fibre is relatively richer in mitochondria, the mitochondria having a subsarcolemmic and intermyofibrillar position. This fibre is a FTH fibre (fast twitch, high oxidative) or a ST fibre (slow twitch). The lower fibre is relatively richer in glycogen and is a FT fibre (fast twitch).
Lead citrate, 9,000 ×.

Figure 2. Control horse. Detail of upper fibre in Fig. 1 (FTH or ST fibre). Note the frequent mitochondria and abundant juxtamitochondrial lipid bodies in interfibrillar spaces.
Lead citrate, 13,500 ×.

Figure 3. Control horse. Detail of lower fibre in Fig. 1 (FT fibre). The interfibrillar spaces are rich in glycogen, but there are few mitochondria. The short I-band indicates contraction.
Lead citrate, 15,000 ×.

Figure 4. One to 4 hrs. after onset of acute "tying-up" symptoms (after o. o. a. "tying-up" s.). Longitudinal section demonstrating homogenization, granularity and segmental disruption of the cytoplasm.
Masson trichrome, 290 ×.

Figure 5. One to 4 hrs. after o. o. a. "tying-up" s. Longitudinal section. Degenerated fibres lack cross-striation and are homogenized and segmentally disrupted. Mononuclear cells are frequent.
H & E, 190 ×.

Figure 6. One to 4 hrs. after o. o. a. "tying-up" s. Cross-section of degenerated fibres among intact ones. Early degenerative changes visible as homogenization of muscle fibres (triangle). Fibres in advanced degeneration are partly excavated and contain granular sarcoplasmic material and frequent mononuclear cells (arrow), but the sarcolemma is retained (arrow head).
H & E, 210 ×.

Figure 7. One to 4 hrs. after o. o. a. "tying-up" s. Intact fibre in centre with numerous lipid vacuoles (arrow head). The lower fibre displays broadening of the subsarcolemmic and interfibrillar spaces (arrow). The myofibrillar architecture is obliterated in the upper fibre which consists of homogeneous (arrow) and granular (arrow head) material.
Thin section, toluidine blue, 520 ×.

Figure 8. One to 4 hrs. after o. o. a. "tying-up" s. Early changes in the form of waving of the myofibrils, broadening of the interfibrillar spaces (arrow head) and sarcomeric disintegration (arrow). Mitochondria are numerous in the left fibre but less numerous in the right one, whereas juxtamitochondrial lipid bodies are more frequent in the right fibre.
Uranyl acetate, 4,500 ×.

Figure 9. One to 4 hrs. after o. o. a. "tying-up" s. Enlarged mitochondria with dilated matrix of low electron density (arrows) and indistinct cristae. The sarcoplasmic reticulum is dilated (arrow heads).
Lead citrate, 30,000 ×.

Figure 10. Eighteen to 24 hrs. after o. o. a. "tying-up" s. Cross-section demonstrating degenerated fibres with subsarcolemmic calcium deposits (arrows).
van Kossa, 200 ×.

Figure 11. Eighteen to 24 hrs. after o. o. a. „tying-up" s. The intact sarcomeres in the centre (stars) are contracted (note the narrow I-bands). The surrounding myofibrils display streaming of the Z-lines (arrows) and filamental dissolution (triangles). The intact sarcomeres are shorter than disintegrated ones.
Lead citrate, 30,000 ×.

Figure 12. Eighteen to 24 hrs. after o. o. a. "tying-up" s. Myofibrillar degeneration (arrows) with streaming of the Z-line and myofilamental disintegration. The mitochondria in the left fibre are condensed. Mitochondria are more numerous in the right fibre than in the left one.
Uranyl acetate, 6,000 ×.

Figure 13. Eighteen to 24 hrs. after o. o. a. "tying-up" s. Detail of fibre with advanced myofibrillar degeneration. Note the electron-dense mitochondrial deposits (arrow heads). The matrix is dilated (arrows). Membranes and cristae are partly indistinct. Occasional mitochondria are condensed with densely packed cristae (triangle).
Lead citrate, 30,000 ×.

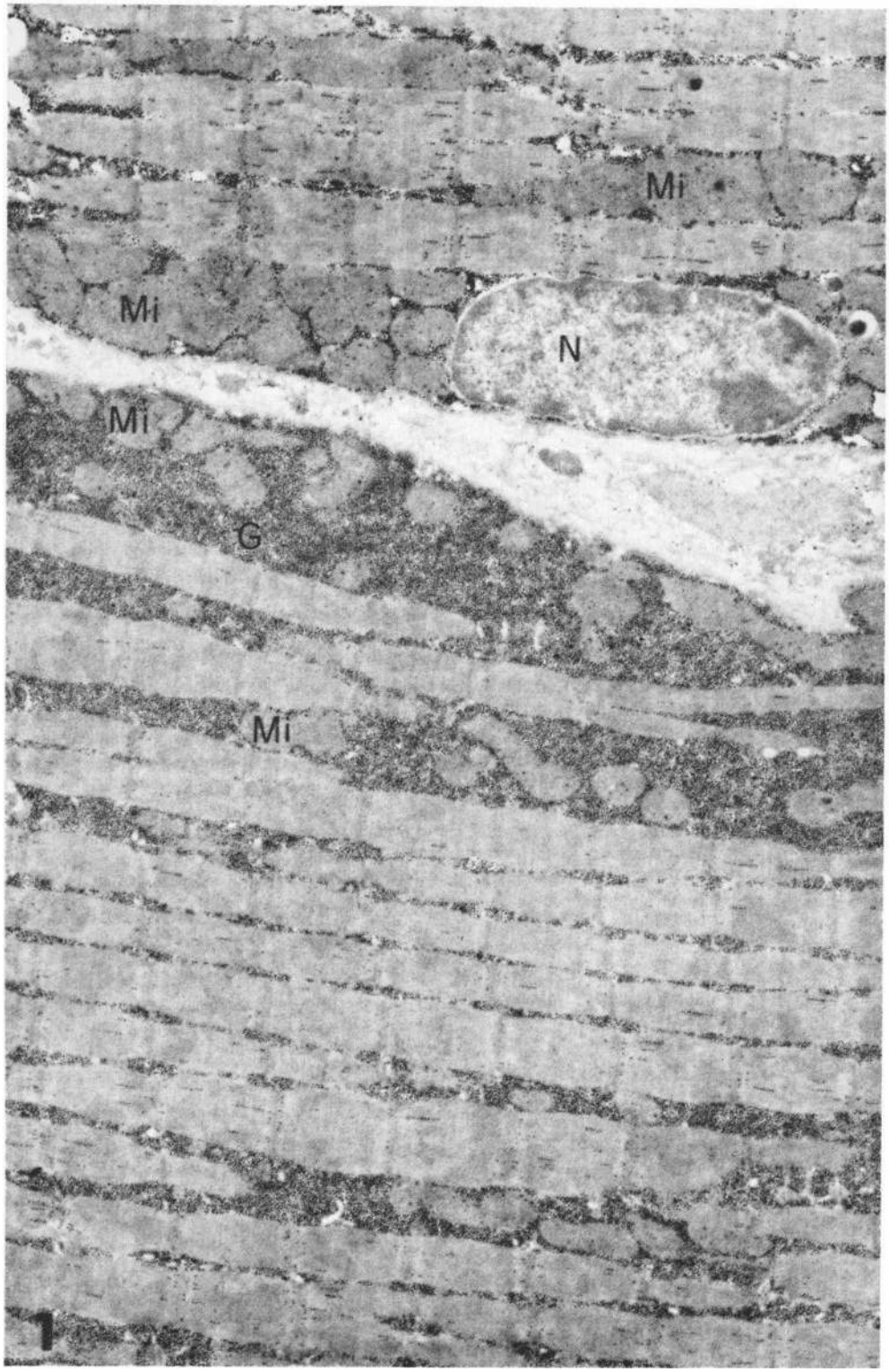
Figure 14. Eighteen to 24 hrs. after o. o. a. "tying-up" s. In the centre, a fibre completely void of myofibrillar architecture. Conglomerates of condensed mitochondria with electron-dense deposits are seen among granular and filamentous material. The fibre in the centre is surrounded by 2 intact fibres.
Uranyl acetate, 4,500 ×.

Figure 15. Eighteen to 24 hrs. after o. o. a. "tying-up" s. Detail of the central fibre in Fig. 14, showing granular and filamentous material (FM), mitochondrial cristae (triangle) and electron-dense mitochondrial deposits (arrow heads).
Uranyl acetate, 30,000 ×.

Figure 16. Eighteen to 24 hrs. after o. o. a. "tying-up" s. A monocyte-like cell in a necrotic muscle fibre.
Uranyl acetate, 11,000 ×.

Figure 17. Necrotic fibre containing a macrophage with abundant phagocytized material.
Uranyl acetate, 4,300 ×.

Figure 18. The panels show 3 different stains of serial sections (× 120) of the gluteus medius muscle of 1 horse 2 days after acute symptoms of "tying-up". The upper panel shows staining for myofibrillar ATPase, the middle for NADH-diaphorase and the lower for alkaline phosphatase. The 3 different fibre types are designated as follows: ST (▽▼), FTH (△▲) and FT (□■). Arrows denote degenerated fibres.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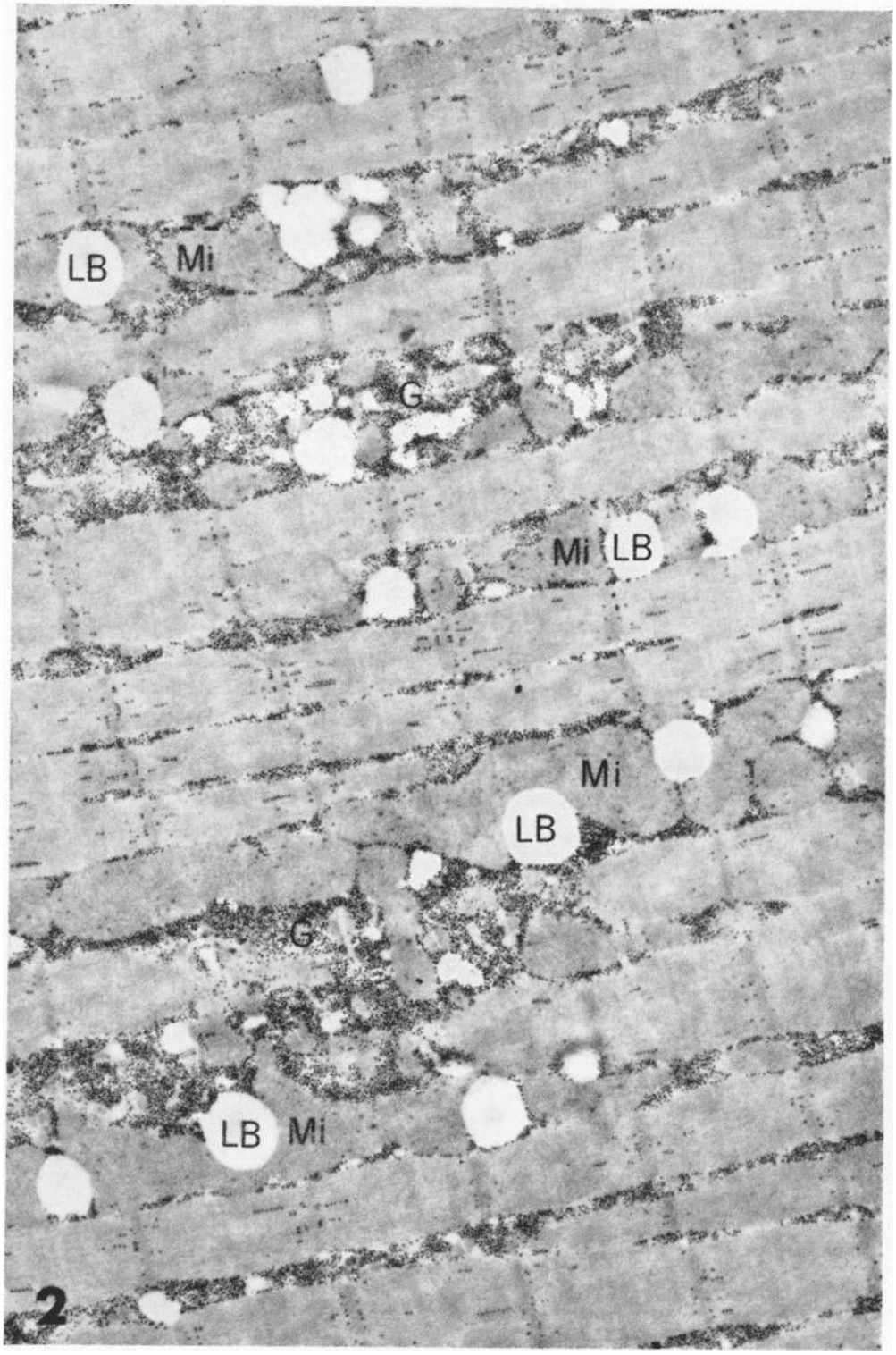
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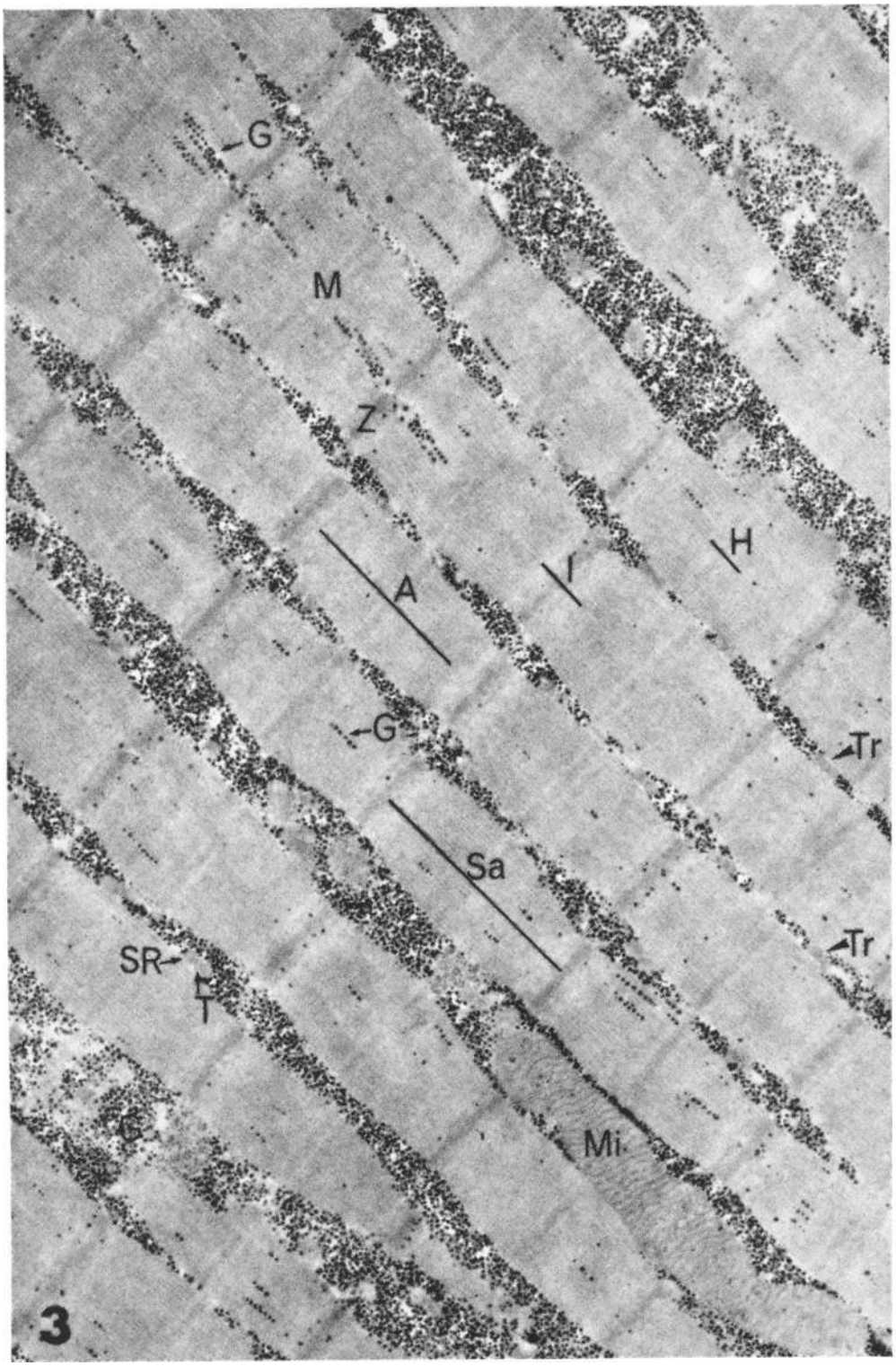
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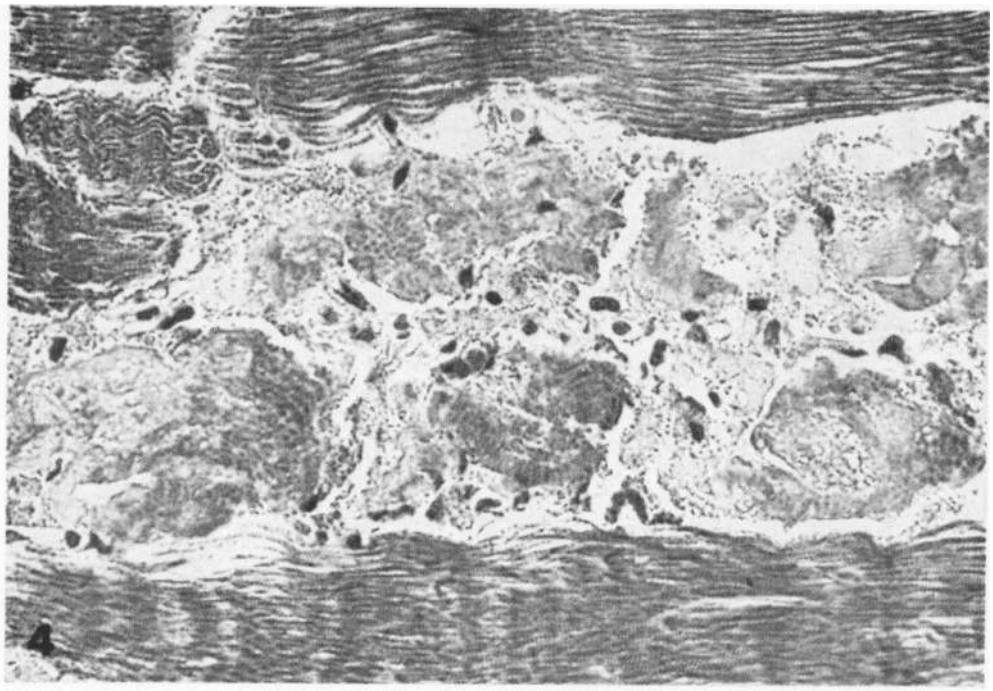

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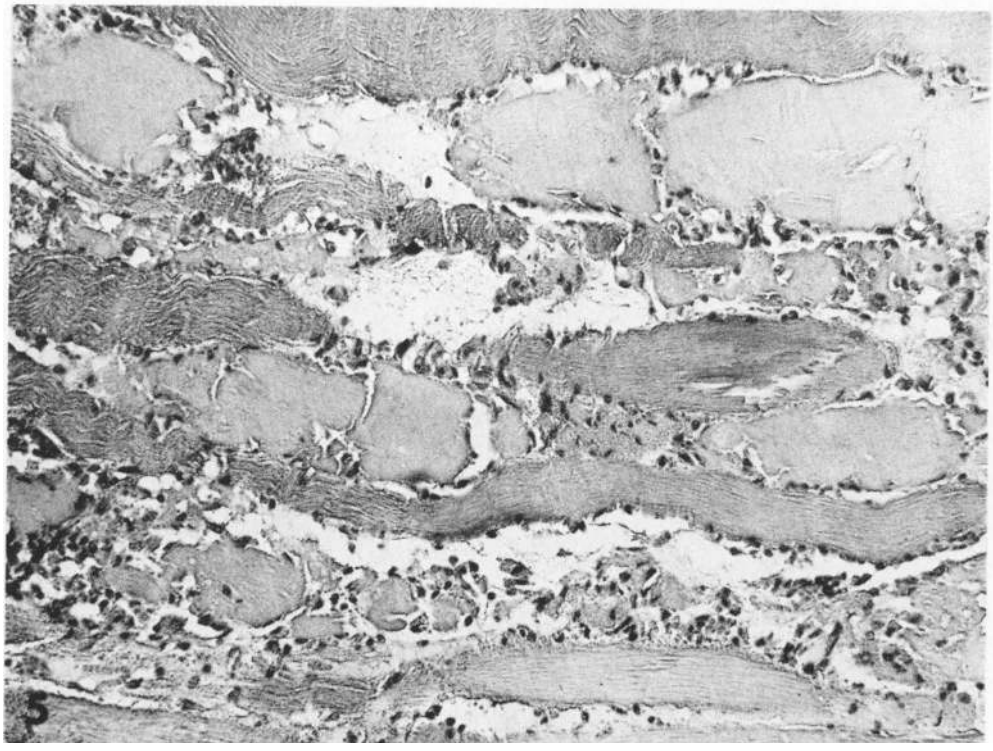

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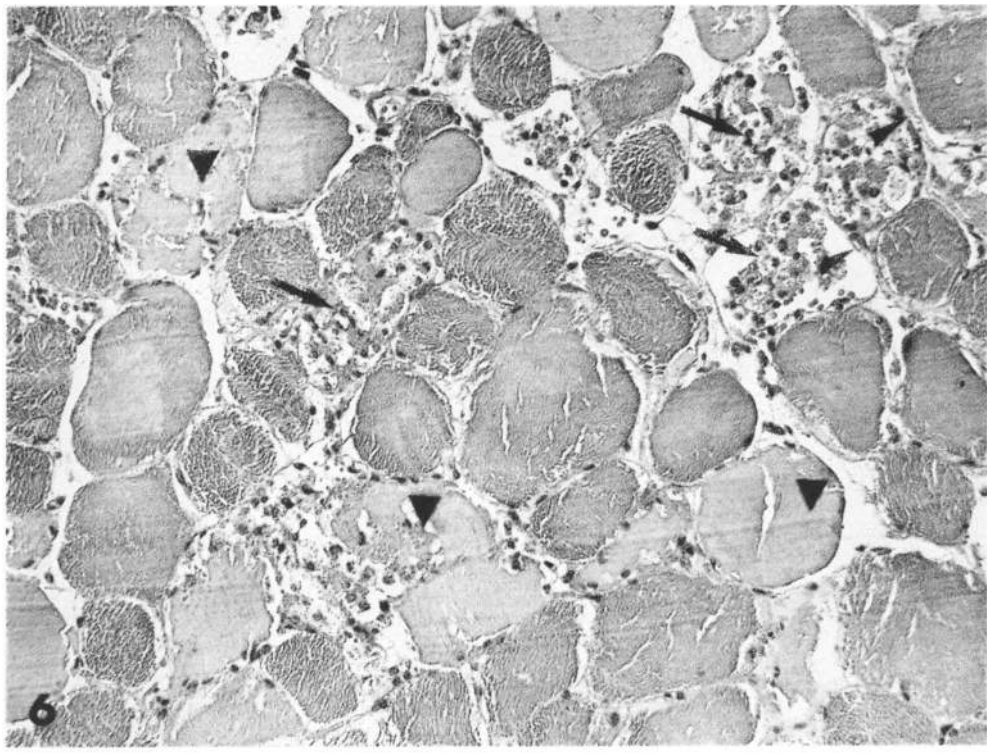
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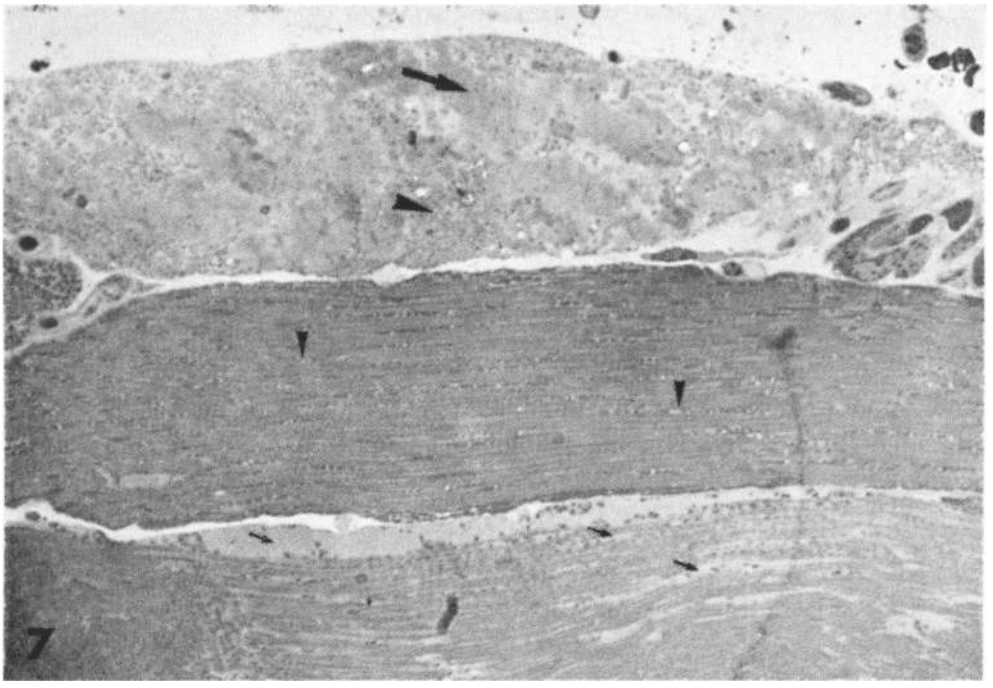
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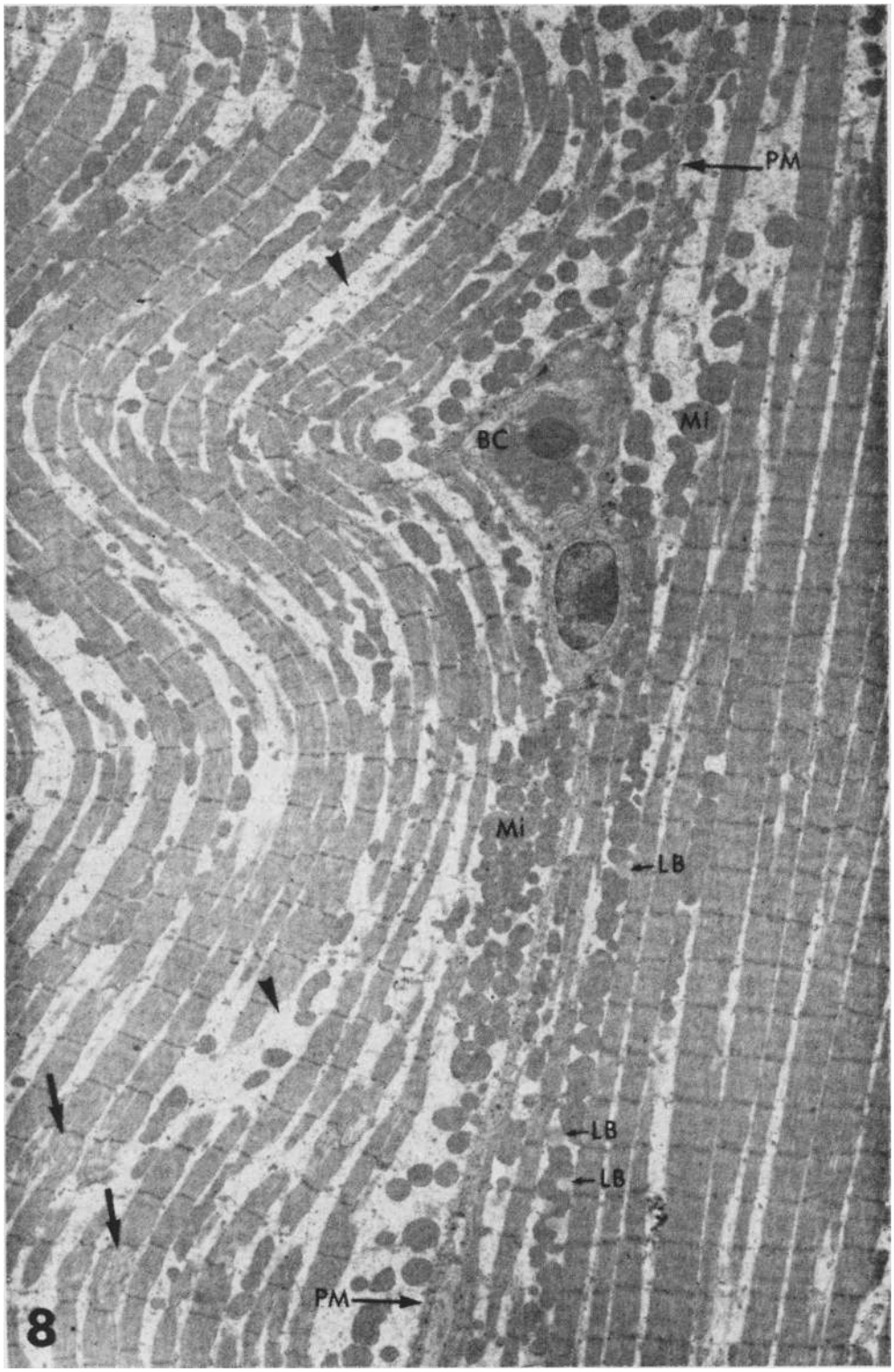
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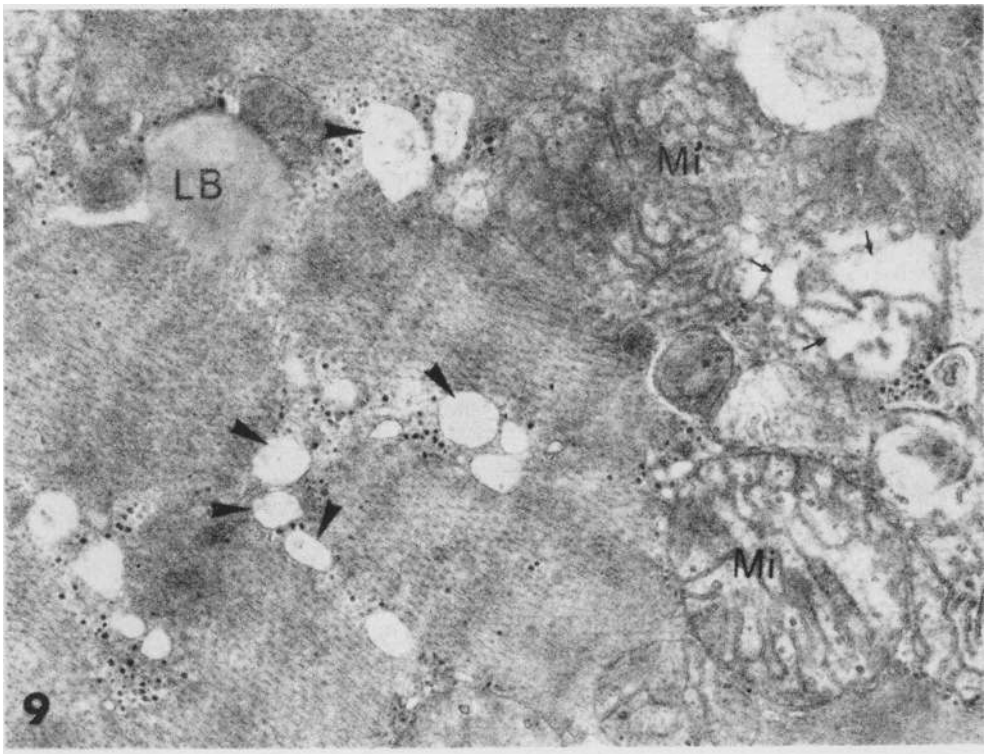
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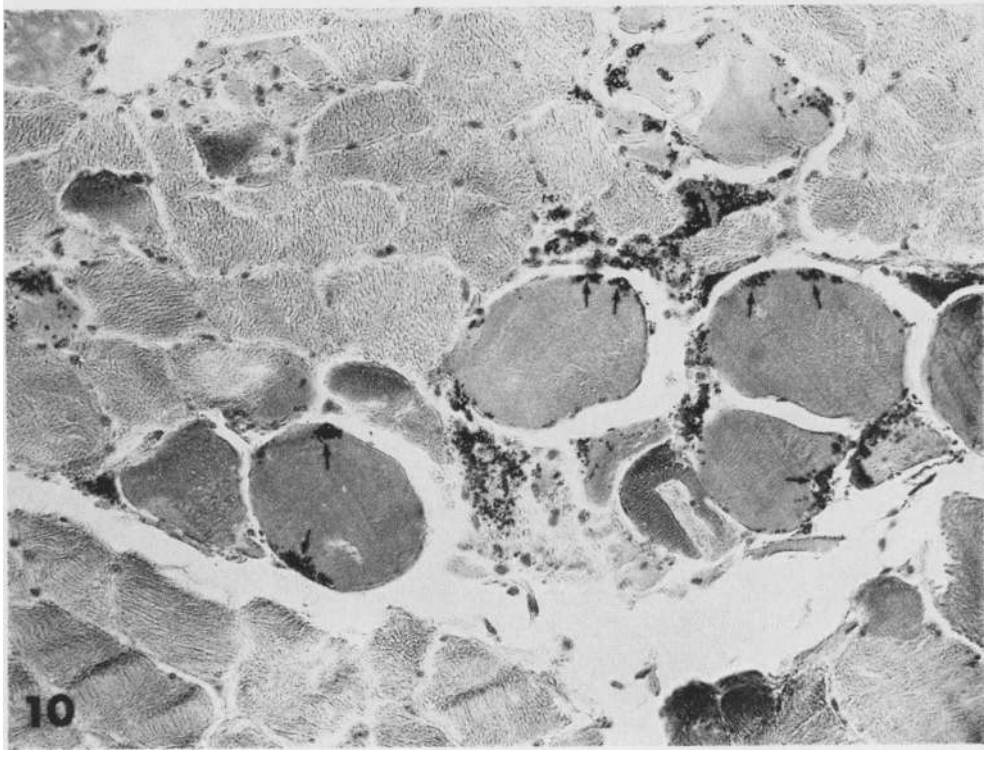
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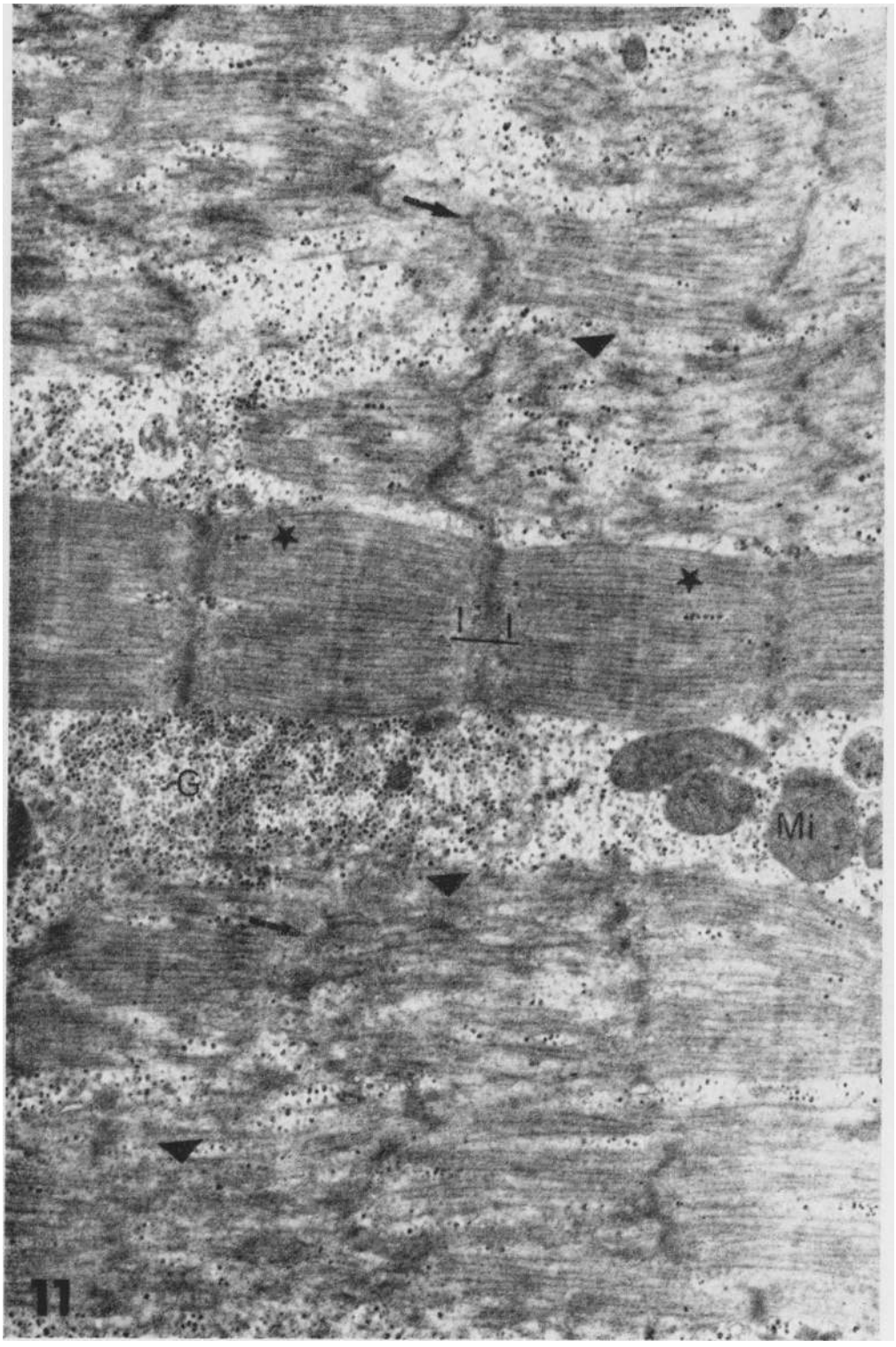
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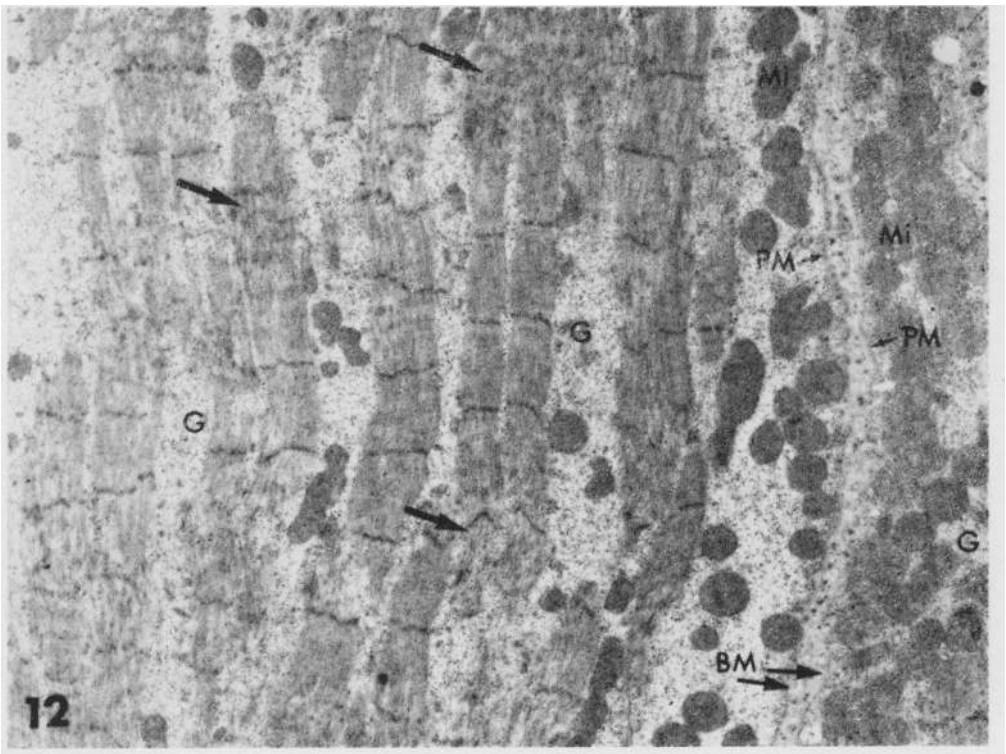

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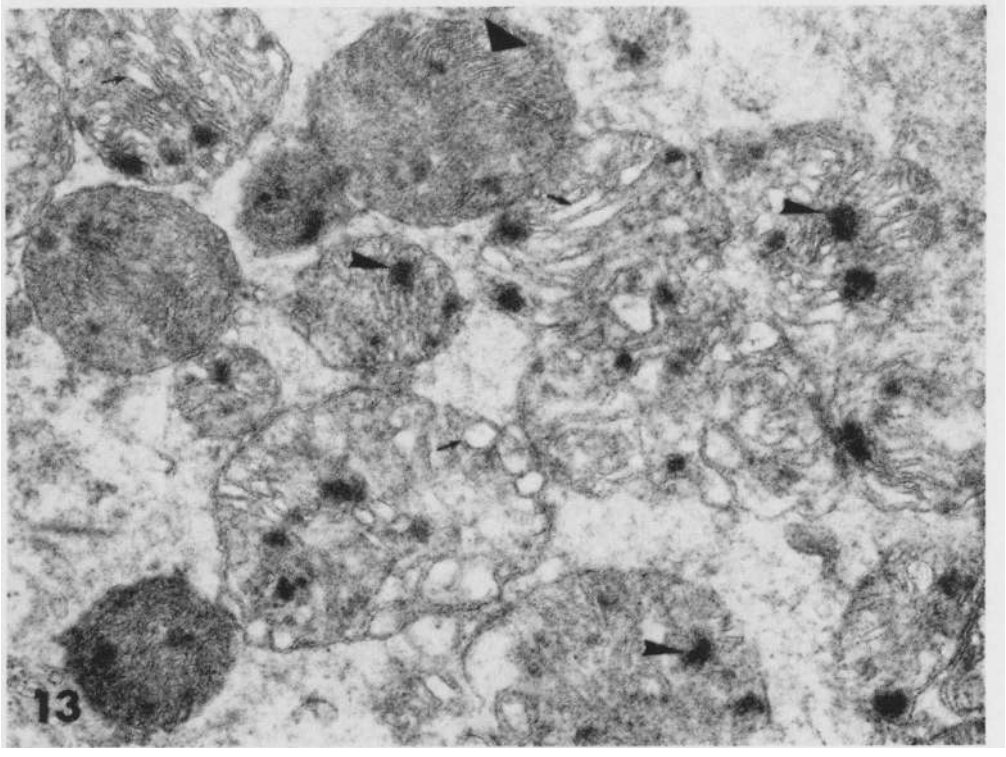
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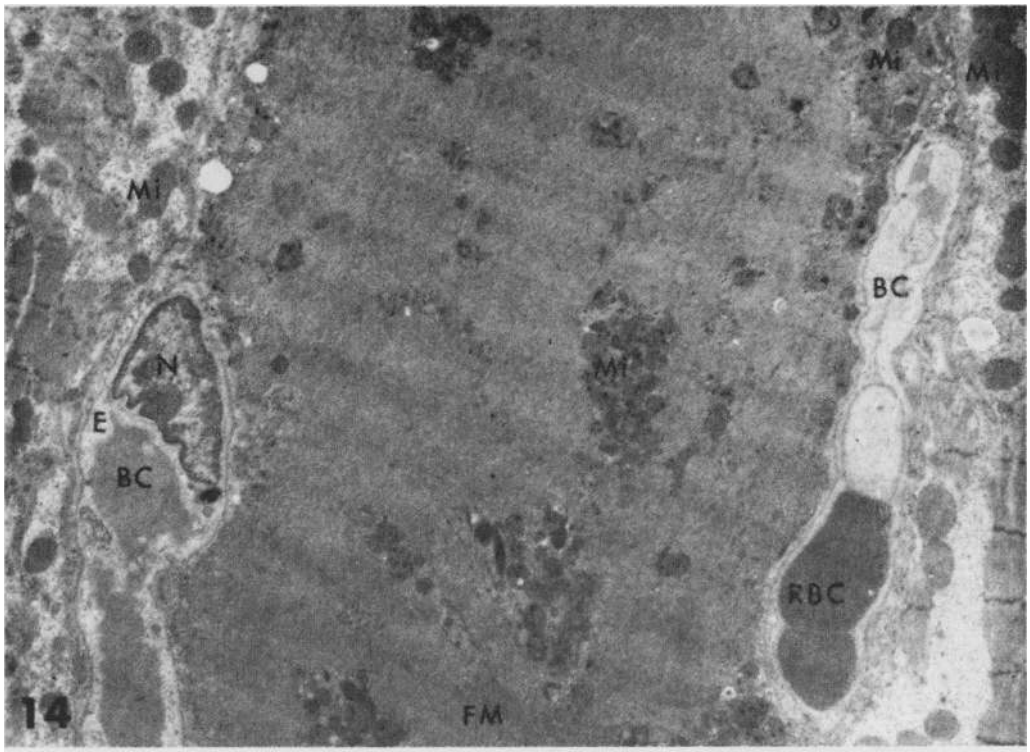

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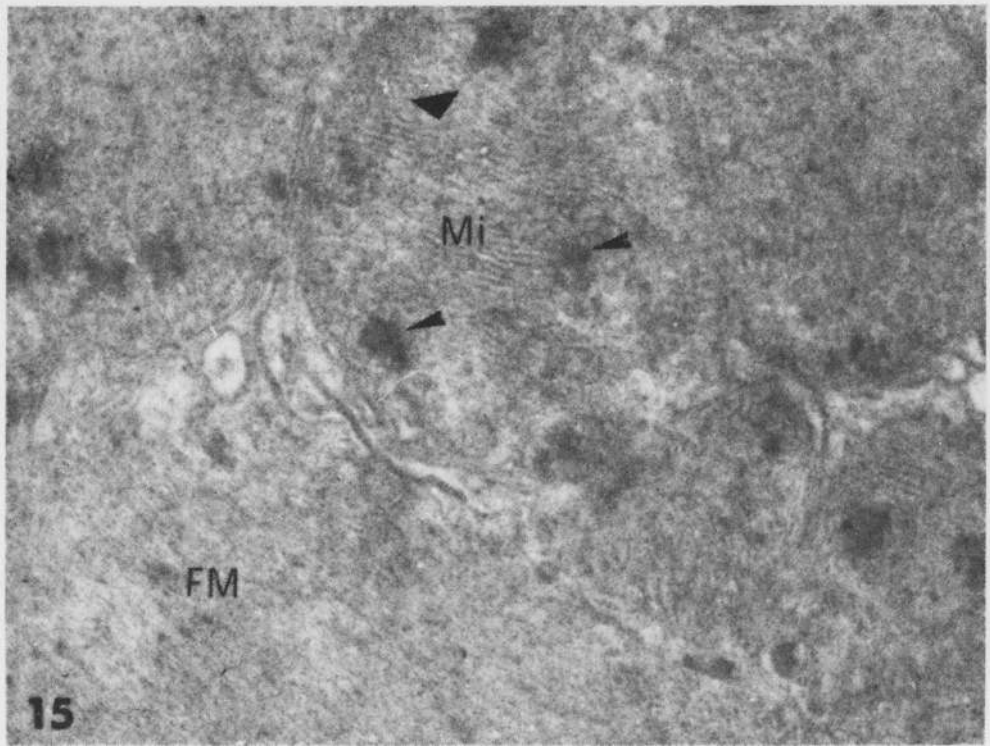

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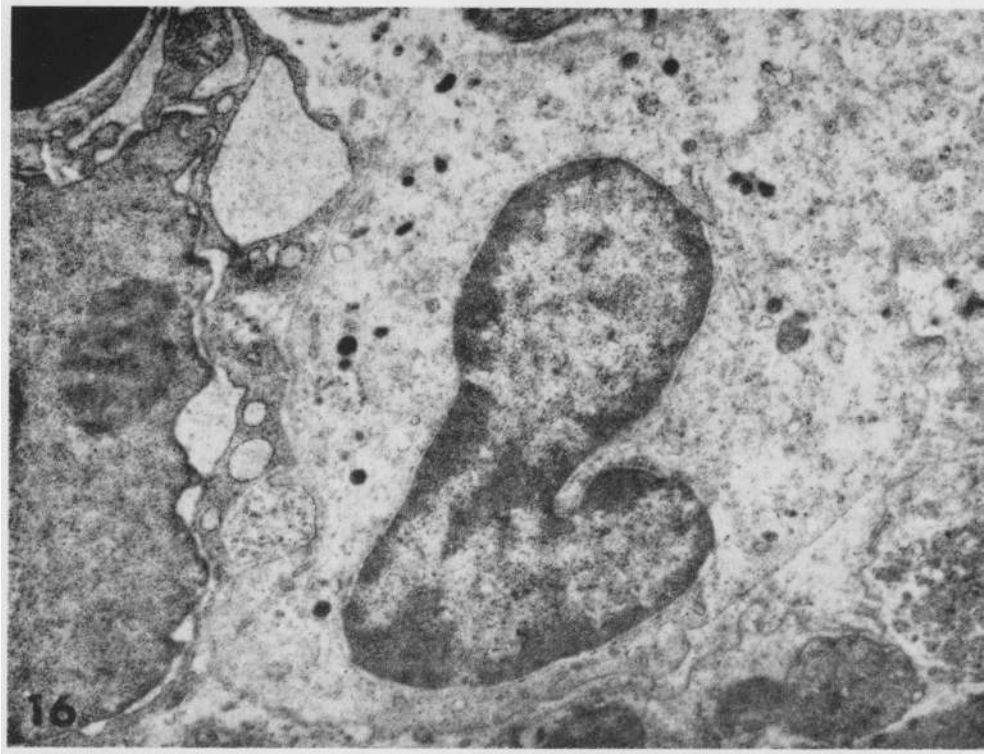

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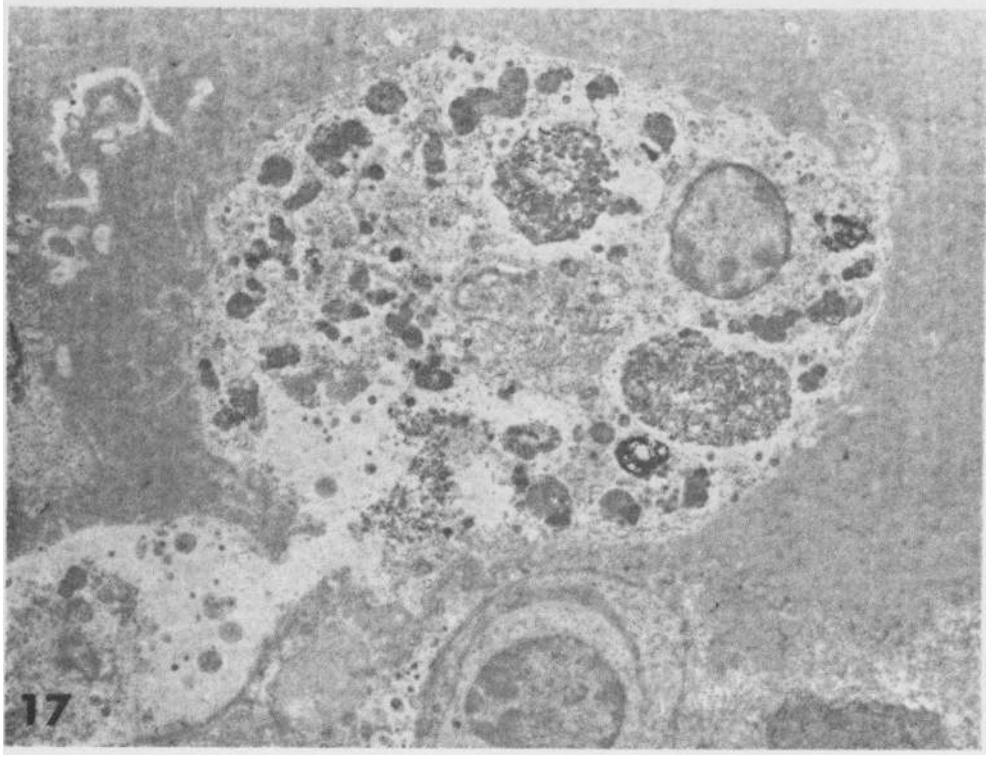

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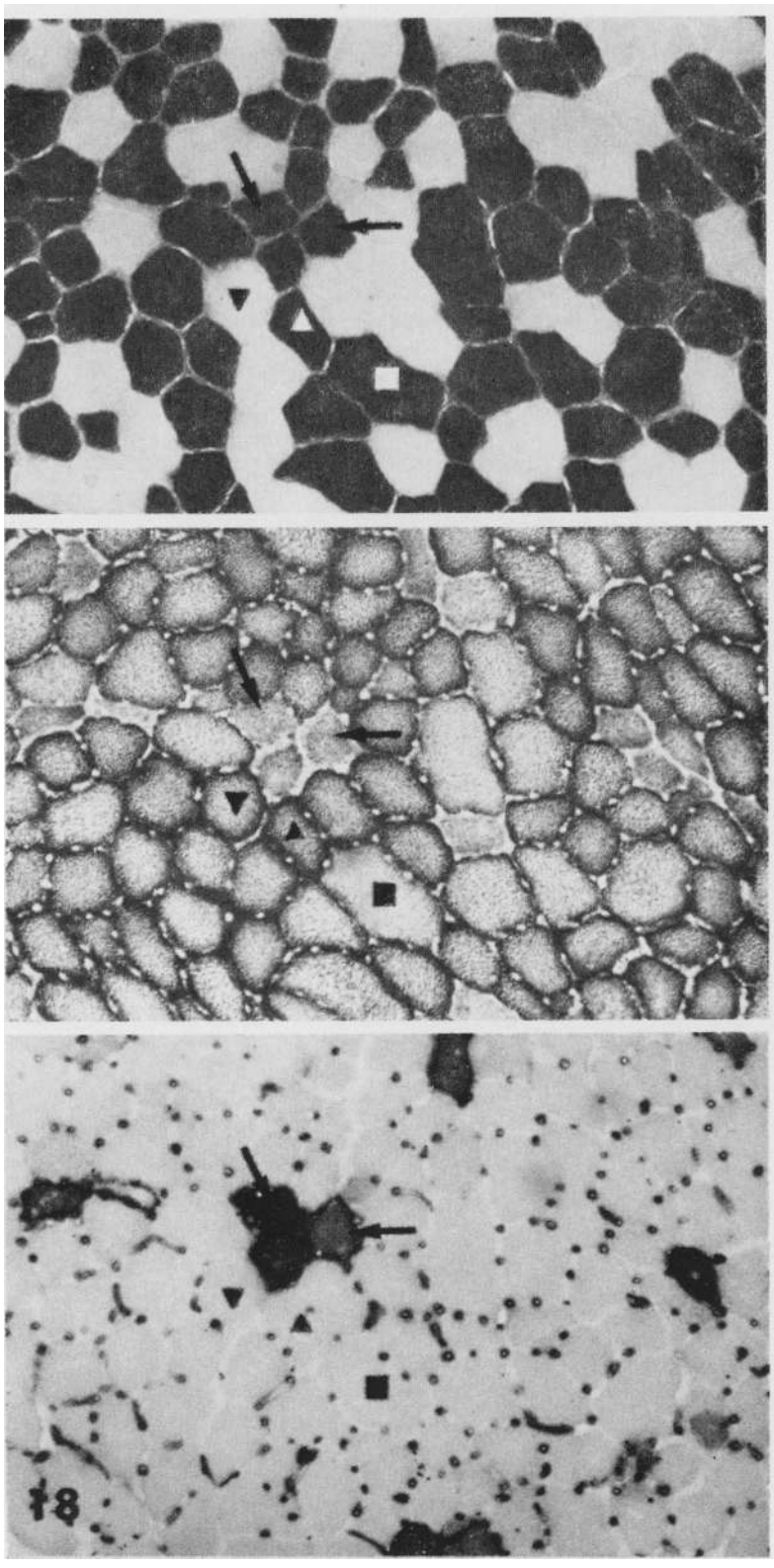
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phosphorylation in skeletal muscle during exercise might cause acute rhabdomyolysis in man.

In addition to idiopathic rhabdomyolysis in man, some well-defined experimental conditions in laboratory animals produce morphological alterations similar to those described in the present investigation on horses. Thus, studies on dogs and mice have shown that experimental skeletal muscle ischaemia (*Stenger et al.* 1962, *Reznik* 1969) produces primary mitochondrial alterations (swelling and disorganization of cristae), swelling of the sarcoplasmic reticulum and, as a late event, dissolution or disintegration of the myofilaments.

Jennings et al. (1965) and *De la Iglesia & Lumb* (1972) have shown that electron-dense deposits occur in the myocardial mitochondria in hypoxic conditions. These deposits have been shown to contain calcium (*Jennings et al.* 1969) and are thought to be an expression of an impaired cellular calcium balance.

Afifi et al. (1968) reported that the characteristic features of steroid-induced myopathy in man consist of myofilamental loss, dilation of the sarcoplasmic reticulum, enlargement and vacuolization of the mitochondria. In triamcinolone-induced myopathies in rabbits *Smith* (1964) described involvement of predominantly white muscle fibres (FT).

Lindholm (1974) has observed a possible relationship in standardbred trotters between corticosteroid treatment and the occurrence of "tying-up". He noted that in 37 % of the examined horses clinical symptoms develop 3—5 days after treatment with dexamethasone or prednisolone. He also noted that most examined horses with "tying-up" were subjected to a stress situation at the time prior to the myopathy.

It is interesting to note that the aforementioned conditions in man and laboratory animals display EM similarities to "tying-up" myopathy in horses. This does not necessarily mean that the triggering factors are the same as in equine myopathy; as for instance strenuous exercise can be ruled out on anamnestic grounds. But the uniformity in morphological alterations points to a possible derangement in mitochondrial function as a primary or early event.

A myopathy similar to the "tying-up" syndrome was described in draft horses by *Carlström* (1931, 1932). He postulated that a carbohydrate-rich diet, in conjunction with a few days of rest, results in an increased glycogen storage, leading to the production

of an abnormally large amount of lactate during subsequent exercise, which in turn resulted in swelling of the affected muscle fibres.

As previously discussed, muscle degeneration may be due to a miscellaneous array of triggering factors. In the present investigation, the involved muscle fibres were richer in glycogen in the acute stage of "tying-up" than in the late stages, pointing to an increased glycogen storage in the early phase of "tying-up" compared with normal healthy trotters. Since most "tied-up" horses, for various reasons, had no speed training a few days prior to onset of the disease but had normal feeding, it is possible that excessive glycogen did accumulate in the muscle. The high glycogen content in most of the examined horses in the 1—4 and 18—24-hr. groups lend support to *Carlströms* theory (1932). He found an increased muscle glycogen concentration in draft horses with myoglobinuria and suggested impaired circulation in the diseased muscle.

During hypoxia increased anaerobic energy demands are imposed on exercising muscles because of decreased oxygen pressure in the mitochondrion (*Linnarsson et al.* 1974). The biochemical muscular alterations of 4 horses in the 1—4-hr. stage (Table 2), and the ultrastructural changes in deseased muscle, suggest a possible primary hypoxic condition as a triggering factor to the myopathy.

In conclusion, "tying-up" disease in horses displays great morphological and biochemical similarities to idiopathic rhabdomyolysis in man. We suggest that this disease in horses should be called "equine rhabdomyolysis". It was found that the muscular alterations displayed focal localization in predominantly FT or FTH fibres. The biochemical investigation suggests a derangement in the carbohydrate metabolism, possibly caused by a local hypoxia.

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SAMMANFATTNING

Akut rhabdomyolys (korsförlamning) hos travhästar. En morfologisk och biokemisk undersökning.

Morfologiska, biokemiska och histokemiska metoder användes för att studera förändringarna i muskelbiopsier, uttagna från m. gluteus medius från 59 varmblodiga travhästar med akuta kliniska symtom på s k "tying-up". Samtliga hästar hade förhöjda serumvärden av enzymerna SGOT och SCPK. Biopsierna togs vid olika tidpunkter efter de kliniska symtomens insättande (1—4 tim., 18—24 tim. respektive 2—20 dagar). Ljusmikroskopiskt (LM) visades att muskelförändring-

arna hade en härdformig utbredning och att de var av typen hyalin degeneration med viss inflammatorisk reaktion och liten tendens till förkalkning. De ultrastrukturella förändringarna manifesterades först såsom en vågighet av myofibrillerna och med förändringar i mitokondrier samt det sarkotubulära systemet. Senare utvecklades myofibrillär degeneration och nekros med invandring av inflammatoriska celler. De inflammatoriska cellerna liknade ultrastrukturellt monocyter och makrofager. De degenerativa förändringarna uppträdde huvudsakligen i de snabba fibrerna (FT och FTH), vilket kunde visas histokemiskt med myofibrillär ATPas- och alkalisk fosfatas-färgning.

Muskelbiopsier uttagna 1—4 tim. efter de akuta symtomens insättande hade låg koncentration av glykogen, ATP och CP medan en hög koncentration av laktat och glykos förelåg. Mot denna bakgrund anses att de iakttagna muskelförändringarna orsakats av en störd kolhydratmetabolism, som i sin tur kan ha orsakats av en lokal hypoxi.

Då muskelförändringarna vid "tying-up" liknar dem vid idiopatisk rhabdomyolysis hos människa, föreslår författarna att namnet "equine rhabdomyolysis" fortsättningsvis används för denna sjukdom.

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