FINAL SUMMARY REPORT

FOR

LIFE SCIENCES FLIGHT MUSCLE EXPERIMENT 303

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"Effects of Microgravity on the Electron Microscopy, Histochemistry, and Protease Activities of Rat Hindlimb Muscle"

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By

DANNY A. RILEY, Ph.D.
Principal Investigator

MEDICAL COLLEGE OF WISCONSIN
Department of Cellular Biology & Anatomy
8701 Watertown Plank Road
Milwaukee, Wisconsin 53226

For

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION
AMES RESEARCH CENTER

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Abstract

The process of skeletal muscle deterioration induced by spaceflight was studied in rats exposed to microgravity (µG) for 9 days aboard Spacelab SLS-1. To control for the effects of the flight caging environment and other experimental treatments, a Delayed Flight Profile Test using a replicate set of animals was conducted in the flight cages at 1G to otherwise simulate the mission. Spaceflight induced significant atrophy (fiber shrinkage) and increased expression of fast muscle characteristics (fast myosin) in the slow antigravity muscles; these slowly adapting changes most likely occurred inflight. The degree of muscle fiber atrophy was affected by caging conditions which influenced hindlimb posture as seen by video recording. The atrophic adductor longus muscles showed increased susceptibility to pathological damage upon resumption of weightbearing contractile activity at 1G. Postflight damage included thrombosis of the microcirculation, interstitial and cellular edema, muscle fiber fragmentation, sarcomere disruptions, activation of phagocytic cells, and elevated ubiquitin conjugation suggestive of increased protein breakdown. Accelerated aging-like involution of neuromuscular junctions was significantly more prominent in rats housed in flight cages at µG and 1G compared to the vivarium housed 1G controls, indicating caging-induced affects. The soleus also atrophied but showed less pathology than the adductor longus which appeared related to greater resumption of loaded contractile activity by the adductor longus. Countermeasures designed to maintain the health of skeletal muscles during spaceflight and upon return to Earth's gravity will have to deal with the multifaceted nature of the problem.

Introduction

Even with daily strenuous exercise during spaceflight, prolonged exposure of humans to microgravity (µG) results in a loss of strength [14], increased fatigability, and postflight soreness of antigravity skeletal muscles. While the exercise regimens utilized may have been suboptimal, the debilitation indicates that the weakness involves deterioration more severe than simple muscle fiber atrophy (cell shrinkage). Postflight recovery of strength does not rule out pathological processes because compensatory growth and repair could occur within the observed period of recovery and mask the true severity of the damage. Understanding the mechanisms of muscle deterioration requires invasive morphological and biochemical studies of both humans and surrogate animal models. Our long term goal is to define the cellular and biochemical basis for spaceflight-induced

muscle weakness by studying the hindlimb muscles of rats flown in space or subjected to simulated µG by hindlimb suspension unloading.

During the past 20 years, rats have been flown in space aboard Soviet biosatellites and the Space Shuttle for durations of 1-3 weeks. Within individual missions, the rats were first killed postflight for tissue sampling as early as 2 hours after landing or as late as 2 days (Table 1). The data indicate that the stress of 1G reloading produces degenerative changes in the atrophic muscles adapted to microgravity. A major contribution of the SLS-1 mission is that it provided tissues from 15 rats killed periodically over a 2-7 hr postflight period. Examination of these tissues has permitted reconstruction of the temporal scenario of postflight degenerative changes. To control for the effects of animal housing conditions during spaceflight, a replicate set of rats was treated identically and put into the flight cages at 1G to simulate the mission environment except for µG. For this Delayed Flight Profile Test (DFPT), rats were processed at R&-65Vï&+65V+0d and R&- 65Vï&+65V+9d. The major
objectives of the present investigation were 1) to define the effects of spaceflight at R+0d on the cellular and biochemical properties of two postural slow muscles, 2) to identify primary (inflight) and secondary (postflight) changes, and 3) to assess at R+9d the degree of recovery from spaceflight-induced deterioration.

Materials and Methods

For the SLS-1 mission and the subsequent Delayed Flight Profile Test, a total of 163 male Sprague Dawley rats (about 300g at launch) were utilized to document changes induced by spaceflight (µG), returning to earth (1G reloading), and caging conditions (Table 2). As required by the experiment animal sharing scheme, the rats were subjected to injections and blood draws as described by the bone and hematology investigations. Rats were housed in Animal Enclosure Module (AEM), Research Animal Holding Facility (RAHF) and standard vivarium cages. The 1G controls were treated similar to the rats in the RAHF and AEM flight groups. All DFPT rats were killed on a schedule accurately matching that of the flight subjects.

Five AEM flight rats were killed 2.3-3.3 hrs after landing, and the 10 RAHF flight rats were killed 4.5-6.8 hrs after landing. In this paper, the animals are segregated for descriptive purposes into three postflight groups of 5 rats each: an early group 2.3-3.3 hrs called the 3 hr group postflight, a 4.5-5.5 hrs group designated the 5 hr group, and a late group 5.8-6.8 hrs referred to as the 6 hr group. Sequential necropsy of these fifteen rats permitted consideration of the temporal nature of muscle changes with

increasing duration of 1G reexposure. On R+0, flight and control rats were removed from their cages and video taped to document patterns of movement.

From these rats, a total of 1490 muscles, including the adductor longus (AL), soleus (SOL), extensor digitorum longus (EDL), plantaris (PLT), lateral and medial gastrocnemii (LG and MG), and the respiratory diaphragm (DIA) were quick frozen in freon 12 cooled by liquid nitrogen for histology and biochemistry for experiments 303 (Riley/Ellis) and 247 (Hoh). Drs. Ellis and Hoh are submitting results in separate reports. A representative subset of the muscles were weighed before freezing to determine wet weights. Frozen specimens were shipped in dry nitrogen shippers (-195∞C) to the Medical College of Wisconsin (MCW) for storage in liquid nitrogen refrigerators until analyzed. For electron microscopy, 300 muscles or portions thereof, including AL, SOL, EDL, and DIA, were immersion fixed and shipped to MCW for completion of processing [10-12]. Histochemical myofibrillar ATPase, SDH and _GPD reactions, H&E, toluidine blue, and indirect immunofluorescence antibody staining for platelets, red blood cells, macrophages, isomyosins (IIA,IIB,IID or 2X,I) and ubiquitin conjugates were conducted as described previously [8-12]. The cross sectional areas (CSA) of muscle fiber types (n=100 fibers) in the caudal (slow fiber-rich) and rostral (fast/slow mixed) regions of AL and the central portion of soleus were measured by computer-assisted digitizing morphometry of fibers in alkaline preincubated myofibrillar ATPase reacted sections [11,12]. The percentages of dark, light and intermediate staining fibers in the ATPase sections were also determined for the same regions of the AL and soleus muscles by counting the numbers of each type of fiber in consecutive fascicles to obtain a representative sample of 100 fibers. For assessment of atrophy and recovery, mean fiber CSA (n=35 fibers/muscle) was measured in H&E sections of soleus muscles from the L+0, R+0, R+9 and R+10 vivarium, DFPT (R+0 only) and flight RAHF groups.

Results

Muscle atrophy The AEM and RAHF flight rats on R+0 showed reduced and unsteady movements compared to 1G controls suggesting muscle atrophy and fatigue. The flight rats moved in ways that worked the AL muscles but the rats did not stand on their hindlimbs and load the soleus muscles. Compared to the DFPT AEM- and RAHF-caged 1G control values (n=15 rats), decreases in the average muscle wet wgt./body wgt. ratios for the AL and SOL muscles of the flight rats were both about 29%, and their average decreases in cross sectional area (CSA) were around 40% for both muscles. For the soleus, average fiber area in the R+0 RAHF flight muscle

was 1589±72 µm≤; this was 37% less than the DFPT R+0 RAHF flight control muscle (2513±µm≤) and 47% less than R+0 vivarium control (2994±45µm≤). The fiber area of DFPT R+0 RAHF housed rats was 16% less than the R+0 vivarium housed rats, indicating a cage-induced atrophy. The R+9 RAHF flight soleus fibers were 23% less than the companion R+9 vivarium control demonstrating that recovery was incomplete at 9 days postflight. Fiber CSA and degree of atrophy were not altered by the 6.8 hr postflight reloading period (Histogram A).

Utilizing alkaline myofibrillar ATPase sections, fiber type specific atrophy was assessed in the caudal (slow-rich) and rostral (fast/slow mixed) regions of the AL muscles of AEM flight and DFPT groups. In the slow region, the CSA of both light fibers and dark/intermediate (lumped) fibers decreased 37%. Atrophy was relatively less in the mixed region where light fibers decreased 30% and dark/intermediate fibers 22%.

For EDL muscles, the muscle wgt./body wgt. ratio for the R+0 AEM- caged flight rats showed no difference from the R+0 DFPT control. However, the EDL wet weight ratio was decreased 11% in the R+0 RAHF- caged flight group. Measurement of EDL fiber CSA is not complete at this time.

Fiber type changes In the AL and SOL muscles of the flight rats, there was atrophy of both slow and fast fibers (Figs. 1,2). In the AL, light fibers atrophied 32% in the mixed region and 38% in the slow region. The alkaline myofibrillar ATPase activities of the R+0 flight SOL and AL muscle fibers demonstrated an increase in fibers with high (fast) ATPase activity (Figs. 3,4). For the AL, there were increases in moderate plus dark fibers in the rostral mixed region (Flight AEM 22%, Flight RAHF 25%) as well as increases in the caudal slow region (Flight AEM 12%, Flight RAHF 19%). Monospecific antibodies against fast and slow isomyosin heavy chains showed increased fast isomyosin (IIA and IID or 2X, but not IIB and embryonic/neonatal), and fewer fibers in the flight muscles expressed slow myosin compared to control (not illustrated). Hybrid fibers containing both fast and slow myosins were common in the flight muscles and rare in the ground controls.

Mitochondrial SDH histochemical activity was decreased in the subsarcolemmal regions of R+0 flight AL and SOL muscle fibers, and intermyofibrillar staining was at or above that of the 1G control muscles. Mitochondrial _GPD activity was generally higher in the subsarcolemmal regions of these flight muscles. These changes are in keeping with the acquisition of fast fiber properties during spaceflight.

Interstitial edema, macrophages and fiber damage While the soleus muscles of space flown rats showed no edema, the adductor longus muscles of rats 3 hrs postflight already exhibited edema. The AL interstitial nonmyofiber area was twice that of controls (Histogram B). There was no change in the level of edema in the 5 hr group, but in the 6 hr group, muscle edema was three times the control level. In addition, there was focal infiltration by mononuclear cells and damaged muscle fibers (Figs. 5,6). Overall, damaged fibers, comprising <0.5% of the fibers, were not more prevalent in muscles of the R+0 flight groups. In the AL, necrotic fibers were confined to the focal regions of greatest edema which tended to occur in the middle third of the muscle.

In the AL, edema of muscle cells was evident by rounding (swelling) of the atrophic muscle fibers (Fig. 6). Rounding was confirmed by a shape factor measurement showing that control fibers were angular or less circular (.608) than the edematous fibers (.751); a value of 1.0 equals a circle, and 0.0 equals a straight line. Mast cell degranulation was common at 5 hrs, and a significant decrease (58%) in the number of detectable mast cells per section (from 16 to 5 cells/section) was noted 6 hrs postflight suggesting complete degranulation of many cells. In the 3 hr group, mast cells numbers (14/section) were at control levels (12/section), and degranulated mast cells were rare. Mast cell degranulation and numbers (each 25/sections) were not different in the soleus muscles of the flight and control animals.

ED1 immunoreactive macrophages were at control (R+0 DFPT) concentrations in the soleus muscles of the R+0 AEM and RAHF Flight groups. The AL muscles of the control rats showed a significantly lower (14%) macrophage concentration compared to the soleus controls. Inexplicably, the concentration of macrophages in the flight AL muscles was 12% lower than in the AL controls. Though difficult to quantitate, in general the macrophages in control AL and soleus muscles and the flight soleus muscles appeared inactive, being small and having few processes; in contrast, macrophages in the flight AL muscles appeared active, possessing more and larger processes.

Immunostaining the AL muscles from the R+0 RAHF 6 hr group with antibodies against red blood cells (Fig. 7) and platelets (not shown) revealed extensive thrombosis, especially in the most edematous regions. In the 3 and 5 hr groups, flight AL muscles did not exhibit thrombosis and were indistinguishable from controls in this regard. Rare extravascular red blood cells were found with electron microscopy in the 6 hr group (Fig. 9). Capillary endothelial cells appeared intact, but pericytes showed more

process formation than normal (Fig. 9). About 60% of the arterioles were open in the DFPT controls whereas 100% were open in the 3 hr flight group. In contrast, 100% of the arterioles were closed in the 5 hr RAHF flight group (Fig. 10).

Sarcomere lesions Fibers with a fragmented appearance and less dense staining in H&E sections were present in the edematous regions of the 6 hr postflight AL muscle (Fig. 6). In serial sections, the damaged fibers had greatly increased immunolabeling for ubiquitin conjugates throughout (Fig. 8). Other fibers, not detectably damaged at the light microscopic level, showed elevated ubiquitin conjugates in discrete patches. These focal sites appeared to correspond to eccentric contraction-like lesions which were detected in the 4 hr postflight AL muscle but not earlier. Viewed in longitudinal section, the sarcomere lesions are areas of sarcomere disruption and hyperlengthening (Fig. 11).

Neuromuscular junctions and mitochondria The neuromuscular junctions of the flight AL muscles exhibited shrinkage and disruption of motor nerve terminals and vacated postjunctional membrane suggestive of a loss of terminals in 72±16% of R+0 flight RAHF and 48±12% of R+0 flight AEM rat junctions sampled (Fig. 13). The DFPT AEM and RAHF caged controls exhibited similar degrees of affected junctions, 65±17% and 88±6% respectively. However, the values for the vivarium housed controls were significantly ($p<0.05$) lower (19±19%) from the above four groups, implicating nonspaceflight effects on junction integrity. Enlarged subsarcolemmal mitochondria with granular inclusions occurred in both control and flight AL muscles of the R+0 AEM flight, R+0 vivarium, L+0 vivarium, and DFPT R+0 RAHF groups, indicating a possible preexisting metabolic problem in the pool of rats utilized for this mission (Fig. 14).

Discussion

Nine days of spaceflight produced marked atrophy of the slow red SOL and AL postural muscles whereas the fast EDL and plantaris muscles were less affected. This agrees with previous spaceflight and suspension unloading findings [1,3,5,8,10-12]. During flight, the AL and SOL exhibited greater atrophy in rats housed singly in RAHF cages compared to those maintained in groups of 5 in AEM cages. A 10-16% RAHF cage-induced atrophy was detected in the soleus muscles of control rats. The EDL also atrophied in the RAHF-caged flight rats; this indicates that factors other than loss of weightbearing influenced the mass of this non-antigravity muscle. Elevation of glucocorticoids may have caused selective lost of EDL fast myosin contractile proteins [13]. Adrenal hypertrophy was present in the

RAHF animals. Reduced movement and higher stress possibly associated with flight cages compared to vivarium cages may have exacerbated atrophy in the µG environment.

Atrophy of the EDL was observed previously for RAHF housed flight rats during Spacelab-3 [6], whereas no EDL atrophy was present in flight rats group-housed during the 14-day-flight of Cosmos 2044 [12]. Group housing may be less stressful than singly housing. Another possible explanation may be hindlimb postural differences influenced by cage type. In µG, the hindfeet of AEM housed rats adopt a "foot drop" posture which lengthens the EDL, stimulates growth, and counteracts weight loss [11]. SLS-1 inflight video indicated that the smaller vertical and lateral dimensions of the RAHF cages compared to AEM cages prevented or reduced foot drop; this would remove the growth stimulus and permit EDL atrophy. Consistent with this interpretation are previous Cosmos 936 results from rats maintained singly in tube type cages which like RAHF cages were more restrictive in size than the gang type cages. Rats in tube type cages exhibited EDL atrophy and shortening of muscle fiber length, consistent with the hindfeet being constrained from plantarflexing (dropping) [1]. Interestingly, humans in µG adopt a "foot drop" plantarflexion posture that may be accelerating the atrophy of the calf muscles [14]. A countermeasure such as the penguin suit with elastic cords to prevent foot drop may be beneficial to reduce muscle atrophy, not so much from restoring workload, but rather from counteracting the foot drop posture.

The slow fibers in the AL and SOL muscles of the flight rats exhibited increased expression of fast fiber properties i.e., production of fast myosin and increased activities of glycolytic mitochondrial _GPD and fast myofibrillar ATPase activities. In the same fibers, the content of slow myosin diminished and mitochondrial SDH oxidative activity was reduced. Upon return to 1G, the antigravity functions of these transforming muscles would be compromised because fast myosin would generate rapid contractions consuming large amounts of energy leading to more rapid fatigue.

A strength of the SLS-1 mission was that 29 rats were flown permitting analysis of multiple postflight time points. Comparing AL muscles from rats in the 3,5 and 6 hr groups demonstrated that increased reloading time yielded progressive increases in thrombosis, interstitial and cellular edema, mast cell degranulation, muscle fiber fragmentation, sarcomere stretch-like lesions, activation and invasion of phagocytic cells and elevated ubiquitin conjugation of fibers.

Interstitial edema was present in the AL muscle of the rat with the shortest postflight reloading (2.3 hrs). This edema may represent a "feet-ward" fluid shift or resumed AL contractile activity during reentry when the environment of the returning Shuttle shifted gradually over a 30 minute period from µG to 1.8G. Inflight tissue removal as proposed for SLS-2 will determine whether interstitial edema occurs inflight or exclusively postflight. Hindlimb suspension unloading studies have shown no edema when suspension unloaded muscles were excised from rats anesthetized in the head down tilt position [6]; rats permitted to bear weight for only 6 hrs showed edema [Krippendorf, unpublished observations]. This indicates that resumption of activity in atrophic muscles leads to edema. The flight AL muscles showed edema whereas soleus muscles did not. The postflight video recording indicated earlier and more strenuous contractile activity of the AL compared to the soleus.

For Cosmos 1887, the integrity of the microvessels was clearly violated with endothelial cell necrosis. A thorough examination of capillaries in the present study did not reveal damaged capillary endothelial cells, although a few red blood cells were extravascular. These red cells may have escaped by diapedesis. The progressive increase in interstitial edema from 2 to 6 hrs was consistent with mast degranulation beginning around 5 hrs and becoming more pronounced with time. Histamine release by mast cells would be expected to increase permeability of post capillary venules and accelerate edema. Our Cosmos 2044 results showed marked thrombus formation in the postcapillary venules and capillaries 8-11 hours postflight [12]. These first day changes predict marked edema and extensive ischemic necrosis by 2 days postflight [10]. Interestingly, for SLS-1 rats, arterioles of the AL were open in the 3 hr group, but by 5 hrs postflight they were constricted. This vasoconstriction likely reduced muscle blood flow and promoted pooling and activation of clotting factors. Thus, resumption of muscle contractile activity in response to reloading and fluid shifting in response to gravity appear to initiate mild interstitial edema. Following arteriole vasodilation as muscle contractions resume upon reloading, reflex arteriole vasoconstriction occurs and reduces blood flow, favoring clotting and ischemic necrosis. Necrotic cells release components that activate mast cell degranulation and further accentuate edema. The rising intramuscular pressure and the vasoconstriction would further reduce blood pressure and together with thrombosis dramatically compromise blood flow leading to ischemic necrosis. Other possible scenarios may exist to explain the observations. Further investigation is needed to explain the increased susceptibility of spaceflown rats to microcirculation failure in slow red muscles.

Thrombosis within antigravity muscles following return to 1G weightbearing is worrisome because emboli may dislodge and pass to the lungs causing life-threatening pulmonary edema. The cause of thrombosis is not understood at this time, but its absence at 3 hrs permits examination of tissue before onset which may offer insights into the primary events. Interestingly, the flight soleus did not show , edema and thrombosis demonstrating that even though the slow AL and soleus atrophied similarly, they did not respond the same postflight. As mentioned previously, the R+0 videotaping indicated that the AL was more active earlier postflight than the SOL because the flight rats were not bearing weight on the hindfeet so that the SOL muscles were not reloaded. The rats maintained their hindlimbs in the normal mid-adduction position which would have required AL contractile activity. These results indicate that spaceflight- induced muscle atrophy renders muscles susceptible to damage during resumption of weightbearing contractile activity. SLS-3 studies are being developed to measure directly with electromyography AL and soleus activity following reloading to test the hypothesis that resumption of active contractions is necessary for postflight tissue damage to occur.

The sarcomere stretch-like lesions in the AL appear to be a postflight reloading phenomenon because they were barely detectable at 3 hrs and pronounced at 5 hrs. A similar temporal onset was found in the atrophic AL muscles of hindlimb suspension rats reloaded for 6 hrs. Disruption of the sarcomere integrity suggests that structural proteins are lost during atrophy, along with contractile proteins, increasing the vulnerability to A and Z band disruption during tension generation. Studies are in progress to identify possible defective structural proteins contributing to sarcomere fragility.

Aberrant neuromuscular junctions, showing motor nerve terminal involution similar to aging, were present equally in both control and flight AL muscles of rats housed in flight cages. This occurrence of abnormal junctions was significantly greater than that in vivarium housed rats, suggesting a caging-related or other nonmicrogravity influence. Further studies are necessary to identify factors that have contributed to accelerated neuromuscular junction involution. We have previously observed extensive neuromuscular junction necrosis in the AL muscles of Cosmos 1887 rats [10]; it appears that this damage 2 days postflight was secondary to ischemia. However, others have suggested that pathological changes occur at neuromuscular junctions inflight [2]. Until the nonmicrogravity factors are sorted out, this issue will remain controversial.

The muscle wet weight data from the R+9d groups of flight rats showed that atrophic muscles had recovered substantial weight. The EDL and PLT weights were near normal, but the AL and SOL muscles remained below control levels, and fiber CSAs were less than controls. Partial recovery 9 days after landing was consistent with the return of normal-appearing weightbearing movements of the flight rats which had been dramatically reduced on the day of landing because of muscle weakness and fatigue. The failure of flight AL and SOL to recover fully by 9 days was expected because, based on Cosmos 1887 results, large scale AL and SOL muscle degeneration secondary to ischemia could have occurred by 2 days postflight [10]. About 1 week after such an injury, muscle fibers can regenerate to the myotube stage [7]. Regenerating fibers at R+9d would be smaller than mature fibers, accounting for the residual lower than normal muscle weights. While fibers of flight rats were smaller in size than controls, they did not show morphological features of muscle fiber degeneration/regeneration as expected. The relatively soft landing of the Space shuttle returning to 1G compared to the higher reentry and impact G forces of Cosmos biosatellites may account for the lack of degeneration for SLS-1 and the major muscle necrosis found for Cosmos 1887 [10,12].

The present findings on rodents are relevant to human muscle atrophy during spaceflight. They predict that antigravity muscles will exhibit simple atrophy and acquire fast muscle characteristics becoming less efficient for weightbearing function. In addition, the skeletal muscles will show an atrophy-related increased vulnerability to a plethora of muscle fiber and microcirculatory pathological changes that will be exacerbated by postflight reloading in 1G. Countermeasures designed to maintain the health of human muscles during spaceflight and upon return to Earth's gravity will have to deal effectively with the multifaceted nature of the problem.

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Figure and Histogram Legends

Histogram A Muscle fiber cross sectional areas for H&E stained fibers in the flight AL (FA), DFPT AL (DA), flight soleus (FS), and DFPT soleus (DS) in R+0 rats. AL and soleus were obtained from 5 AEM housed rats (2.3-3.3 hrs postflight), 5 early RAHF housed rats (4.5-5.5 hrs) and 5 late RAHF housed rats (5.8-6.8 hrs). The asterisks indicate that the mean flight muscle area was significantly ($p<0.05$) less than the corresponding DFPT control. Error bars are s.e.m. Both the flight AL and soleus muscles exhibit about a 40% decrease in cross sectional area at all three postflight time points.

Histogram B The percentage of a muscle cross section not occupied by muscle fibers represents the interstitial connective tissue area % in R+0 rats. Abbreviations are as in histogram A. At 2.3-3.3 and 4.5-5.5 hrs postflight, the non-myofiber area % in the flight AL is twice that of the DFPT control ALs, indicating interstitial edema. By 5.8-6.8 hrs postflight the flight AL interstitial area % is nearly three times that of the DFPT control AL. No morphological evidence for edema is evident in the flight and DFPT control soleus muscles.

Figure 1 Cross section of an R+0 soleus muscle from a DFPT rat simulating the flight in a RAHF cage at 1G. Histochemical alkaline actomyosin ATPase staining reveals that the muscle is composed primarily (_85%) of lightly staining slow fibers with low ATPase activity. Darkly staining fast fibers with high ATPase activity comprise most of the remaining fibers. A few transitional fibers with intermediate levels of ATPase activity represent fibers with both slow and fast myosin; this was confirmed by immunostaining serial sections with antibodies specific for fast and slow myosins. X96.

Figure 2 Cross section of an R+0 soleus muscle from a rat flown 9 days aboard SLS-1 in a RAHF cage. Decreased fiber cross sectional areas of both

slow and fast fibers is striking. Spaceflight-induced elevated expression of fast actomyosin activity is indicated by the increased percentages of darkly and intermediately staining fibers. Immunostaining adjacent sections for slow and fast myosins confirmed the large number of transitional fibers containing both types of myosin. X96.

Figure 3 Cross section of the caudal portion of an adductor longus muscle from a R+0 DFPT flight simulated animal housed at 1G. Alkaline actomyosin ATPase activity shows that this region of the ground control muscle is very rich in slow fibers. Fast fibers and transitional fibers are much less common than in the control soleus in fig. 1. X96.

Figure 4 The caudal region of an adductor longus of an R+0 rat flown in microgravity for 9 days exhibits a dramatic increase in transitional and fast fibers in the normally slow region. The increase in hybrid fibers containing both slow and fast myosins was confirmed in immunoreacted sections adjacent to this actomyosin ATPase stained section. As in the soleus of the flight rat, there is marked fiber atrophy. X96.

Figure 5 An H&E stained cross section of an adductor longus muscle from a R+0 rat housed in the RAHF at 1G. The normal appearance of interstitial connective tissue is illustrated by the thin light areas (endomysium) encircling individual muscle fibers and the wider light areas (perimysium) surrounding groups of muscle fibers forming fascicles. Arterioles, venules, nerves and mononuclear cells are most common in the perimysium, and capillaries reside in the endomysium. X165.

Figure 6 An H&E section of an adductor longus muscle from a R+0 RAHF flight rat that experienced _30 minutes of reentry G force and _6 hours of 1G exposure following landing of the Space Shuttle. The region shown is comparable in location to that in the control muscle in fig. 5. Prominent endomysial and perimysial edema is evident by the wider than normal interstitial regions. An increase in mononuclear cells has occurred, and immunostaining of serial sections with cell marker antibodies demonstrated that many of these cells were activated monocytes and macrophages. X165.

Figure 7 In this low magnification micrograph of an edematous region in an R+0 flight adductor longus muscle section, immunostaining for red blood cells shows larger than normal collections of brightly fluorescent red cells. A serial section immunostained for platelets revealed indicated thrombosis in this region. The red cell antibody also labels blood vessels and the muscle fiber basal lamina in flight and control muscles. X103.

Figure 8 A section serial to that in fig. 7. immunostained for ubiquitin conjugates shows brightly immunofluorescent muscle fibers restricted to the thrombotic region. Elevated ubiquitin conjugation of muscle fibers is indicative of increased protein degradation by the ATP dependent nonlysosomal pathway. Some of the brightly stained fibers may have been damaged by ischemic necrosis, and others may contain stretch contraction lesions of the type shown in fig. 9. X103.

Figure 9 Electron micrograph of a cross section of an AL muscle from an R+0 RAHF flight rat. Within the enlarged endomysial region, are two capillaries with normal appearing endothelium partially surrounded by pericytes showing greater than normal process formation. An extracellular red blood cell and processes from macrophages and fibroblasts are present. X13,000.

Figure 10 A constricted arteriole in a longitudinal section of an AL muscle from an R+0 RAHF flight rat. The lumen of the vessel is completely closed. X10,000.

Figure 11 A toluidine blue-stained longitudinal section (0.5μm) of an AL muscle from a R+0 RAHF flight rat returned to 1G for ~4 hours before tissue removal. Eccentric (stretch) contraction-like lesions, representing disruption of the contractile proteins and abnormal widening of the sarcomeres, are present in two muscle fibers at the center of the field. Muscle atrophy induced by spaceflight appears to increase the susceptibility of muscle fibers to myofibril microinjury following transition from μG to 1G. X940.

Figure 12 The sarcomere lesion in an AL flight muscle of an R+0 RAHF rat shows loss of A band thick filaments, diminished and excessively wavy Z lines. X25,000.

Figure 13 A neuromuscular junction with three motor nerve terminals synapsing on an AL fiber from a R+0 RAHF flight rat. Shrinkage of the upper-most terminal and accompanying schwann cell is indicated by the associated empty basal lamina. The preterminal axon, partly visible in the lower right, appears intact. X16,500.

Figure 14 A cross section of an AL fiber from a L+0 vivarium rat. Unusually large mitochondria with amorphous inclusions are present in the subsarcolemma region. X19,600.

TABLE 1

Mission	Launch Date	Duration	Rats Sex	Rats Wgt	Time Killed Postflight	Soleus Net Wgt	Cage
Cosmos 1514	12/83	5.0 days	female	109g 290g	6 hrs	11% 16%	group
STS-48	9/91	5.5 days	female	62g	2 hrs	38%	group
Spacelab-3	4/85	7.0 days	male	250g 380g	12-16 hrs	33% 17%	single
Cosmos 1667	7/85	7.0 days	male	330g	4-8 hrs	23%	group
Spacelab SLS-1	6/91	9.0 days	male	334g 330g	2-3 hrs 4-6 hrs 9 days	22% 33%	group single
Cosmos 1887	9/87	12.5 days	male	300g	48-56 hrs	11%	group
Cosmos 2044	9/89	14.0 days	male	340g	5-11 hrs	25%	group
Cosmos 936	8/77	18.5 days	male	210g	4.5-9 hrs	36%	single
Cosmos 1129	9/79	18.5 days	male	290g	7, 36 hrs 6, 29 days	39%	single group
Cosmos 782	11/75	19.5 days	male	210g	11 hrs 25 days	38%	single
Cosmos 690	10/74	20.5 days	male	223g	2, 27 days	25%	single
Cosmos 605	10/73	22.0 days	male	225g	2, 27 days	32%	single

TABLE 2

SLS-1 PREFLIGHT AND POSTFLIGHT DATA COLLECTION

FLIGHT AND VIVARIUM GROUPS (74 rats)

L+0d - vivarium 1G baseline controls; 10 singly- and 5 group-housed rats

R+0d - launch, ÂG, reentry, 1G readaptation; 10 RAHF and 5 AEM flight rats

R+9d - recovery, 1G; 9 RAHF and 5 AEM spaceflown rats
controls; 10 singly- and 5 group-housed control rats

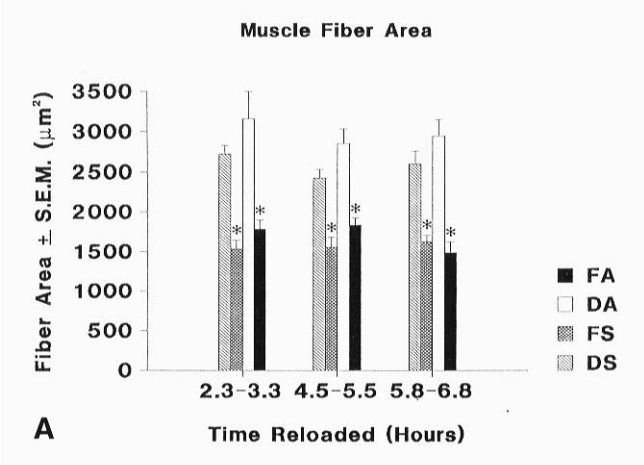
1G DELAYED FLIGHT PROFILE TEST GROUPS (89 rats)

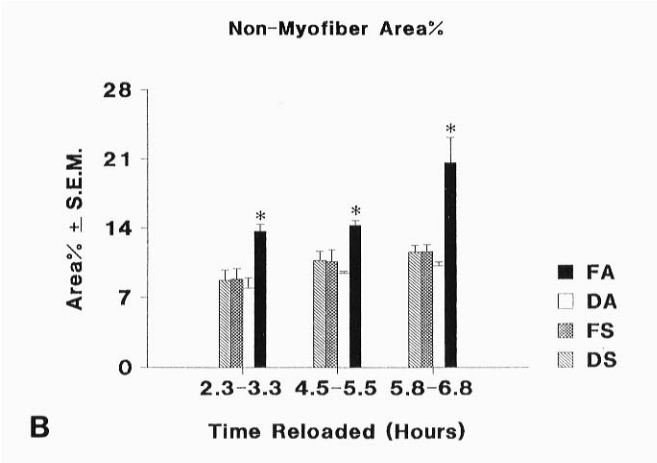
L+0d - vivarium baseline controls; 10 singly- and 5 group-housed

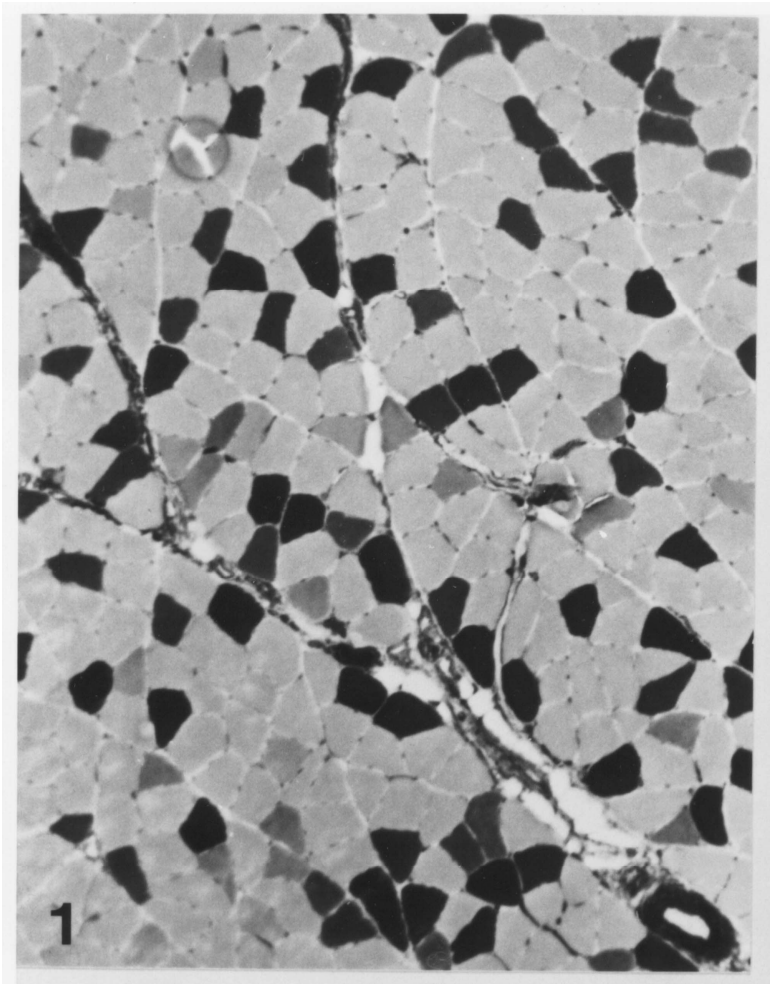
R+0d - caging environment; 10 RAHF and 5 AEM flight-caged rats
controls; 10 shoebox singly- and 5 AEM group-housed rats

R+9d - recovery, flight to shoebox caging; 9 RAHF, 5 AEM rats
controls; 10 singly- and 5 group-housed rats

R+10d - non postflight-injected vivarium; 10 singly, 5 grouped


Muscle Fiber Area
Fiber Area ± S.E.M. (μm²)
3500
3000
2500
2000
1500
1000
500
0
2.3-3.3
4.5-5.5
5.8-6.8
Time Reloaded (Hours)
FA
DA
FS
DS
A


Non-Myofiber Area%
28
21
14
7
0
Area% ± S.E.M.
*
*
*
2.3-3.3
4.5-5.5
5.8-6.8
Time Reloaded (Hours)
FA
DA
FS
DS
B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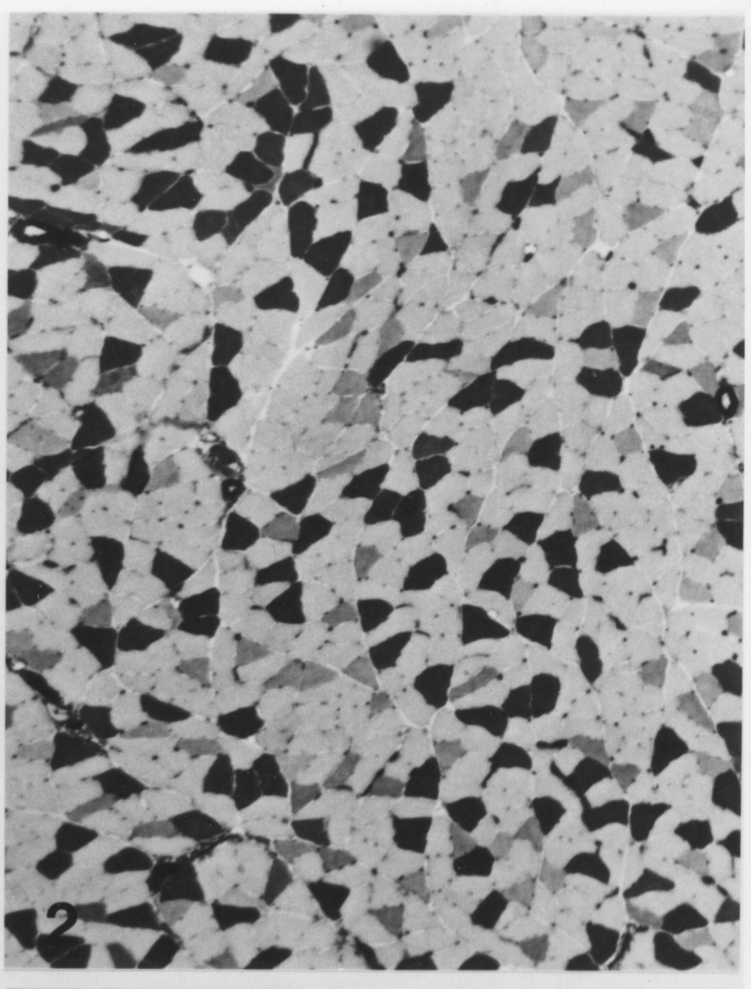
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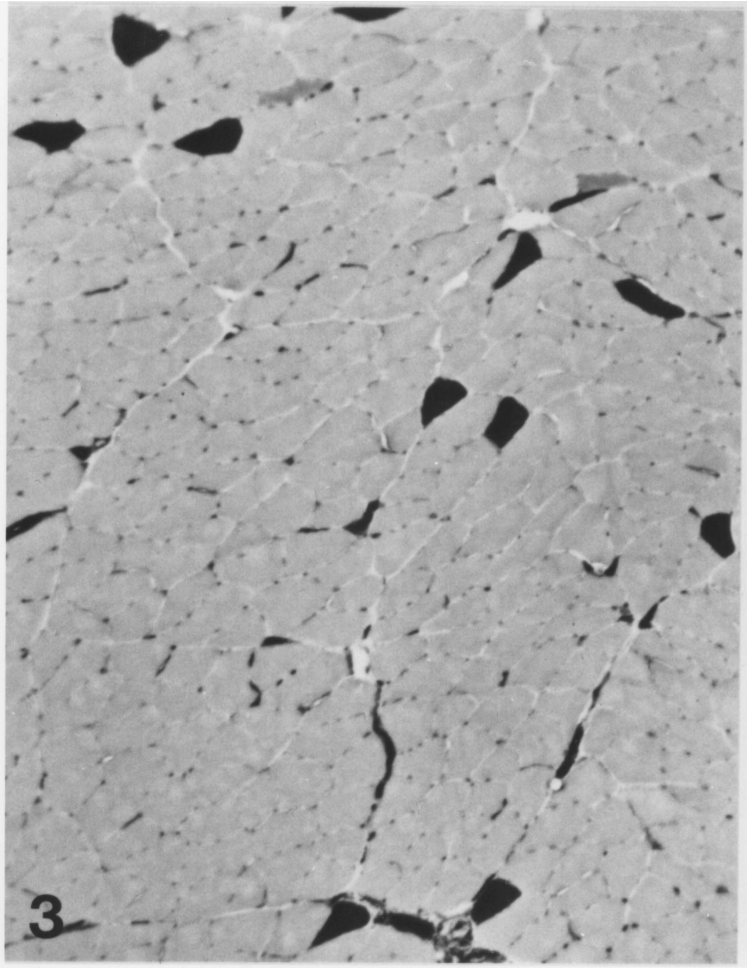
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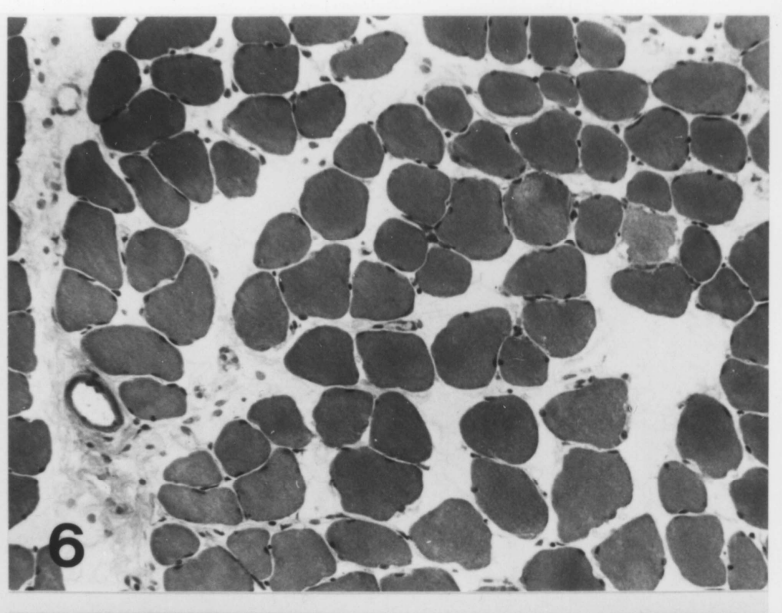
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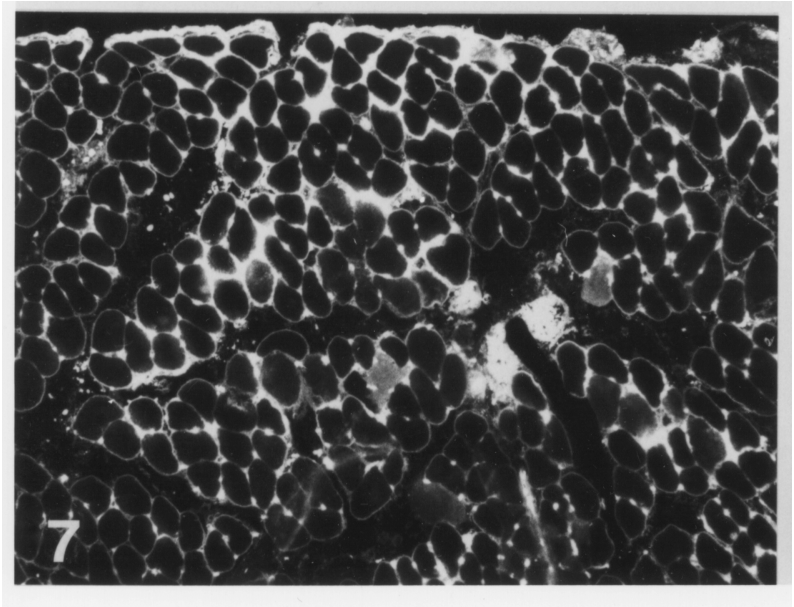
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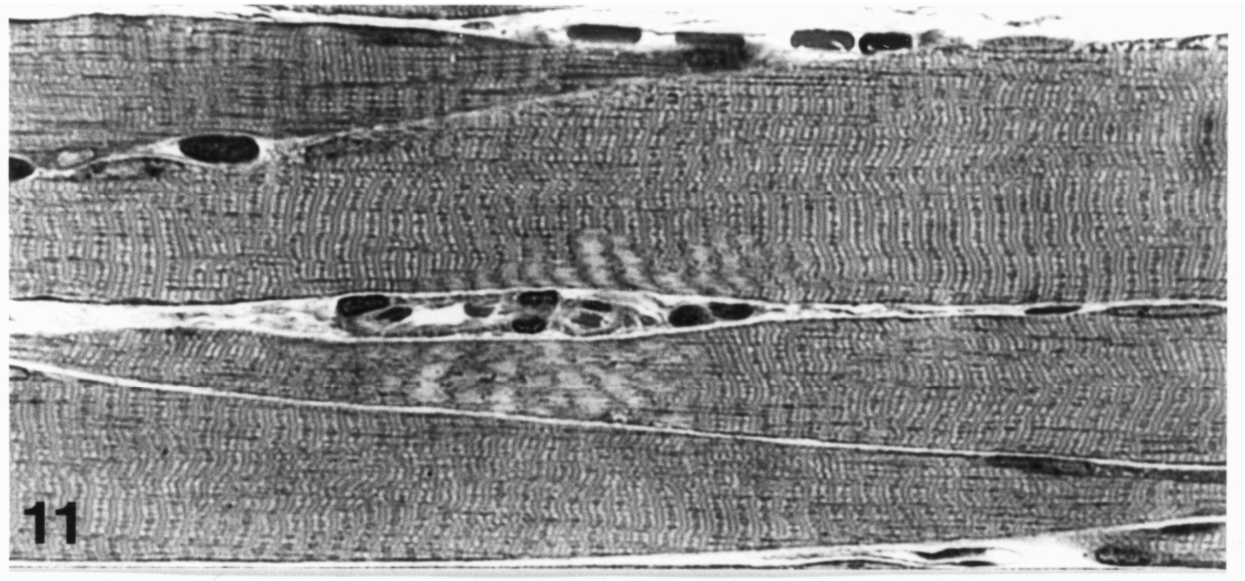
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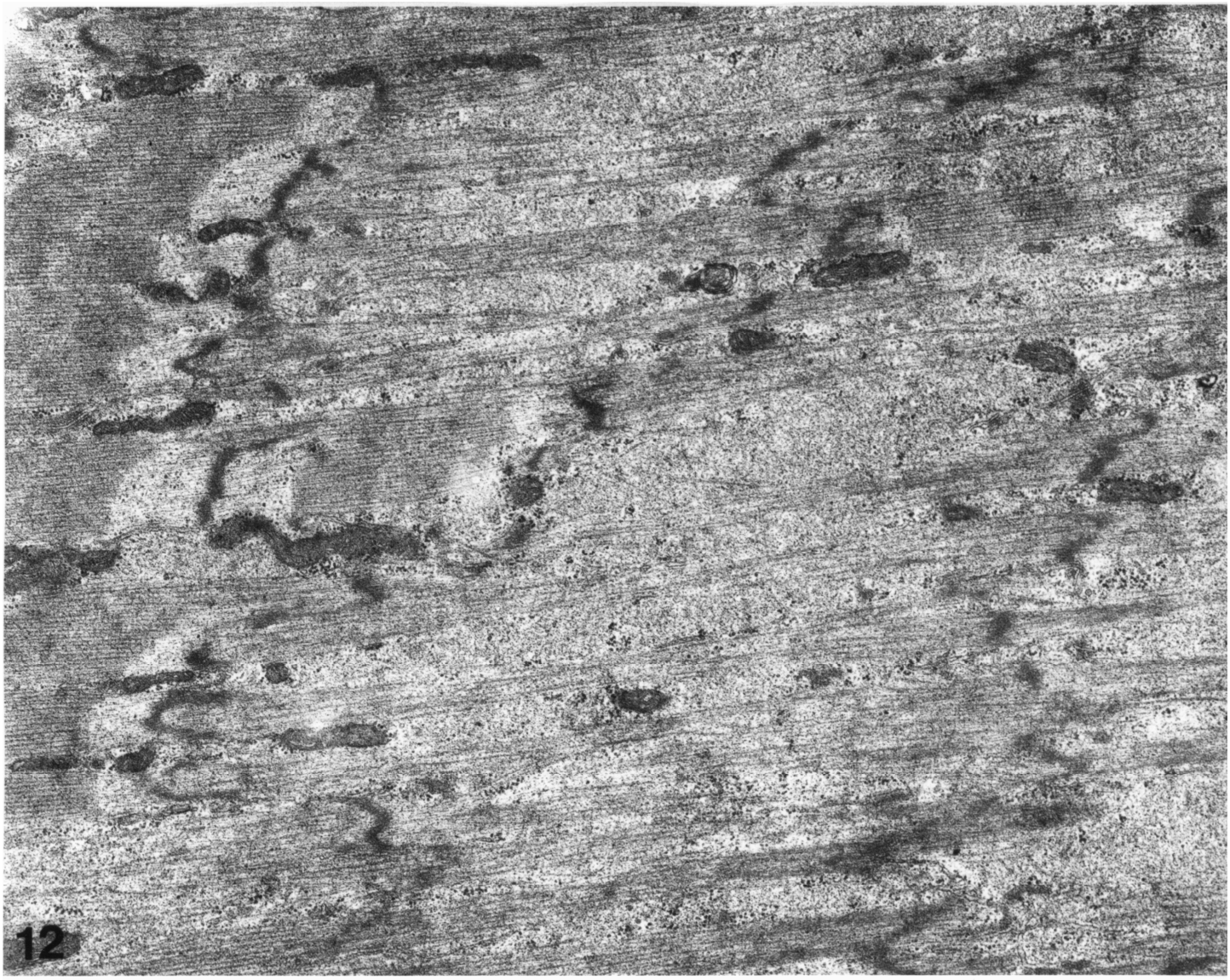
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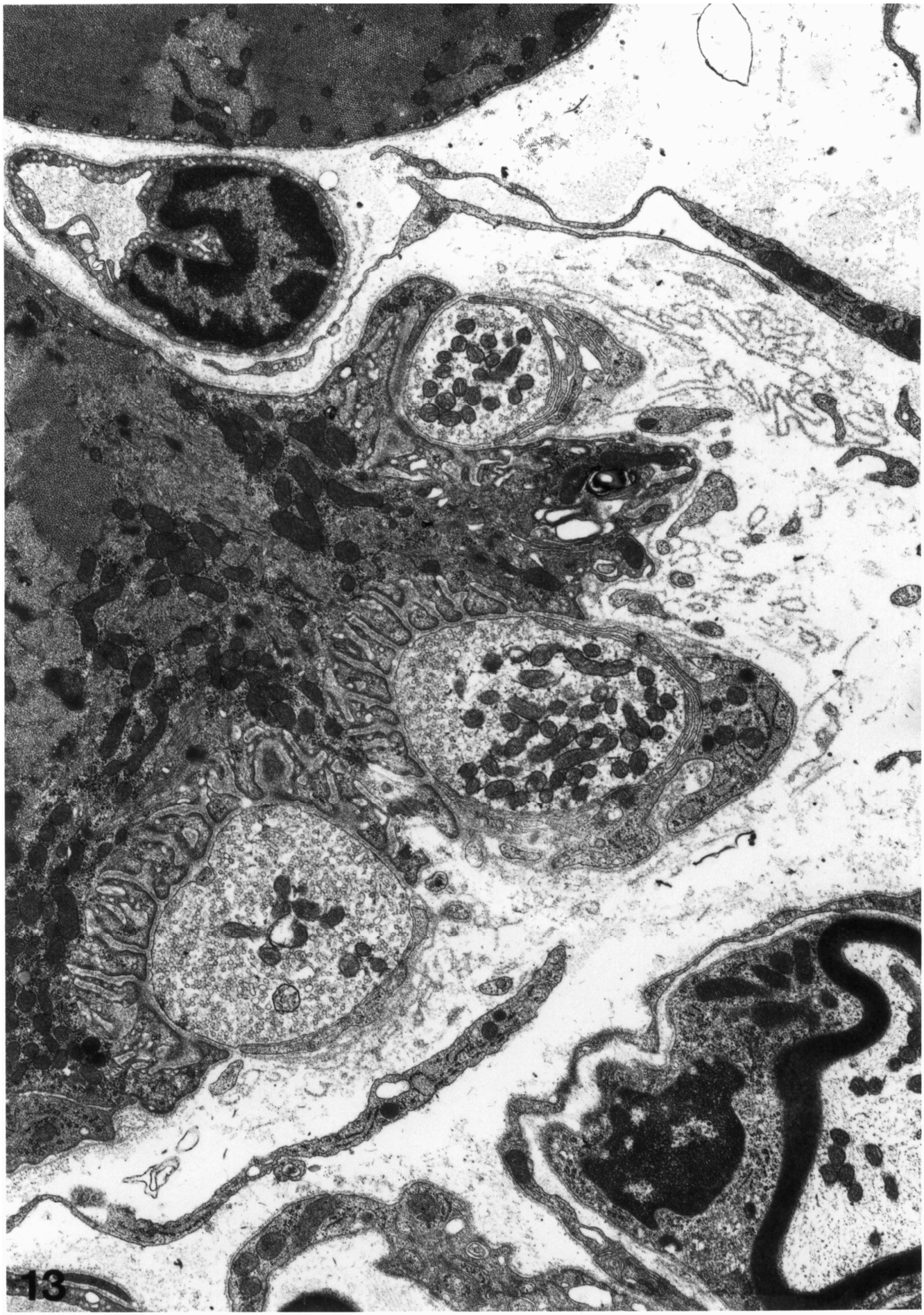
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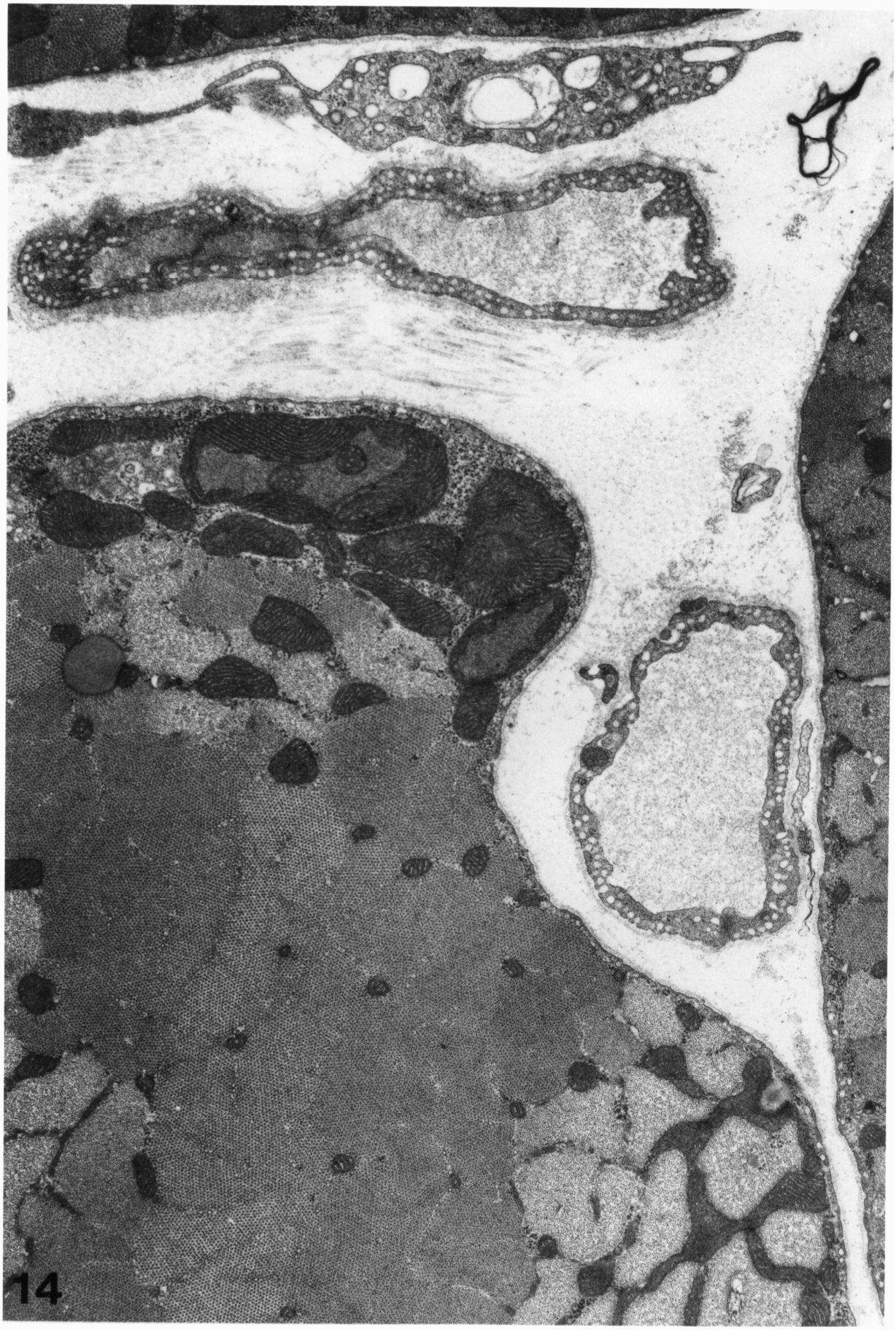
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