Soft Tissue Behavior During Limb Lengthening: An Experimental Study in Lambs

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SUMMARY

The effect of femoral elongation on skeletal muscle, nerves and vessels was studied. Three groups of five lambs were used. After the intervention, the animals were killed at 2. 3. and 4 months. A left femoral elongation of 6 cm was practiced on all of them by means of callotasis, with a distraction rate of 0.5 mm every 12 hours. The femoral elongation process was evaluated by monthly x-ray films. The nucleic acid and protein levels in the muscular tissue were quantified at the level of the elongation focus and in the control extremity. The motor conduction velocity of the sciatic nerve was measured in both posterior limbs before the intervention and immediately before the lambs were killed. The arterial blood flow of both subsequent extremities was measured at the moment of death. A histological study of quadriceps muscle, sciatic nerves, artery, and subsequent femoral vein were examined histologically at the level of the elongation focus of both extremities.

After elongation, no significant differences were observed in the muscle protein and nucleic acid levels with respect to the control extremity. No significant changes of the nerve conduction velocity were observed in any animal among the different groups. The arterial blood flow of the elongated extremity showed a progressive increase, reaching its maximum value 1 month after the distraction had terminated, with subsequent normalization. This increse of the blood flow was also observed in the control extremity, suggesting a possible systemic effect. The histological study revealed a comparative thickening of the endomysium and perimysium in the elongated muscle tissue, present at the end of the distraction and which was later normalized. No histological changes of the nerve stems undergoing distraction were observed either. During elongation, the arteries showed minimal histological changes. On the other hand, the veins showed areas of endothelial damage accompanied by thrombosis phenomena, especially at the end of the distraction period. The vascular morphology presented progressive normalization after the distraction phase.

KEY WORDS

External fixation; Limb lengthening; Soft tissues.

INTRODUCTION

Bone lengthening can be limited in its extent by the response of the soft tissues, of acute or insidious presentation, to the progressive increase of tension. Muscle contracture and loss of strength (8,27,41,46,61) have been mentioned among the effects of bone elongation on muscle tissue. Signs of muscle tissue growth have been observed, which disappear after distraction (30-32,53,57). One of the most important complications during limb lengthening is nerve injury. This injury may appear intraoperatively by direct injury of a nerve stem (40,45), by an extemporaneous elongation of the member (6,36, 43), or during the progressive distraction phase (10.46,58). In human clinical practice, fractionating of the distraction has been recommended, adapting its rhythm to the biological response of each patient, thus significantly reducing the risks of nerve injury from traction (37,46). Among the effects that bone elongation can cause on the cardiovascular system, arterial hypertension (16,46), alterations of the blood flow (BF) of the extremities (22,23,25), and structural alterations of the blood vessels (19,20,26,35,48) have been cited. In the present work we study the functional and histological response of muscle, nerves, and vessels during extremity elongation.

METHOD

Fifteen 10-week-old lambs with an average weight of 15.4 kg were used. They were divided into three groups of five animals according to the time of external fixation: 2 months (Group A), 3 months (B), and 4 months (C). A percutaneous diaphyseal osteotomy of the left femur was performed under general anesthesia. By means of a Wagner's external fixator, a femoral elongation of 6 cm was achieved, the distraction beginning on the first postoperative day at a rate of 0.5 mm every 12 hours. X-ray films of the left femur were taken in a standardized anteroposterior projection intraoperatorily and later monthly. The free movement of the animals was permitted postoperatively with full load on the extremity intervened. The right extremity served as control.

Under sedation with 1 mg of intramuscular flunitrazepam, the sciatic nerve motor conduction velocity of both posterior limbs was measured preoperatively and immediately before death using a Medelec Ms 92a apparatus. The needle electrode of proximal stimulation was implanted between the medium and deep gluteal muscles at the level of the hip joint. The needle electrodes of distal stimulation were placed at the level of the popliteal cavity. The distal electrodes were implanted in the lateral flexor muscle of the toes at the level of the most proximal section of the tibia. Under general anesthesia, immediately before death, the BF of the common femoral artery (CFA), anterior femoral artery (AFA), and anterior tibial artery (ATA) was measured in both posterior extremities. This measurement was carried out by means of gauged probes attached to a Cliniflow II FM7OIDE (Carolina Medical Electronics Inc.) apparatus before the dissection of these vessels. Under general anesthesia, immediately before death, samples were taken of the external vast muscle, sciatic nerve, posterior femoral artery (PFA), and posterior femoral vein (PFV) at the level of the elongation focus in both posterior extremities. Muscle specimens were processed for nucleic acid and protein measurement according to methods previously published in detail (7,9). The histological preparations were stained with hematoxylin-eosin and Masson's trichrome. The animals were killed with an intravenous overdose of C1K. The statistical study was carried out by means of the Mann-Whitney U test.

RESULTS

Fractures were not produced, although a mild varixation of the elongation focus was observed in the final phase (Fig. 1). No clinical manifestations of neurological deficiency of the limb intervened were observed in any of the animals. The lambs presented a stiffness of the left knee during the distraction phase, which disappeared progressively.

Nucleic acid and protein measurement

Tables 1 and 2 show the protein and nucleic acid values. No significant differences were observed in the values obtained from both extremities at the moment of death in any of the animal groups.

Nerve conduction velocity

Tables 3 and 4 show the data of the motor conduction study. No significant differences in the motor conduction velocity values were observed between both limbs preoperatively or immediately before death. No significant differences were observed between the latency values either.

Blood flow

Table 5 shows the results of the BF measurement. All the animals showed a greater BF in the elongated extremity at the three levels of measurement with respect to the control extremity. The differences were significant at the three levels: CFA ($p < 0.005$), AFA ($p < 0.05$), and ATA ($p < 0.001$). The BF reached its maximum values in Group B, killed a month after the distraction terminated. There were no significant differences between the values of Groups A and C.

Histological changes

The muscle tissue of the elongated extremities (Fig. 2) showed a thickening of the endomysium and perimysium at the end of the distraction (Group A), which was progressively normalized in the animals of Groups B and C. Sciatic nerve of the elongated limbs presented no histological differences with respect to that of the control limbs (Fig. 3). No alterations of the epineural and perineural connective tissue were seen. In one Group B animal, the presence of an inflammatory infiltration was observed surrounding the intraneural vessels in the sciatic nerve undergoing distraction (Fig. 4). No histological differences were observed between the wall of the elongated PFA and the control in the three groups. Occasionally, erythrocyte aggregates were observed in the proximity of the arterial endothelium both in the elongated arteries as well as in the controls of Group A (Fig. 5). These findings were not observed in Groups B and C. The elongated PFV showed arcas of endothelial loss accompanied by thrombosis phenomena in Group A (Fig. 6). Once the distraction terminated, normal morphology recovered progressively; small arcas of endothelial damage were observed in some animals of Group C.

DISCUSSION

Because of the progressive tension of the soft tissues, the lambs presented a stiffness of the left knee during the distraction phase, which disappeared progressively. This fact is frequently observed in humans as a response to the contracture of the strongest muscle group of the elongated bone segment (11,46).

Nucleic acid and muscle protein measurement

No significant differences were observed between the nucleic acid and protein values of the elongated muscle with respect to those of the control muscle. This finding suggests the absence of hyperplasia or muscular hypertrophy at the level of the central section of the elongation focus. This observation constrasts with those of Ilizarov (19,20), who considered that the muscular tissue subjected to progressive distraction grows by means of fusion in myotubes from myoblasts proliferating throughout the muscle if the length increase does not exceed 20%. To this respect, Yasui et al. (59) stated that they cannot specify how this process, also observed by Schumacher et al. (53) in rabbits, is carried out. The fact that in our animals the final length increase reaches 50% would justify these differences.

Schultz (52) considered that, during the growth, the process of myogenesis is carried out from a subpopulation of myoblasts, which continues throughout the lifetime. Their proliferation produces a progressive increase of the nucleic acid contents of the myofibrils, that stabilizes on reaching maturity. This method can be reactivated, for reparative purposes, in response to a muscular injury, although its intensity is inversely proportional to the age and muscular weight of the individual. Our results suggest that this phenomenon would not be carried out at the level of the elongation focus because of no increase in the nucleic acid or muscle protein contents at said level.

For Kochutina (30-32) this phenomenon takes place at the level of the tenomuscular joints when an elongation by means of double corticotomy is performed. Griffin et al. (12) considered that muscle growth is carried out in the tenomuscular joints by means of the addition of new sarcomeres at this level and not all along the growing myofibrils. Ilizarov (20,21) established that, during extremity elongation, the proliferative, metabolic, and biosynthetic changes in the cellular activity of the elongated tissues are similar to embryonic and fetal histogenesis as well as to postnatal extremity growth. Although other authors (2,17,49,57) claimed that progressive distraction provokes skeletal muscle hypertrophy, our results do not support this fact at the level of the osteotomy without denying the fact that this phenomenon is at the level of the tenomuscular joints.

Nerve function study

The absence of clinical and functional manifestations in our study contrasts with those reported by other authors. Thus, Wall et al. (62) together with Kwan et al. (34) reported in vivo an impairment of the function of the tibial nerve in rabbits after subjecting them to different percentages of maintained tension. The time between the application of the tension and the appearance of the functional impairment was in direct relation to the magnitude of the applied tension as well as the capacity of functional recuperation. Similar findings were observed by Lundborg and Rydevik (38,51) in their in vitro models as well as by Yong Lee et al. (66) using evoked potentials in rabbits. Brown et al. (4), indicated the wide variety of tension limits in the biomechanical studies of the peripheral nerves, most of which use in vitro models.

Mizumoto et al. (44) reported differences in the latency values if a motorized distraction is used with respect to a distraction in two sequences, which did not exceed 1 mm/day, observing less damage to the nerve function with the motorized distraction. Simpson and Kenwright (55) reported an impairment of the nerve function after carrying out a progressive distraction in rabbits, if a tension of 2% a day is exceeded. Kenwright (29) calculated the magnitude of the tension necessary to cause functional damage to be 15%. Battiston et al. (3) did not find functional nerve damage in rats if the distraction velocity did not exceed 1 mm/day, coinciding with that observed in our results. However, Strong et al. (58) observed, in dogs undergoing femoral elongation, normality in the nerve conduction study with electromyographic signs of denervation, locating the nerve damage at a proximal level to that of the elongation.

In human clinical studies, Galardi et al. (10), together with Davis et al. (8), observed a reduction of the motor conduction velocity as well as a partial motor denervation without clinical effects in patients undergoing tibial elongation following Ilizarov's technique. Cañadell et al. (5) also observed electromyographic alterations in 85% of patients undergoing limb elongation, although without clinic repercussion.

Young et al. (67), as well as Cañadell et al. (5), observed a fundamentally neuropathic pattern in their patients. For Young et al. (67), the cause was the rise of progressive tension associated with a rise of intracompartmental pressure, capable of altering the intraneural blood flow without a real compartmental syndrome being established. This hypothesis, already suggested by Galardi et al. (10) and Davis et al. (8), is supported by the observations of Mizumoto et al. (44), who reported a gradual decrease of the intraneural blood flow in relation to progressive distraction in rabbits. In this way, the pathogenesis of functional nerve damage during limb elongation would be explained by the association of mechanical damage with the ischemia provoked by a subacute compartmental syndrome. On the other hand, Brown (4) and Makarov (40) and their colleagues considered that the presence of scar tissue, which changes the elastic behavior of the nerve stems undergoing distraction, may facilitate the appearance of functional damage in the same.

Blood flow measurement

Our results showed a mean BF increase of 151% in the elongated extremity. This increment represents an intermediate value between the findings of Lavini et al. (35) and Schwartsman (54) in patients undergoing extremity elongation. Their results range widely, from a 43 to 330% increase of the BF, measured by different methods. Ilizarov (19-21) suggested the possibility of improving the clinical condition of some patients affected by occlusive vascular diseases by means of techniques of bone elongation.

The maximum values of BF were observed in the killed animals a month after the distraction ended (Group B); the values reached in Groups A and C were similar. This fact suggests that progressive distraction may have a transient and deferred effect in BF increase. Our findings agree with those observed in rats by Grundnes and Reikerås (13,14). Using labeled microspheres, they reported that, between 4 and 8 weeks after performing an osteotomy, the blood flow of the bone and of the peripheral musculature increased significantly, later recovering its normal values. Kelly et al. (28), along with Grundnes and Reikerås (13,14), suggested that this increase of BF indicates a metabolic requirement of the bone repair process, although this response may be conditioned by other factors. Similar observations have been reported by Aalto (1) with rabbits and Zdeblick et al. (68) with dogs. Wallace et al. (63), in their study of lambs, observed that the BF presents a similar behavior at the level of a diaphyseal osteotomy stabilized with a dynamic external fixator if the adjacent soft tissues were preserved. In contrast to Wallace's study, our system of external fixation was rigid. Kofoed et al. (33) explained the increment of BF as a response to the tissular hypoxia provoked by the osteotomy followed by an angiogenesis stimulus.

The BF increase was also observed in the control extremities, perhaps as an expression of a systemic effect that the distraction would cause on the peripheral BF. Although the BF increase is able to stimulate bone growth (15,42,47,64), the final length difference of the femurs of our animals did not exceed 6 cm. An explanation of this fact is that said stimulus might have been the same on both extremities; therefore, the final difference of femoral length did not exceed that provoked by elongation.

Histological changes

Muscle

The elongated muscle showed a comparative thickening of the perimysium in the animals of Group A, which disappeared progressively in Groups B and C. This finding coincides with the observations of Kochutina et al. (30-32) in dogs and those of Yong Lee et al. (60) and Simpson et al. (56) in rabbits. According to Yong Lee et al. (60), these findings after a 20% length increase suggests irreversible muscular damage. Povkov et al. (49) asserted that these histological changes disappear progressively after the distraction ends and the system of external fixation is removed. For Simpson et al. (56), the histological changes of the muscle tissue subjected to distraction are minimal if a distraction rate of 1 mm/day is not exceeded. Kochutina et al. (30-32) reported dystrophic and degenerative changes accompanied by regenerative phenomena when an elongation by means of double osteotomy is performed and at a rate of 2 mm/ day. On the other hand, Pouliquen et al. (50) in rabbits and Peretti et al. (48) in calves did not observe morphological changes in muscle subjected to different distraction rates.

Matano et al. (41) reported differences between the percentage of muscle and bone elongation. In their observations, this percentage is higher in the muscle than in the bone. They, therefore, suggest the necessity of considering this fact in bone extremity elongations to avoid excessive tension on muscle tissue. The histological changes observed by different authors might be related to the transitory functional loss observed by Mafulli et al. (39) and Käljumae et al. (27) in patients subjected to bone elongation, who, according to Holm et al. (18), tend to recover completely.

Nerve

Endoneural and perineural integrity after subjecting a nerve to tension has been considered a positive factor with respect to the maintenance of the nerve function (34,51,62). This observation would support the absence of functional disorders in our animals in relation to the conservation of the endoneural and perineural structures observed in the histological study. Our results contrast with those of Pouliquen et al. (50), who observed nerve fiber rupture and vacuolar degeneration images in rabbits undergoing femoral elongation at a distraction rhythm greater than 1 mm/day as opposed to our experimental model. Battiston et al. (3) observed proliferation of connective tissue and of Schwann's cells of the sciatic nerve of rats undergoing progressive distraction, with an absence of functional impairment if the distraction rate did not exceed 1 mm/day.

For their part, both Peretti et al. (48) and Ippolito et al. (26) observed, in calves undergoing metacarpal elongation, that in the first stages of the distraction injuries were already appearing in the myelin sheath in the fibers of greater diameter. These injuries were associated with images of axonal degeneration when the elongation obtained was greater. According to their observations, were the myelin fibers of smaller diameter together with the amyelin fibers which best support progressive distraction (26,48). Similar findings were reported by Kochutina et al. (32) in dogs undergoing femoral elongation: the degenerative and regenerative phenomena of the nerve fibers coexisting during the distraction phase, with prevalence of the regenerative at the end of this phase.

The intraneural vessels of our lambs did not present any alterations. This observation contrasts with the physiopathology of nerve injury caused by traction suggested by Lundborg et al. (38) and Rydevik et al. (51), considering that progressive tension would lead to an intraneural vascular occlusion with secondary ischemia. Other authors have tried to explain functional nerve impairment by the mechanical deformation caused by tension (34,62) and by a possible disorder of calcium metabolism at the level of the nerve ends (65).

Vessels

Ilizarov (19,20) established that, during the extremity elongation, the proliferative, metabolic, and biosynthetic changes in the cellular activity of the elongated tissues are similar to embryonic and fetal histogenesis as well as to postnatal extremity growth. Just as Pouliquen et al. (50) observed in rabbits, we found minimal tissue changes in the vessels present at the end of the distraction stage after the initial femoral length had increased by 50%, and which later disappeared. However, Peretti et al. (48) and Ippolito et al. (26) observed atrophy of the arterial middle tunic in calves with duplication of the internal elastic membrane, vacuolization of the muscular fibers, and partial loss of the contractile proteins when the elongation exceeded 20%. They also observed, as did Ilizarov (19-21), transitory thinning of the venous wall, with the presence of degenerative and regenerative phenomena. In the same way as in our observations, such alterations were normalized 2 months after the distraction stage ended. Our results, together with those of these authors, suggest that arterial vessels are more resistant than venous vessels to progressive distraction.

The observation of thrombosis phenomena was previously reported by Ilizarov et al. (19,22-25) in dogs as a systemic effect. Ilizarov attributed this observation to an alteration of the physiological balance of coagulation provoked by too rapid a distraction or by the instability of the system of external fixation. Our observations support this possibility because phenomena of thrombosis could be observed in the control vessels. The endothelial damage described by Ippolito et al. (26) and Peretti et al. (48) and observed in our animals 2 months after the distraction is another explanation of this thrombosis phenomena. Pouliquen et al. (50) found in rabbits that a more rapid distraction provokes greater venous endothelial damage. However, the distraction rate in our animals did not exceed 1 mm daily in two steps.

These histological findings may partly explain certain clinical observations during bone elongation such as the edema of the member, which has been attributed to venous deficiency or lymphatic alterations (26,46).

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Table 1. Protein values (mg/g)		
Animal	**Control**	**Lengthened**
A1	109.16	70.46
A2	86.85	76.78
A3	87.89	100.00
A4	85.14	85.08
A5	117.81	I 17.07
BI	101.25	118.26
B2	89.04	77.39
B3	91.20	80.90
B4	103.00	94.20
B5	91.50	87.10
C I	58.10	59.50
C2	106.19	76.04
C3	90.20	81.07
C4	87.80	98.92
C5	99.30	106.40

Table 2. Nucleic acids values (mg/g)				
	DNA		**RNA**	
Animal	**Control**	**Lengthened**	**Control**	**Lengthened**
Al	1.85	2.02	2.23	1.69
A2	1.22	0.04	2.13	1.16
A3	1.01	0.78	3.00	3.80
A4	0.65	0.38	3.70	3.00
A5	1.80	0.38	1.60	2.09
B1	3.42	1.70	1.92	1.41
B2	1.59	1.92	1.28	1.23
B3	2.50	2.00	1.91	1.31
B4	1.33	1.37	1.71	0.98
B5	2.06	1.76	1.52	2.11
C1	1.50	1.78	1.44	2.22
C2	1.05	1.82	1.72	2.26
C3	1.57	1.75	2.01	2.10
C4	1.80	1.95	1.53	1.98
C5	I.61	1.70	1.60	1.09

DNA, deoxyribonucleic acid: RNA, ribonucleic acid.

Table 3. Preoperative nerve conduction study

	Unoperated extremity						Operated extremity					
Lambs	**TI**	**T2**	**Δ**	**D**	**A**	**V**	**TI**	**T2**	**Δ**	**D**	**A**	**V**
Al	3.4	1.8	1.6	150	1	93.00	3.2	2.0	1.2	120	2	100.00
A2	6.6	5.0	1.6	150	3	93.00	3.0	1.6	1.4	150	3	107.00
A3	3.4	1.6	1.8	150	2	83.00	3.0	1.4	1.6	150	1	93.00
A4	5.8	3.6	2.2	180	3	81.00	5.4	3.2	2.2	180	3	81.00
A5	3.2	5.2	2.0	180	2	90.00	3.0	5.0	2.0	180	1	90.00
B1	2.4	4.0	1.6	180	2	112.50	2.4	4.0	1.6	180	1	112.50
B2	4.0	6.4	2.4	180	1	75.00	0.8	3.2	2.4	180	2	75.00
B3	5.7	3.5	2.2	180	3	81.00	5.5	3.3	2.2	180	3	81.00
B4	2.4	0.8	1.6	150	1	93.00	3.6	2.6	1.0	120	3	120.00
B5	2.4	4.0	1.6	160	3	100.00	2.4	4.0	1.6	160	1	100.00
C I	3.5	2.1	1.4	120	3	85.70	6.9	5.1	1.8	160	3	88.80
C2	6.4	4.8	1.6	150	1	93.00	3.2	1.8	1.4	150	2	107.00
C3	1.6	4.0	2.4	190	1	79.16	2.4	4.8	2.4	190	2	79.16
C4	2.4	4.8	2.4	170	3	70.80	1.6	4	2.4	180	1	75.00
C5	5.8	4.2	1.6	150	2	93.00	5.8	3.9	I.9	150	3	84.00

Table 4. Postoperative nerve conduction study

	Unoperated extremity						Operated extremity					
Lambs	**TI**	**T2**	**Δ**	**D**	**A**	**V**	**TI**	**T2**	**Δ**	**D**	**A**	**V**
Al	0.9	2.7	1.8	180	2	100.00	2.2	4.2	2.0	210	1	105.00
A2	2.0	4.2	2.2	210	1	95.45	2.4	4.6	2.2	200	3	90.90
A3	1.8	3.2	1.4	150	3	107.14	2.0	4.4	2.4	210	2	87.50
A4	5.4	3.4	2.0	200	1	100.00	3.4	6.0	2.6	210	2	81.00
A5	2.0	3.7	1.7	180	3	105.00	2.6	4.6	2.0	200	1	100.00
B1	4.4	2.7	1.7	150	1	88.23	6.1	4.2	1.9	165	2	86.84
B2	1.7	4.1	2.4	190	3	79.16	2.3	4.7	2.4	190	1	79.16
B3	3.6	2.2	1.4	120	1	85.70	6.8	5.0	1.8	160	2	88.80
B4	6.6	5.0	1.6	150	3	93.00	3.4	2.0	1.4	150	1	107.00
B5	0.9	2.7	1.8	180	3	100.00	3.2	2.0	1.2	120	2	100.00
C I	4.3	2.6	1.7	150	1	85.23	6.0	4.1	1.9	165	1	88.84
C2	2.1	4.3	2.3	210	1	95.45	2.4	4.4	2.0	210	1	105.00
C3	5.8	3.6	2.2	180	3	81.00	7.6	5.6	2.0	150	2	75.00
C4	3.4	5.4	2.0	150	1	75.00	2.3	4.7	2.4	180	3	75.00
C5	7.6	5.6	2.0	150	2	75.00	8.8	6.4	2.4	200	1	83.30

Table 5. Blood flow measurements (mL/min)

	CFA		AFA		ATA	
Animal	**Control**	**Lengthened**	**Control**	**Lengthened**	**Control**	**Lengthened**
Al	59	79	42	54	12	26
A2	80	150	67	100	19	39
A3	130	140	65	75	14	38
A4	40	60	29	42	11	25
A5	65	80	55	60	19	29
B1	100	125	75	86	23	34
B2	100	120	71	83	21	35
B3	80	120	65	90	24	34
B4	72	105	53	68	18	31
B5	65	92	55	85	21	36
C1	95	120	62	81	15	33
C2	59	84	47	53	13	31
C3	65	90	50	59	14	27
C4	73	98	64	70	18	32
C5	60	110	50	85	16	35

CFA, common femoral artery; AFA, anterior femoral artery; ATA, anterior tibial artery.

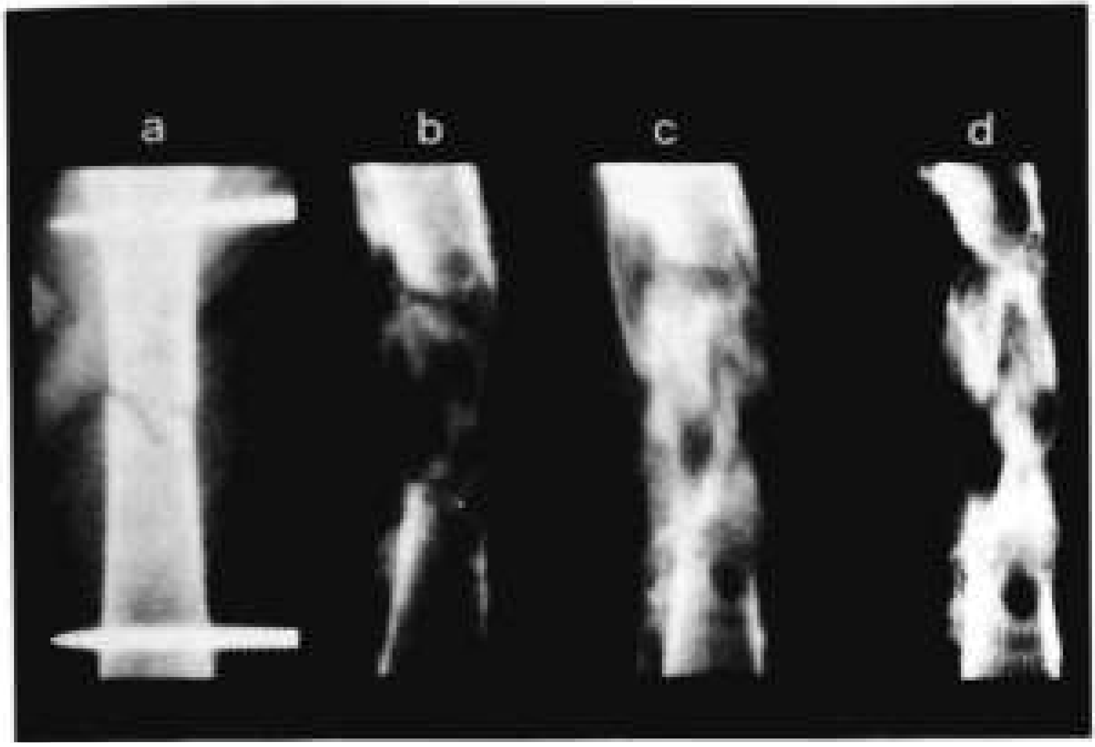

Figure 1. Radiographic image of lengthening callus: (a) immediately postoperatively; (b) 1 month postoperatively; (c) end of distraction period; (d) 3 months postoperatively.

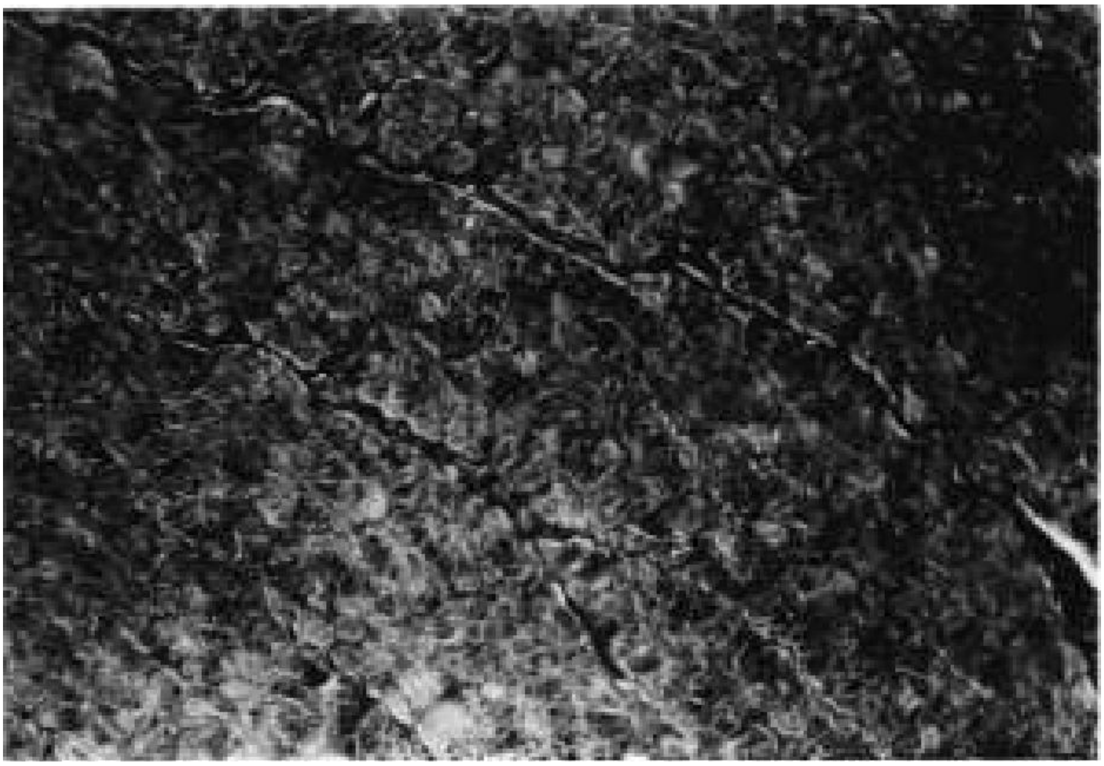

Figure 2. Lengthened muscle at end of distraction. (Masson's trichrome x300.)

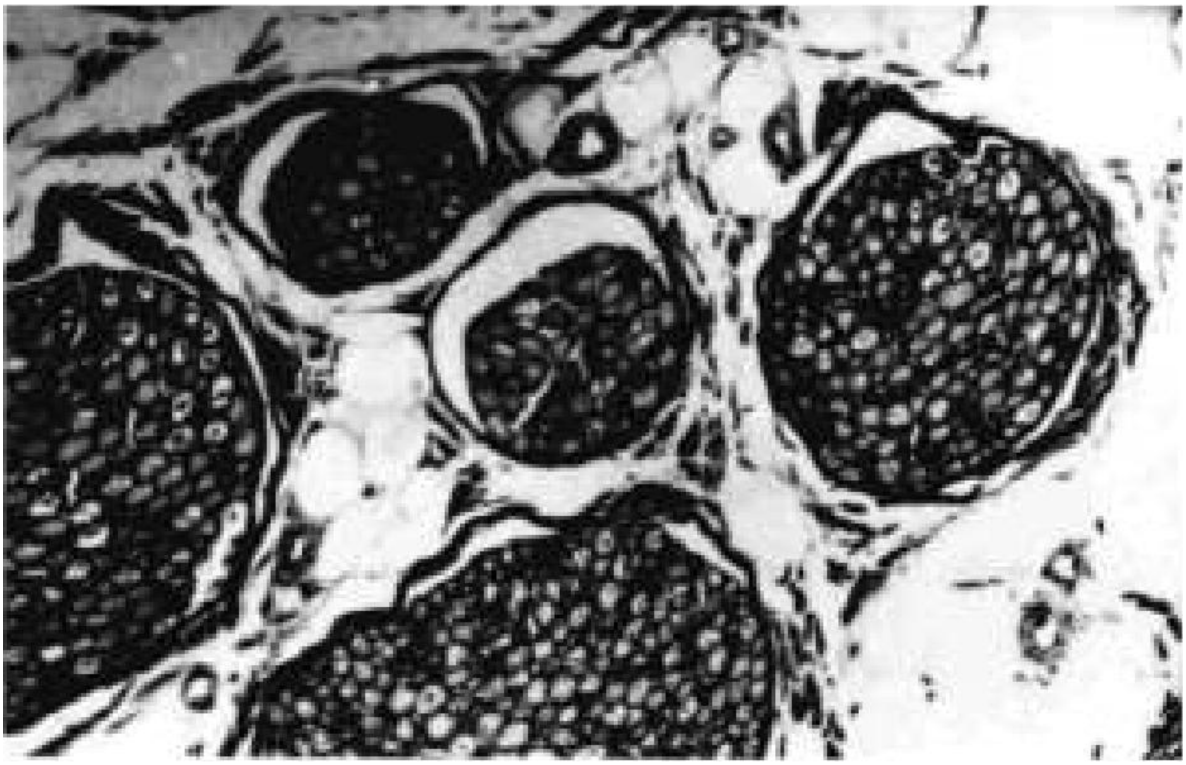

Figure 3. Lengthened nerve 2 months after distraction. (Masson's trichome x300.)

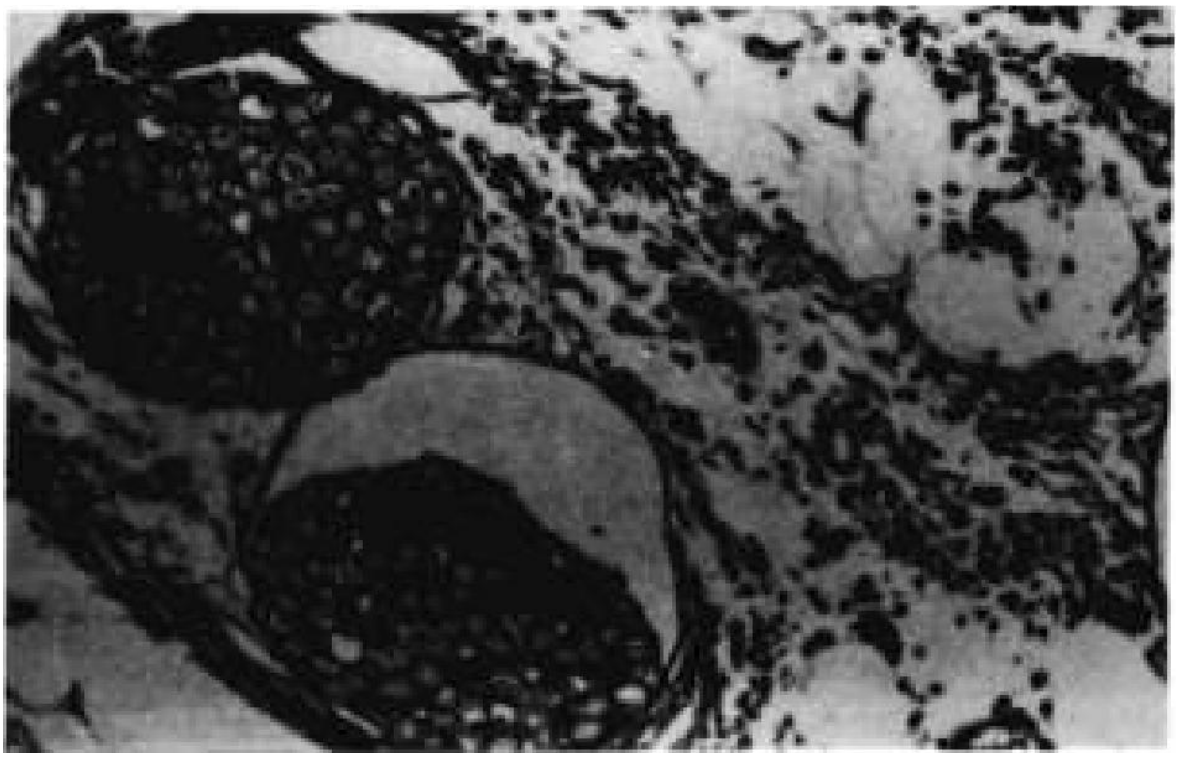

Figure 4. Lengthened nerve 1 month after distraction. (Masson's trichrome x300.)

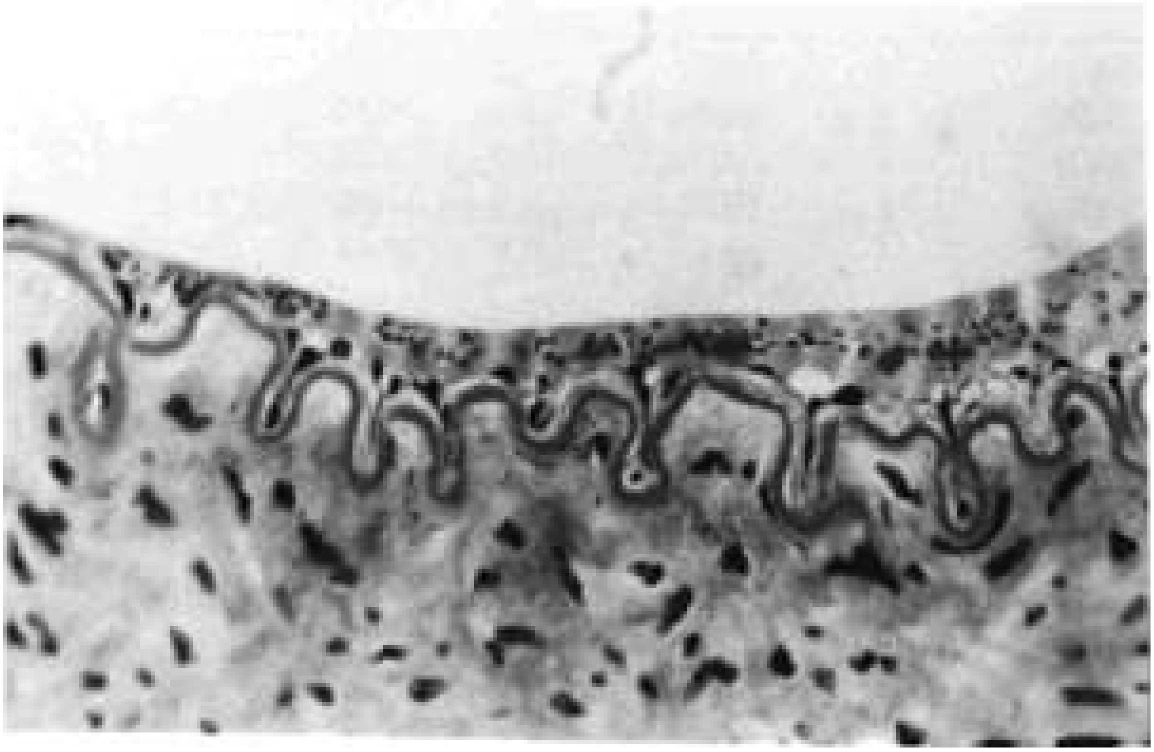

Figure 5. Lengthened posterior femoral artery showing hematic aggregates near endothelial cells. (Hematoxylin & eosin x1200, before 25% reduction.)

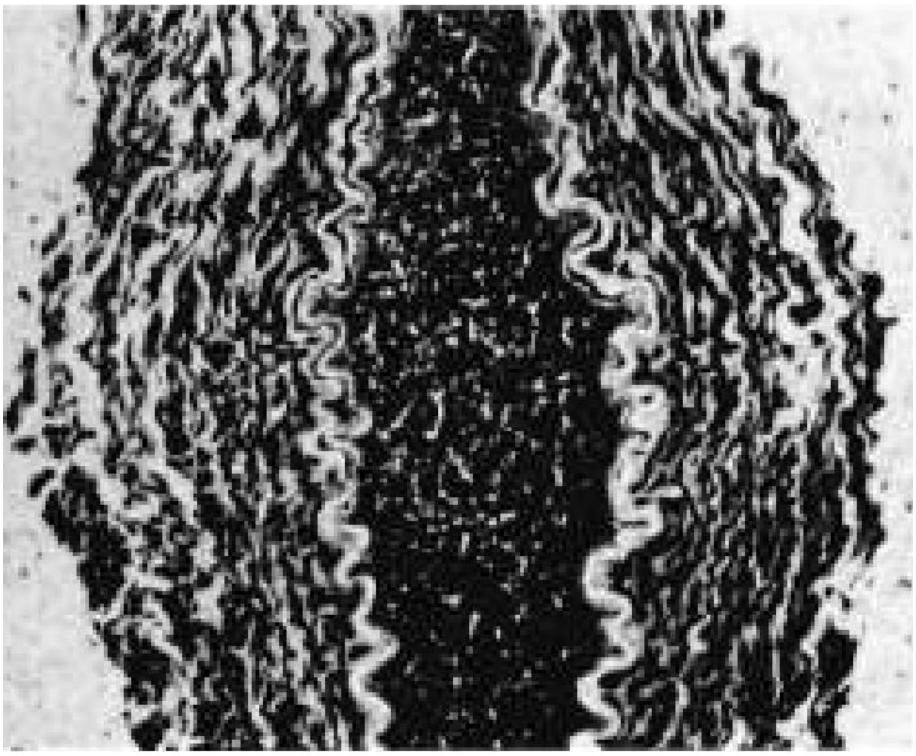

Figure 6. Lengthened posterior femoral vein showing thrombosis phenomena. (Masson's trichrome x800.)