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STRUCTURAL AND FUNCTIONAL CHANGES IN THE MICROVASCULATURE OF DISUSED SKELETAL MUSCLE

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1. ABSTRACT

Skeletal muscle and its microvasculature can exhibit remarkable plasticity in response to decreased functional demand (i.e., muscle disuse). Since the microvascular adaptation to disuse does seem to not depend solely on reduced demand, this review examines the various factors that may be responsible for the observed regression of microvascular structure and function during disuse. There are several animal models of muscle disuse; their common feature is that they are associated with a variety of confounding effects that make the interpretation of the "pure" disuse effect challenging. As well, in clinical studies, the effect of disuse can be difficult to separate from that of various pathologies. Regardless of methodological difficulties, degeneration of the capillary wall, capillary loss, arteriolar remodelling, reduced resting state blood flow, and reduced arteriolar responsiveness to acute vasodilative and vasoconstrictive stimuli have all been observed in disused muscles. The level, and presence/absence of these changes may depend on many factors including the duration of disuse, degree of muscle atrophy, residual muscle activity, microvascular blood flow, release of vasoactive agents from the degenerating muscle, muscle type, and the particular pathology associated with the muscle withering in humans. It is the present challenge to discover the presence/absence of key agents (possibly originating at the interface between the blood stream and the vascular wall, within the extracellular matrix, or the muscle fibres themselves) that alter the intra- and/or inter-cellular signalling to explain the mechanism of adaptation of the microvasculature to skeletal muscle disuse.

2. INTRODUCTION

Skeletal muscle can exhibit remarkable plasticity in its biochemical composition in response to increased or decreased functional demand. The skeletal muscle microvasculature, viewed as a route of delivery of life-supporting materials to the muscle fibres, can also exhibit such plasticity. During a chronic increase of functional demand (e.g., exercise) this plasticity is manifested by microvascular adaptation (i.e., increased capillarity) that meets the increased demand (1). During a chronic reduction in functional demand (e.g., disuse), however, it is not clear whether microvascular adaptations in the degenerative direction depend solely on reduced demand. Rather than to provide an exhaustive survey of the literature [topic reviewed earlier by Hudlicka (1)], the purpose of this chapter is to present the authors' view on the intriguing question of the regression of the microvasculature in skeletal muscle during disuse.

3. EFFECT OF DISUSE ON SKELETAL MUSCLE MICROVASCULATURE

3.1. Models of disuse

Prior to discussing the effect of disuse on microvascular structure and function, it is important to highlight the various models of skeletal muscle disuse that have been used to collect structural and functional data. In terms of animal models, denervation (1,2), nerve crush (3), nerve conduction block via chronic tetrodotoxin application (4) and tenotomy (5) have all been employed to produce

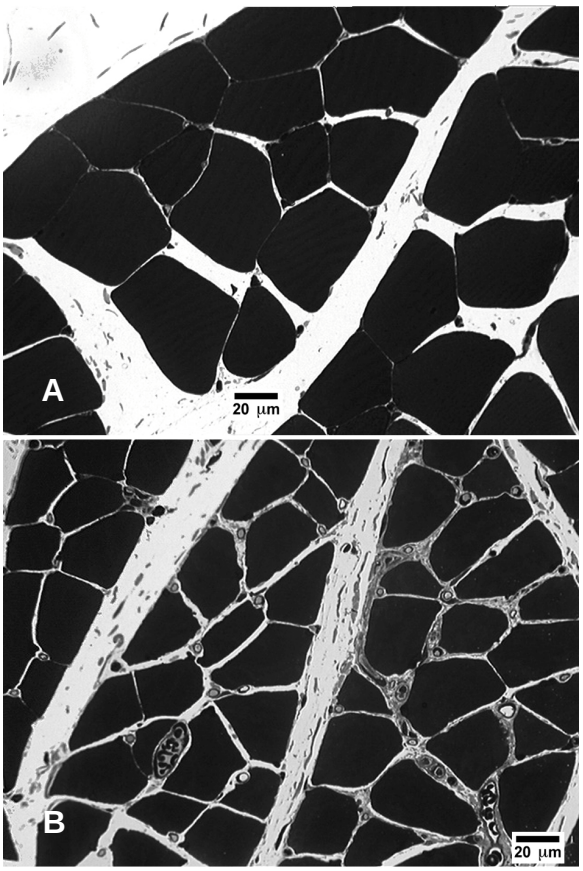

Figure 1. Light micrographs of portions of cross-sections of gastrocnemius muscle from a control rat (A), and from a rat subjected to a 2-wk hindlimb disuse caused by a functional sympathectomy of the sciatic nerve (B). Note marked fibre atrophy and increased interstitial collagen after disuse. Capillary-to-fibre ratio decreased 39% with disuse (26).

rapid, severe muscle atrophy and the corresponding microvascular change since the majority of muscle activity is eliminated in these models. Muscles in animals subjected to immobilization via casting (6), hindlimb (7) or whole body suspension (8), as well as space flight (8) retain residual activity and therefore, in general, yield a less pronounced atrophy and microvascular response. Hibernation, a "natural" model of disuse, also falls into the category of less severe atrophy (9). While the majority of models show a shift in fibre types towards fast-twitch muscles (55,56), a common feature of these models is that they are associated with a variety of confounding factors that make the interpretation of the effect of "pure" disuse on the microvasculature challenging. These factors include, for example, the effect of sympathectomy during denervation, the effect of behavioural stress and change in the animal feeding pattern during suspension, or the effect of temperature during hibernation.

Clinical/human studies have also examined the effect of disuse on skeletal muscle microvasculature. These include studies of denervation (10), ruptured tendon (11), immobilization (12), disuse atrophy, inflammatory myopathies, neurogenic disorders (13), mitochondrial myopathy (14), peripheral vascular disease (16), space flight (17) and bedrest (18). Although the effect of disuse is difficult to separate from that of various pathologies, space flight and bedrest offer an opportunity to study disuse in terms of a diminished muscle load.

3.2. Capillarity

All three components of the microvasculature (arterioles, capillaries, venules) participate in the transvascular exchange of materials in skeletal muscle. Although the large total surface area of capillaries makes the capillary bed the major site of oxygen supply to the muscle fibres, in resting muscle a significant amount of oxygen diffuses from arterioles to the tissue (19), and stimulated venules can provide the major route of transport for large solutes (20). While the gross structure of larger blood vessels (e.g., arterioles) appears to be preserved during disuse, media thickening and decreased arteriolar diameter were found after long term denervation (23). The major adaptation to disuse occurs at the capillary level. The anatomical density of capillaries in skeletal muscle has been used as a structural index of blood/oxygen supply to tissue. However, since muscle disuse is associated with muscle fibre atrophy, changes in capillary density can reflect changes in the total number of capillaries, changes in fibre size, or both. Thus, the capillary-to-fibre ratio (C/F) or the number of capillaries around a fibre have been used as the appropriate indices of capillarity in disused skeletal muscle.

In general, there is a significant correlation between (i) the C/F ratio and the metabolic characteristics of the muscle (i.e., a larger C/F in oxidative than in glycolytic muscles) and (ii) C/F ratio and fibre diameter (i.e., larger C/F for larger fibres; 13). In mammalian animal models of disuse with residual muscle activity, a modest reduction in fibre size (15-30 % atrophy) is accompanied by an unchanged C/F ratio. This lack of change has been reported for hibernation (9), 7-day space flight (8), 7 and 14-day whole body suspension (8), and 7-day tail suspension (7). In an amphibian model, even a severe atrophy (64%) did not alter C/F ratio (29). In clinical studies, unchanged capillarity has been reported in calf muscle of patients with intermittent claudication (16), and in deltoid and vastus lateralis muscles of healthy subjects after 37 to 42-day bedrest (18,30). Extending the duration of disuse in the models with residual activity, or employing models without this activity, results in a reduced C/F ratio (figure 1). A 5-wk rat tail suspension (associated with a 63% atrophy in soleus muscle) caused a 49% reduction in C/F in this tissue (7). In animal models of minimized or eliminated muscle activity, a 1 to 3-wk immobilization (5,21), 3-wk to 18-mo denervation (22,23), 7-wk nerve crush (24), 2 to 8-wk nerve conduction block (26) or 1 to 3-wk tenotomy (5) of various hindlimb muscles caused a 10 - 90% reduction in C/F ratio. For the same duration or type of disuse, C/F reduction was more

pronounced in oxidative than mixed muscles (5). A similar

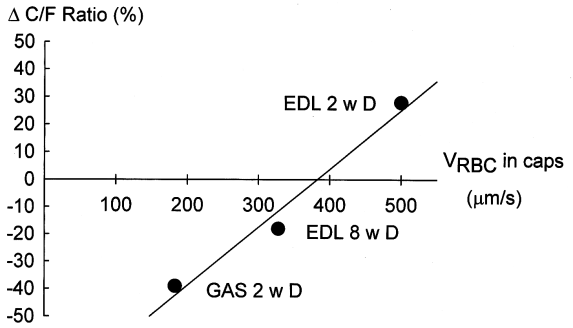

Figure 2. Change in capillarity in disused muscle may depend on blood flow in the capillary bed. Y-axis: change in capillary-to-fibre ratio (delta C/F Ratio) in rat extensor digitorum longus and in the medial portion of gastrocnemius muscle muscles after 2wk functional sympathectomy (EDL 2wD and GAS 2wD points, respectively), and in the EDL muscle after 8wk functional sympathectomy (EDL 8wD point). X-axis: red blood cell velocity measured in capillaries of the disused muscles. The line is the best fit through the three points. EDL 2wD point indicates angiogenesis after disuse, while EDL 8wD and GAS 2wD points demonstrate capillary loss (26).

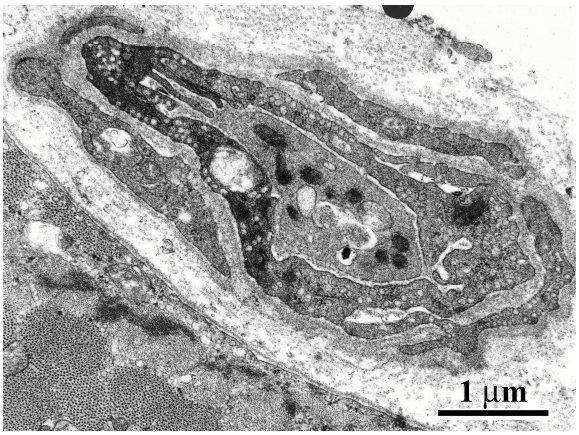

Figure 3. Degeneration of the capillary wall in the rat extensor digitorum longus muscle after a 2-wk disuse caused by a functional sympathectomy (4).

range of C/F reduction was reported in clinical/human studies of space flight (11 days; 31), denervation (10), ruptured tendon (11), disuse atrophy, inflammatory myopathies, neurogenic disorders (13), and mitochondrial myopathy (14).

To our knowledge, the mechanism of the capillary removal process from skeletal muscle during disuse is not known. In particular, it is not known whether fibre atrophy and capillary loss depend on the same mechanisms (23). Increased interstitial collagen around vessels and muscle fibres with disuse (15, 23) further separates the muscle fibres from their reduced capillary supply, and can contribute with the capillary loss to the hypoxia-induced atrophy of the muscle fibres (23). The characteristic interstitial fibrosis with disuse can also impair the

microcirculation and cause capillary loss, which in turn can trigger further fibrosis (15). While capillary loss preceded morphological evidence of muscle fibre degeneration in denervated muscle, substantial spatial heterogeneity was found in both capillary and fibre degeneration in the tissue (23).

Clearly, the confounding effects associated with the various models make it difficult to set up an experiment where only disuse is manipulated and the removal process studied under controlled conditions. Our recent experiments demonstrated that confounding effects can modulate the capillary removal process, and that reduction in capillarity may not depend solely on the duration of disuse, the presence/absence of residual activity and/or the resulting degree of muscle atrophy. Using a nerve conduction block model (tetrodotoxin, TTX, application on sciatic nerve) in the rat extensor digitorum longus (EDL) muscle, a 2-wk TTX application caused a severe muscle fibre atrophy (52%) and, surprisingly, an increased C/F ratio (4). Since this model is associated with a substantial drop in mitochondrial volume density (4) and citrate synthase activity (27), there appears to be a clear dissociation between oxygen supply and demand in this disused muscle. The model had the ability to reduce C/F, as the gastrocnemius muscle (medial portion) in the same hindlimb exhibited both atrophy and a 39% C/F reduction (26). Measurements of red blood cell velocity in the capillary bed of both muscles revealed that TTX-induced disuse at 2 wks was associated with an elevated velocity in the EDL but not gastrocnemius muscle. Analysis of change in capillarity versus velocity indicated that the outcome of disuse may be modulated by capillary blood flow (figure 2), and that flow-related angiogenesis (28) may be superimposed on the capillary removal process during disuse (26). A similar modulation of capillarity during disuse (nerve crush model) was reported by Zaida and coworkers (24) who noted an increased capillarity in EDL muscle after a chronic elevation of blood flow.

3.3. Capillary ultrastructure

At the ultrastructural level, several studies have documented the capillary degeneration process in various animal models of muscle disuse (4,23,26,29) and in clinical study of denervation atrophy (10). In rat soleus muscle, Oki and coworkers (25) observed endothelial fenestrations and pores in 8-10% of capillaries after 4 to 12-wk immobilization of the ankle joint. In frog sartorius muscle after 7 to 14-mo of laboratory captivity, 34% of capillaries were associated with signs of endothelial damage (i.e., wall thickening, increased size and frequency of both cytoplasmic folding and projections; 29). In rat EDL muscle subjected to 2 wks of TTX-induced disuse, 11% of capillaries were found damaged in 12-mo old rats (4) (figure 3), while negligible damage was seen in the same muscle in 2 to 4-mo old rats (26). Negligible capillary damage was also found in the EDL muscle in 2 to 4-mo old rats after 8-wk TTX application (26). This contrasts with the report of Borisov and coworkers (23) who documented progressive capillary damage and capillary loss in the rat EDL muscle after 1 to 18-mo denervation. From these studies it appears that a snap-shot of capillary ultrastructure at a particular time of muscle disuse may not be sufficient to determine the mechanism of capillary removal, since capillary damage does not correlate with C/F reduction [i.e., damage without C/F reduction (4,29), no damage with reduction (26), and damage with reduction (23) have all been reported].

It is the present challenge to determine which tissue stimuli generated by the normal use/physical loading of the muscle are necessary for the maintenance of the vascular bed, which stimuli are responsible for the capillary removal during disuse, and whether these stimuli are produced by forces originating at the interface between the blood stream and the vascular wall (28), within the extracellular matrix, within the muscle fibres themselves (1), or at all of these sites.

3.4. Microvascular blood flow in resting skeletal muscle

From the standpoint of optimal blood flow control in skeletal muscle (i.e., matching oxygen/nutrients supply to tissue metabolic demand), one would expect that disuse is accompanied by a reduced resting blood flow. However, as indicated above, a tight coupling between supply and demand may not occur during disuse as indices of lowered metabolic activity (i.e., reduced mitochondrial volume density and citrate synthase activity) can be accompanied by indices of increased oxygen supply (i.e., increased capillarity). Thus, it is not surprising that many studies report no reduction in the resting blood flow (i.e., relative flow, expressed in ml/min per 100 g) in a variety of muscles subjected to disuse. The relative flow is more suitable for the assessment of blood flow control than the absolute flow (ml/min) to the organ, since the relative flow takes into account the volume of metabolically active tissue that the supply of oxygen/nutrients to the organ subserves. It should be noted, however, that in disused muscle the relative flow depends on the blood pressure drop across the vascular bed of the muscle, the resistance/geometry of the vasculature, and the degree of disuse-induced muscle atrophy. Thus, based on reports that disuse does not alter the mean systemic blood pressure (4,26,33), the absolute flow may be more useful from the hemodynamic point of view to assess changes in the vascular resistance/geometry during disuse. Hudlicka (2) has shown that disuse caused by sectioning of ventral roots in the cat resulted in a steady increase in the relative flow in the gastrocnemius muscle as atrophy increased with the duration of disuse, but in no change in the absolute blood flow. Thus the apparent mismatch between oxygen supply and demand in this muscle was likely accompanied by no net change in the vascular resistance during disuse.

Much of the present knowledge of microvascular flow adaptation in disused muscle comes from animal studies, as direct measurement of microvascular flow in clinical/human studies is difficult. In rats subjected to 2-wk hindlimb suspension, no change was observed in the relative flow to the majority of disused muscles (32,33). However, the soleus muscle exhibited lower relative (33,34) and absolute (33,36) flows, indicating that in the presence of an unchanged blood pressure (33) an increase in the soleus vascular resistance had occurred. This increase could be due to a smaller resting

diameter of arterioles of this muscle (35,47,48) since arteriolar resistance is the major component of the organ vascular resistance (40). Interestingly, McDonald and coworkers (33) reported an increase in both absolute and relative flows in the vastus lateralis muscle after 2-wk suspension, suggesting a lowered vascular resistance in this particular muscle. Recently, Delp (48) provided a plausible explanation for this lowered resistance by observing that suspension reduced the development of spontaneous tone in arterioles of muscles composed of fast-twitch type IIB fibres.

Extending suspension to ~13 wks results in reduced relative and absolute flows in both soleus and gastrocnemius muscles (33). This may be not only due to the smaller resting arteriolar diameter but also due to an increased total capillary bed resistance, since a substantial loss in capillarity during extended disuse could contribute to an increase in vascular resistance.

The lack of reduction in the relative blood flow has also been reported in muscles subjected to immobilization (12,37). The atrophy associated with this model implies that the absolute blood flow in these muscles was reduced and the vascular resistance likely increased. The absence of tight coupling between oxygen supply and functional demand of disused muscle is also evident from the comparison between denervation and tenotomy models. Although denervation can cause a similar degree of muscle atrophy as tenotomy (38), the relative blood flow in rat hindlimb muscle increased 1 wk after denervation (3) but it decreased over a similar time period after tenotomy (38). Clearly, the confounding effect of sympathectomy and the subsequent reduction of vascular tone (39) could mask the blood flow response to a denervation-induced disuse. Our recent experiments using a nerve conduction block model (i.e., a functional sympathectomy via 2wk TTX application) indicated that the absolute flow (assessed in terms of red blood cell velocity in capillaries) in the rat EDL muscle was indeed elevated (4,26). A reduced vascular tone could have been responsible for this elevated flow (26). Surprisingly, our experiments also showed that the velocity in capillaries of the gastrocnemius muscle of the same hindlimb remained unchanged at 2 wks of disuse. These data implied a differential adaptation of vascular resistance/ geometry in the two muscles as they both underwent atrophy (26). The mechanism of this differential adaptation is unknown, but it could involve differences in alpha-adrenergic receptor sensitivity and/or density in the vasculature of the two muscles (41).

In an amphibian model, ~60% of available capillaries in the sartorius muscle were perfused with red blood cells after 7 to 14-mo of disuse. The resting state blood flow in these capillaries remained unchanged when compared with those of the control muscle (~85% of available capillaries perfused), suggesting that any increase in the capillary bed resistance could have been compensated by a reduction in resistance of larger microvessels.

Little of direct data is available to characterize the adaptation of resistance vessels (i.e., vessels responsible for setting the resting state flow) in disused muscle. From available indirect data, a complex picture emerges as it appears that both increases and decreases in arteriolar tone can occur, and that these changes may be muscle-specific. Adaptation of arteriolar tone could result from a number of processes, including a change in the profile of vasoactive metabolites that impinge on the arteriole (e.g., vasoactive agents could be released from degenerating skeletal muscle), a change in the receptor sensitivity/density and intracellular signalling, or vascular wall remodelling known to occur in larger conduit blood vessels (42,47,49). A reduction in capillarity could also affect this adaptation process as the capillary bed and arterioles are functionally coupled through blood flow.

3.5. Microvascular responsiveness to acute stimuli

A key functional response of the microvasculature in skeletal muscle is the ability to increase blood flow (via vasodilation) to the muscle during contraction. A number of studies have examined the effect of muscle disuse on the vasodilative responsiveness. In the hindlimb suspension model (15 day), no difference in running-induced exercise hyperemia was found in various hindlimb muscles of control versus suspended rats (32). Using the same model, hyperaemic absolute blood flow during a graded-intensity running was reduced in disused soleus and plantaris muscles (36). Electrically induced muscle contractions yielded a significantly smaller hyperemia in all disused muscles tested (33). Recently, Schrage and coworkers (35) also demonstrated a reduced exercise-induced hyperemia in the soleus muscle of suspended rats. *In vitro* testing of arterioles from this muscle showed that disuse reduced the vasodilative response to acetylcholine (57) and to a step-increase in flow (50), partially due to a lowered endothelial nitric oxide synthase expression in these arterioles (50,57). A similar animal model and approach were used by McCurdy and coworkers (43) who showed that arterioles from disused soleus and white gastrocnemius muscles dilated normally after isoproterenol, but less than controls after stimulation with adenosine or sodium nitroprusside. Arterioles from the soleus muscle were affected more by disuse than those from the gastrocnemius muscle.

In models of more severe disuse, a 3-wk immobilization of dog gastrocnemius muscle also resulted in reduced contraction-induced hyperemia (44). However, a 3-wk denervation of the rat tibialis anterior muscle resulted in no reduction in hyperaemic flow (3).

The vasodilative ability in disused muscle has also been assessed in terms of the reactive hyperaemic response following a temporary blood vessel occlusion. Following a prolonged immobilization of ~10 wks, the reactive hyperemia in human calf muscles was reduced (12). An absence of reactive hyperemia was also found after long-term disuse in amphibian skeletal muscle (29). Because of an extensive endothelial damage found in this muscle, this absence could be due to an impaired endothelium-dependent arteriolar function (29). In rat hindlimb muscles subjected to 2 to 8-wk TTX-induced

disuse, no reduction in reactive hyperemia was observed (26). Thus, similar to mechanical denervation (3), the TTX functional denervation model of disuse also failed to reduce vasodilative responsiveness. It appears therefore that the ability of the vasculature to dilate may be compromised by disuse, depending on the strength of the vasodilatory stimulus, the severity or duration of disuse, and the presence of confounding effects of the model of disuse (e.g., denervation).

Because of the importance of fluids shifts in astronauts during space flight and their orthostatic intolerance after landing, the vasoconstrictive ability of blood vessels has also been examined in muscles after disuse. In general, a reduced vasoconstriction was reported for aorta of rats subjected to 2 to 4-wk hindlimb suspension (51,52,53). For smaller conduit vessels, normal vasoconstriction was seen in mesenteric artery (54), but both normal and reduced vasoconstriction were reported for femoral artery, depending on the rat strain (53). A differential vasoconstrictive ability was also reported for arterioles in hindlimb muscles of suspended rats (48). In the white portion of the gastrocnemius muscle, the arteriolar sensitivity to KCl and norepinephrine (NE) was reduced, whereas it remained unchanged in the soleus muscle. A relevant study of forarm subcutaneous vascular resistance in humans showed an increased resistance responsiveness to a lower body negative pressure step after a 10-day space flight (17). Contrary to this adaptation of the subcutaneous circulation, Sayet and coworkers found that space flight (12 days) reduced the vasoconstrictive responsiveness of the rat vena cava to NE, via a protein kinase C-dependent desensitization of $alpha_{1B}$-adrenoceptors (45). Using myocytes from portal vein in rats suspended for 2 wks, the same group has further suggested that the reduced vasoconstrictive responsiveness could be due to reduced intracellular calcium signalling via a decreased number of functional calcium channels in the myocyte sarcoplasmic reticulum (46). Thus the process of disuse has the potential to impair the vasoconstrictive component of microvascular blood flow control in both tissue- and species-specific manners.

4. SUMMARY

The regression of the microvascular structure and function in skeletal muscle during disuse has been studied in a variety animal models and in a number of clinical/human investigations. Capillary loss, arteriolar remodelling and altered arteriolar responsiveness to acute stimuli have all been observed in disused muscles. Since there appears to be dissociation between oxygen supply and demand in disused muscle, the process of regression of the microvascular bed may depend on many factors including duration of disuse, degree of muscle atrophy, presence/absence of residual muscle activity, microvascular blood flow, release of vasoactive agents from the degenerating muscle, muscle type, animal species, and the particular pathology associated with the muscle withering in humans. It is the present challenge to discover the presence/absence of key agents that alter the intra- and/or inter-cellular signalling responsible for the "large scale" features of regressed microvasculature characterized in disused skeletal muscle so far.

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Abbreviations: C/F capillary-to-fibre ratio, EDL extensor digitorum longus, NE norepinephrine, TTX tetrodotoxin

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