transport[12,13]. Large molecules were found to pass more rapidly through fenestrated than through continuous capillaries indicating that fenestrations represent the preferential pathway for rapid transcapillary exchange[12-15]. The regenerating vessels are 3–4 times more permeable than normal skeletal muscle vessels to macromolecules[16]. The diaphragms may permit selective molecular sieving during transcapillary exchange[7]. The presence of fenestrated capillaries in injured tissues may have an important role in facilitating the marked transcapillary exchange occurring in areas proximal to injury[7]. Although the significance of fenestrated capillaries in the venous patch adventitia has not been determined according to McKinney et al.[7], they probably exist transitorily during the repair period ad integrum of the suture edges between the rat common carotid artery and the venous patch, since they have not been detected after the 2nd postoperative week (manuscript in preparation). More attention should be directed to investigation of transcapillary exchange in wound capillaries and their surrounding environment.

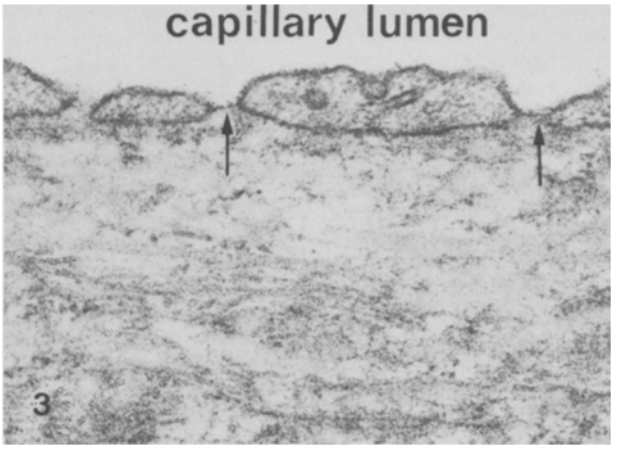

Figure 3. Capillary endothelium of vasa vasorum patch graft 14 days after surgery. The endothelial wall is thin and contains 3 fenestrae. 2 diaphragms are present (arrows). The trilayered cell membrane is visible. ×67,500.

1 The technical assistance of M. Guerricabeitia is gratefully acknowledged.

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The morphology of the Schwann cells and the unmyelinated fibers of a nerve supplying an immobilized muscle

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Summary. Hind limbs of cats were immobilized in the resting position for varying periods and the nerve supplying the medial head of the gastrocnemius muscle was studied while it was undergoing immobilization atrophy. Degenerative changes in the unmyelinated fibers and the Schwann cells, followed by an abundant increase in collagen, were noticed after prolonged immobilization. Electron microscopic evidence that Schwann cells produce the collagen is discussed.

The effects of immobilization of a limb on the morphology, physiology and biochemistry of the skeletal muscle have been thoroughly investigated and widely reported, whereas similar studies on the nerve supplying the immobilized muscle are not numerous. After tenectomy, in albino rats, the mean nerve fiber diameter and the number of myelinated fibers present in the nerve supplying the inactive muscle were found to be significantly reduced[1]. Further, it was reported that, in young animals, immobilization of a limb retards the myelination of the nerve supplying the atrophied muscle[2]. In a similar study, Eisen et al.[3] observed a significant reduction in the diameter of the larger myelinated nerve fibers in the nerve to the immobilized muscle. But further studies at the electron microscopic level are lacking and the present experiment reveals changes, as observed under the electron microsope, in the morphology of the Schwann cells and of the unmyelinated fibers of a peripheral nerve supplying an immoblized muscle.

Materials and methods. Adult cats, *Felis domestica,* weighing 2–3.8 kg were used for the study. 8 cats, irrespective of sex, were weighed and anesthetized using I/P sedium pentothal (30 mg/kg b.wt). The right or left hind limb was cleaned and immobilized in a plaster cast, keeping the knee and ankle joints in the resting position. The tissues of the contralateral hind limbs and also of these of another group of animals, not immobilized but subjected to the same dose of anesthesia and kept in identical surroundings, served as controls. The casts were removed under anesthesia from 2 cats at a time, at the end of 4, 6, 8, and 10 weeks of immobilization. The gastrocnemius muscle and the nerve to its medial head were exposed. Approximately 1 cm length of the nerve, just proximal to its entry into the muscle, was cut, stretched on a filter paper and fixed initially in Karnovsky's fixative[4]. The specimen was further washed with buffer (phosphate buffer, pH 7.4) and cut into smaller pieces and then fixed in buffered 6% glutaraldehyde for 2 h

at 4 °C, followed by post-fixation in 1% buffered osmic acid at 4 °C in the dark for 2 h. The pieces were then washed, dehydrated in acetone and embedded in Durcupan. Simultaneously, the control specimens were identically processed. Semithin sections (0.5–1 µm, in thickness) were cut, using a Reichert OM U2 ultramicrotome, stained with 1% toluidine blue and examined under a light microscope with a view to selecting suitable areas. The blocks were then trimmed further and ultrathin sections of about 50 nm (silver and golden) thickness were cut. These sections, picked on the copper grids, were stained with uranyl acetate and lead citrate and scanned using a Philips 300 EM and appropriate areas were photographed.

Results. As in any peripheral nerve, both myelinated and unmyelinated fibers were present in the nerve supplying the medial head of the gastrocnemius muscle in the control specimens. The myelinated fibers had larger diameters and were more numerous than the unmyelinated ones. Both types of axons were arranged quite compactly. A thin rim of Schwann cell cytoplasm could be seen around the myelin sheath and in between the myelinated fibers usually 4–5 unmyelinated fibers were present, surrounded by a single Schwann cell.

After 4 weeks of immobilization, a noticeable reduction in the number of unmyelinated fibers was observed and there was an increase in the amount of collagen surrounding them. The enclosing Schwann cell was not well delineated (fig. 1). These changes were particularly well pronounced in the nerves of animals immobilized for 6 and 8 weeks. Degenerative changes were observed in the unmyelinated fibers as well as in the Schwann cells enclosing them (fig. 2). There was a marked increase in collagen and in some instances, collagen-like structures were seen in the Schwann cell cytoplasm surrounding the myelinated fibers as well (fig. 3). The Schwann cell cytoplasm appeared vacuolated.

In the specimens obtained after 10 weeks of immobilization, no unmyelinated fibers could be traced at all. Collagen completely filled the spaces between the myelinated fibers and was also found around the myelin sheath.

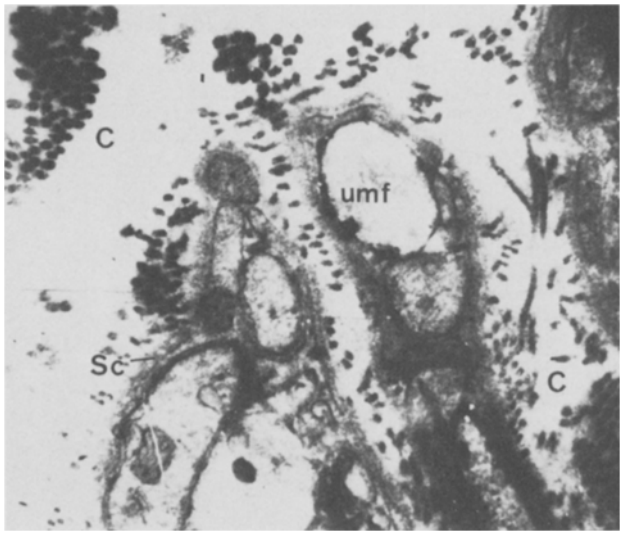

Figure 1. Electron micrograph showing unmyelinated fibers in the early stages of degeneration surrounded by increased amounts of collagen. C, Collagen; dnf, degenerated nerve fiber; ms, myelin sheath; Sc, Schwann cell; umf, unmyelinated fiber. × 57,750.

Discussion. The electron microscopic observations of Zacks[5] and Price[6] brought out the fact that the Schwann cells lie in close proximity to the sarcolemma of the muscle fibers, on either side of the terminating axens and thus are closely related to the muscle fibers. While the muscle fibers are undergoing such atrophic changes as the infolding of the sarcolemma[7] due to immobilization, the Schwann cells could also have been affected, owing to their proximity. The observations of the present study, namely, the initial vacuolation of the cytoplasm and the subsequent complete disappearance of the Schwann cells enclosing the unmyelinated fibers in their final stages of degeneration, confirm the involvement of these cells in immobilization atrophy. Similar results have also been reported in certain cases of peripheral neuropathy, where the degeneration of both Schwann cells and unmyelinated fibers was observed[8].

Nathaniel and Pease[9] suggested that an increasing amount of collagen in a degenerating nerve could be formed by the Schwann cells. Jacobs[10], from her study on the Schwann

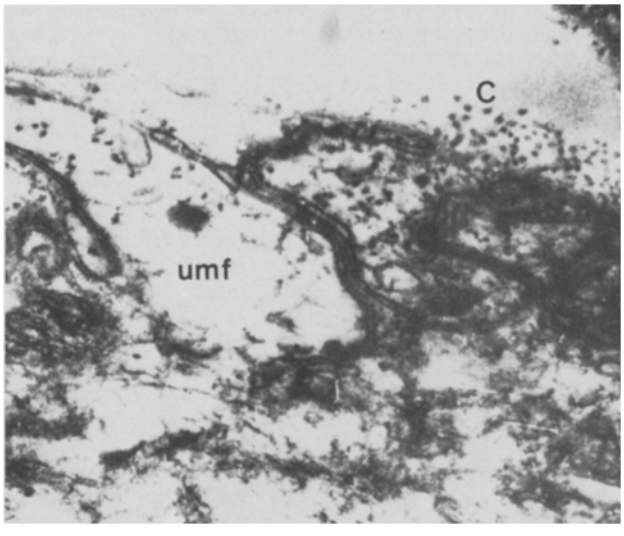

Figure 2. Electron micrograph of an unmyelinated nerve fiber in an advanced stage of degeneration (arrow). × 57,750.

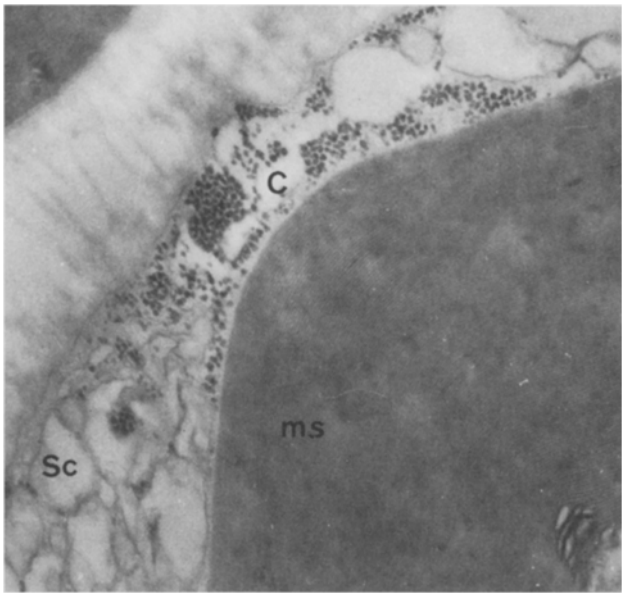

Figure 3. Electron micrograph of a myelinated nerve fiber. The peripheral Schwann cell cytoplasm is vacuolated and contains collagen. × 28,350.

cells of spinal roots, concluded that these cells were capable of producing and polymerizing tropocollagen into collagen and a recent report shows that the Schwann cells produce the collagen found very near to their basement membrane[11]. This is in accordance with the observations of Friede et al.[12] in their in vivo studies on Schwann cells.

It is thus evident that the Schwann cells may be, in some way, connected with the synthesis of collagen under certain conditions of stress or degeneration of nerves. In fact, in the present experimental work, collagen has been found to be located actually within the Schwann cell cytoplasm. In the light of the current observations, it is, therefore, obvious that immobilization of a muscle, besides affecting the unmyelinated fibers, also leads to an increased production of collagen by the Schwann cells.

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Evidence for differences in alpha-adrenergic receptor affinity in stress susceptible swine

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Summary. pA_2-Values were determined using phentolamine-methoxamine. The mean pA_2-value on aortic strips from stress susceptible swine was 7.81 and 7.39 for control. The α-adrenergic receptor from stress susceptible swine has a higher affinity than that of control.

The role of the sympathetic nervous system in the course of the hyperthermia induced by halothane or succinylcholine in stress susceptible swine is unclear. The stress susceptible swine is used as an animal model since a similar drug induced malignant hyperthermia syndrome exists in humans[2]. At present there are primarily two thoughts concerning the site of action of drug induced malignant hyperthermia. One site is thought to be skeletal muscle and another site the sympathetic nervous system[3-9]. Alpha adrenergic agonists promote whereas α-adrenergic receptor antagonists and adrenergic neuron blockers inhibit the development of malignant hyperthermia[7-9]. The purpose of this study was to obtain additional information concerning the possible role of the sympathetic nervous system in malignant hyperthermia induced in stress susceptible swine.

Methods. Yorkshire or Yorkshire crossbred swine were obtained from the Department of Animal Science's herd. The pigs in this herd are screened at 7–11 weeks of age for stress susceptibility using halothane, blood typing and measurement of creatinine phosphokinase levels[10,11]. Pigs weighing 55–70 kg were electrically stunned and exsanguinated. The terminal aorta was removed and placed in a modified Krebs-Henseleit (Krebs) solution containing indomethacin (5×10^{-7} M) which was used throughout the experiment[12]. In the initial studies the aorta strips were observed to increase in tone after being immersed in Krebs solution for 60–90 min despite frequent bathing fluid changes. This increase in tension progressed over several hours. The addition of indomethacin to the Krebs solution prevented the increase in tone and permitted reliable dose-ratios to be determined. The aorta was helically-cut into strips approximately 2 mm wide and 15 mm long. The strips were placed in 10-ml isolated organ baths containing Krebs solution maintained at 37 °C and aerated with 95% O_2: 5% CO_2. The strips were placed under 4 g tension and responses were isotonically recorded. Strips were allowed to equilibrate for 90–120 min prior to adding agonists. Using methoxamine as the agonist and phentolamine as the antagonist, pA_2-values were obtained. The phentolamine concentration ranged from 10^{-8} to 10^{-6} M and was allowed to equilibrate with the tissue for 100–120 min prior to adding methoxamine. All dose ratios were appropriately corrected for changes in sensitivity during the course of the experiment by running a 'time control' tissue, which received only methoxamine[13]. Schild plots were used to obtain the pA_2-values[14]. pA_2 is equal to $-\log K_B$ where K_B is a quantitative measure of the dissociation of the receptor-antagonist complex. The dissociation constant (K_B) is inversely related to affinity and sould be the same for any one antagonist reacting with a common receptor[15].

Results and discussion. The pA_2-values from control and stress susceptible swine are presented in the table. The mean phentolamine pA_2-value for the stress susceptible swine was significantly different from control. The affinity of the α-adrenergic receptor in stress susceptible pigs was 2.6-fold greater than that of control pigs.

pA_2-Values for phentolamine on aortic strips from control (C) and stress susceptible swine (SSS)

	C	SSS
	7.7	8.0
	7.3	7.2
	7.2	
	7.2	8.2
	7.4	7.5
	7.5	8.1
	7.3	7.7
	7.5	7.9
	7.4	7.9
$\bar{x}$	7.39	7.81
SEM	0.12	0.05

The mean pA_2-value for SSS is significantly different from C ($p < 0.01$) using the 2-tailed t-test.