

Strain, stress and stretch of peripheral nerve

Rabbit experiments in vitro and in vivo

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Mechanical stretching is known to cause morphologic and functional changes in peripheral nerve. The points at which these changes occur, however, are not clearly defined and reported data are conflicting. The studies presented in this paper provide a basic understanding of the biomechanical properties, stretch-injury patterns, and changes of conduction properties of peripheral nerves due to stretching. Our studies showed that peripheral nerves exhibited non-linear stress-strain characteristics when placed under tension. Initially, under tension, the nerve had a low modulus that increased gradually with increasing strain until reaching a maximal value. When the nerve failed under tension, the perineurium inside the nerve ruptured, but the exterior of the nerve remained

intact. Our results also show that a peripheral nerve in situ was under significant strain, but minimal in situ stress (less than 0.05 MPa). The in situ strain might vary with limb position, but did not appear to exceed the limit beyond which substantial tension or stress would be developed in the nerve. The time-dependent viscoelastic behavior of peripheral nerves were also characterized. The maintenance of small in situ stresses suggests that sustained increases in tension could be adversely affecting the electrophysiologic properties of the nerve. Indeed, marked alteration of conduction properties resulted from even a small stretch of 6 percent beyond the in situ length of the nerve, or stress less than 10 percent of the ultimate strength of the nerve.

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To provide qualitative, as well as quantitative, data on nerve stretch injuries, we have conducted a series of in vitro and in vivo studies of peripheral nerves. The in vitro studies included 1) the in situ stress and strain, 2) the stress-strain characteristics, and 3) the viscoelastic properties. These findings are correlated with histologic observations to determine the injury patterns of stretched peripheral nerve. In the in vivo studies, we have assessed the changes in conduction properties of the peripheral nerve subjected to various elevated levels of strain and stress in it.

Materials and methods

In situ stress and strain. We have recently developed a new method to measure the in situ stress and strain in the rabbit tibial nerve (Kwan et al. 1989). The experimental procedure was as follows: with the rabbit knee and ankle joints flexed at 90°, the tibial nerve was exposed. This was to standardize the length of the nerve, because, generally, the length of the nerve varies with the angle of flexion at these joints. Care was taken to avoid trauma to the nerve. The nerve was

irrigated with 0.9 percent normal saline solution to prevent dehydration during the preparation. The nerve was then marked by placing two parallel black lines (using Verhoeff's stain), approximately 1 cm apart, on its exposed surface. A reference measuring scale was placed alongside the nerve specimen and on the same plane as the nerve surface. The distance between the black lines, representing the in situ length of the nerve, was then measured by using a video dimensional analyzer (VDA) and the reference scale. The VDA system is an accurate and noncontacting method for length or strain measurement, and has been effectively used in testing of soft connective tissues, such as articular cartilage, tendons, and ligaments (Woo et al. 1983). A nerve specimen, about 5 cm long, between the ankle and the knee was then excised. The width and thickness of the isolated nerve specimen were measured for computation of cross-sectional area. One end of the specimen was wrapped in saline-soaked gauze and mounted in a self-tightening clamp. The clamped nerve was then suspended and allowed to hang freely for 5 minutes. The length of the nerve at this point with no external loads (defined as zero-load length) was measured by the VDA and used as gauge length. The amount of retraction in length was used to

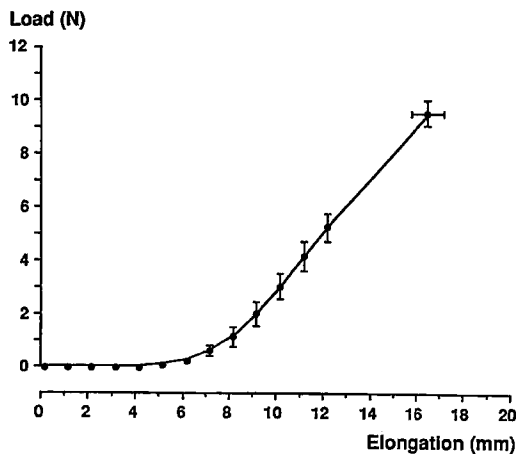


Figure 1. Load-deformation curve for rabbit tibial nerve. It can be seen that, initially under loading, the nerve can be stretched a large amount before substantial tension has developed.

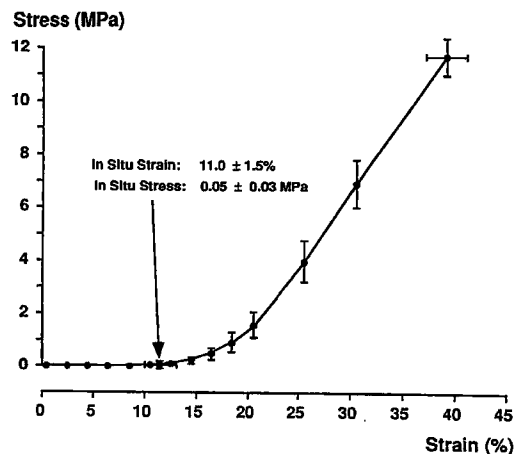


Figure 2. Stress-strain curve for rabbit tibial nerve, which is found to be nonlinear. In situ, the nerve is under large strains, but minimal stresses.

calculate the in situ strain: (in situ length – gauge length)/gauge length.

The in situ strain of the rabbit tibial nerve was 11.0 ± 1.5 percent (n 9), which was relatively large compared with many other soft connective tissues. The in situ stress, on the other hand, determined as the stress corresponding to the in situ strain on the tensile stress-strain curve, was small. The in situ stress will be discussed with the stress-strain characteristics of peripheral nerve in the next section. The existence of in situ stress and strain is believed to be, not only relevant to the strength and integrity of tissue, but also important for its homeostasis, i.e., maintenance of size, mass, shape, and function (Fung 1988).

Stress-strain characteristics. In this study, we have determined the stress-strain characteristics and structural damage of rabbit tibial nerves (n 9) in a tensile test (Kwan et al. 1988, 1989). The excised nerve was clamped and set at its gauge length (i.e., zero-load length) as previously described, and then loaded to failure at an extension rate of 1 cm/min or strain rate of 0.5 percent/sec. By measuring the load with a sensitive load cell (accuracy: ± 0.005 N) and tensile strain with the VDA, the load-elongation curve (structural properties) and stress-strain curve (mechanical properties) were determined.

Our results showed that the peripheral nerve was easily extensible initially; only a minimal amount of tension was required to stretch it (Figure 1). The rabbit tibial nerve could be elongated approximately 6 mm with very little tension. With further elongation, the nerve stiffened with the slope of the stress-strain curve increased steadily. The rabbit tibial nerve had an

ultimate elongation of 16.3 ± 0.7 mm and an ultimate load of 9.52 ± 0.47 N. For human ulnar nerve the ultimate load was reported to be 65 N to 155 N, and for human median nerve, 73 N to 220 N (Sunderland 1978). We noted that chronically injured nerves might have altered biomechanical properties, such as increased stiffness (Beel et al. 1984).

Peripheral nerve was found to exhibit highly nonlinear stress-strain behavior (Figure 2), contrary to what often described in the literature—a linearly elastic material. The stress-strain behavior followed the general characteristics of the load-elongation curve. It had a large initial toe region, demonstrating that the nerves might stretch up to 15 percent strain under minimal stress. Much of the strain within the toe region (i.e., low modulus region) was due to the removal of the slack in the nerve fascicles, as well as in the nerve trunk as a whole. With continued straining, nerve fibers and endoneurial tubes, as well as the perineurium, were recruited and began to lengthen, resulting in gradual increases in the modulus of the nerve. The modulus reached a maximal value at about 20 percent strain, and then remained fairly constant until failure. At the point of failure, rupture of the perineurium of the nerve fascicles occurred. For rabbit tibial nerve, the tensile strength and ultimate strain were 11.7 ± 0.7 MPa and 38.5 ± 2.0 percent, respectively.

We also found that the toe region, as seen in Figure 2, extended well past the in situ strain of the nerve (i.e., 11.0 ± 1.5 percent), which corresponded to a very small in situ stress of 0.05 ± 0.03 MPa. While in situ the nerve is under relatively large strain, it is under minimal stress. The in situ strain might vary with

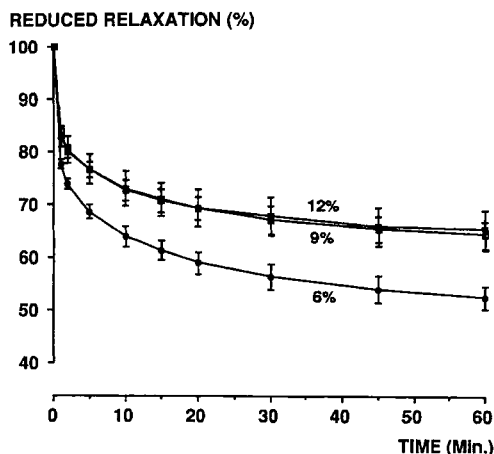


Figure 3. The stress relaxation behavior of rabbit peripheral nerve when stretched to fixed strain levels of 6 percent, 9 percent, and 12 percent (above the in situ length). The reduced relaxation is defined as the actual stress value normalized by the initial peak stress.

angles of flexion of the joints, yet it appeared not to exceed the toe region; thus, the in situ stress in nerves remained small. In other words, within physiologic range of motions, peripheral nerve is flexible without developing significant tension in it. The maintenance of such a small in situ stress suggests that sustained increases in stress could be an important factor adversely affecting the functional properties of nerves. This suggestion, in fact, led to our in vivo studies presented in the following sections.

Another observation we had was that the peripheral nerve remained grossly intact when it failed mechanically (Kwan et al. 1988). This implies that gross examination of a nerve after a stretch injury may not reveal the significant underlying intraneural disorganization. Indeed, histologic evaluation of failed specimens revealed that while there were multiple ruptures of perineurial sheaths along the course of failed nerve specimens, there was no noticeable structural damage of any other connective tissue components of the peripheral nerve (Kwan et al. 1988, Rydevik et al. 1990). This suggests that the perineurium is a major load-carrying connective tissue component. The perineurial sheath was found to rupture not at one given point in the nerve, but rather along the nerve over some distance, indicating that stretch injuries might not be a localized phenomenon. Moreover, nerve fibers within the fascicles in failed nerves appeared intact. These fibers did not seem to contribute to the tensile strength of the peripheral nerve, a finding supported by an earlier report that the distal segment of a severed nerve, with its nerve fibers manifesting

Wallerian degeneration, had the same mechanical properties as a normal nerve (Sunderland and Bradley 1961).

Viscoelastic properties. In this study, we examined the stress relaxation behavior of rabbit tibial nerves (n 8) at various strain levels (Wall et al. 1990). The nerves were excised and mounted on the INSTRON machine. The nerve was first stretched to its original in situ length. After preconditioning, the nerves were stretched at 3 percent/sec, held at either 6 percent, 9 percent, or 12 percent strain above their in situ lengths, and then allowed to stress relax for 1 hour. The results demonstrated that the peak tensions for the 6 percent, 9 percent, and 12 percent groups were respectively 0.86 ± 0.49 N, 2.17 ± 0.64 N, and 3.14 ± 0.79 N. The tension in the nerves decreased rapidly during the first 10 minutes to 64 to 74 percent of the peak value (Figure 3), and thereafter more slowly for the remainder of the experiment, to 52 to 67 percent at the end of 1 hour. Our results showed that less amount of stress relaxation occurred at higher strain levels. The amounts of stress relaxation of the 9 percent and 12 percent groups are both significantly less than that of the 6 percent group ($P < 0.001$). However, the difference between 9 percent and 12 percent were statistically insignificant.

Effects of stretching

Two series of animal studies were conducted. In these studies, the tibial nerves of 30 mature New Zealand white rabbits were used. The rabbits were preoperatively medicated 15 to 20 minutes before induction. The rabbits were then intubated endotracheally and administered inhalation anesthesia with halothane (2-3 percent induction and 0.5-1.0 percent maintenance) and intravenous morphine. Normal arterial blood pressures and heart rates of the animals were monitored and maintained throughout the experiment. With the rabbit limb fixed in full extension, the sciatic and tibial nerves were surgically exposed under microscopic observation, with care not to damage any muscular and vascular structures. The in situ length and cross-sectional area of the nerves were then measured. A bipolar stimulating cuff electrode was placed on the tibial nerve, approximately 1 centimeter distal to the knee, while a recording electrode was placed around the sciatic nerve. The nerves at both exposure sites were immersed in a bath of mineral oil and covered to eliminate dehydration and contact with the atmospheric environment. The temperature of the oil bath was maintained at 35 °C. The nerve was then stimulated at maximum threshold, and baseline compound nerve action potential (CNAP) was recorded by using

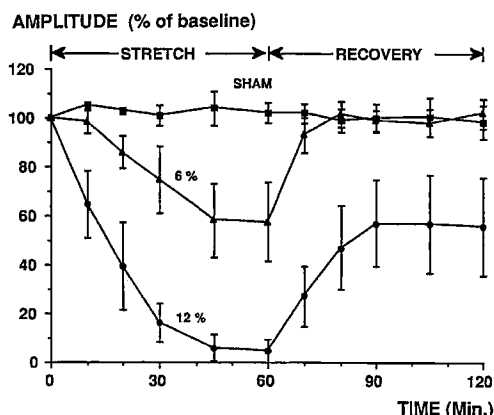


Figure 4. Effects of strain on the CNAP amplitude of rabbit tibial nerve. The strain levels indicated are measured above the in situ length of the nerve.

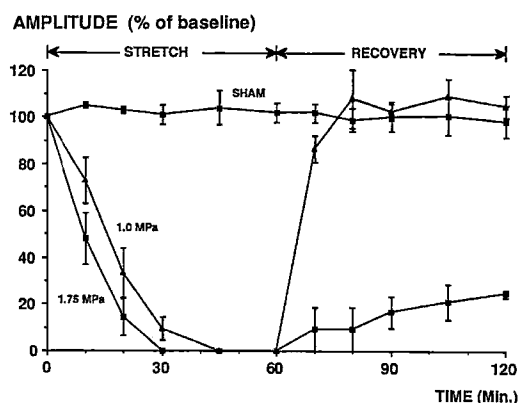


Figure 5. Effects of stress on the CNAP amplitude of rabbit tibial nerve. The applied stress levels represent small elevated stress as compared with the ultimate strength of the nerve.

a Cadwell 5200 unit, with CNAP amplitude expressed as percentage of baseline values before nerve stretch. The nerve was then transected near the ankle, and the distal end of the nerve was clamped to a custom-designed stretch device. In the first series of the study, 18 nerves were stretched uniaxially at a strain rate of 5 percent/min to either 0 percent (SHAM), 6 percent, or 12 percent beyond the in situ strain for 1 hour. After which the nerve was returned to its original in situ length for an additional one hour recovery period. In the second series, a constant stress of either 0 (SHAM), 1, or 1.75 MPa was applied to 12 nerves for 1 hour, and then the nerve was allowed to relax for an additional hour. These applied stresses were small, representing 9 and 15 percent of the ultimate strength determined from our in vitro biomechanical studies (i.e., 11.7 ± 0.7 MPa), and were chosen to determine the effects of small elevated stresses on nerve properties. During the stretch and recovery periods in both series of studies, the nerve was stimulated and CNAP recorded at 10-minute intervals. For the SHAM groups in both series, the CNAP amplitude and latency remained unchanged throughout the 2-hour duration of the experiment.

Effects of strain. At 6 percent strain, the CNAP amplitude remained stable for 20 minutes, and then gradually decreased to about 60 percent of the baseline value at the end of the 1-hour stretch (Figure 4). Upon release of the strain, the CNAP amplitude recovered to the baseline value within 20 minutes. At 12 percent strain, the CNAP amplitude decreased acutely to 65 percent of baseline by 10 minutes, with near conduction block by 1 hour. After release of the stretch, the

amplitude recovered to 58 percent of baseline in 30 minutes and plateaued thereafter. The CNAP amplitudes at 1 hour of stretch were significantly different between the SHAM, 6 percent, and 12 percent groups ($P < 0.001$, one-way ANOVA). At the end of the recovery, only the 12 percent group CNAP amplitude remained significantly depressed compared with the controls ($P < 0.01$).

Effects of stress. After the application of both 1 MPa and 1.75 MPa, the CNAP amplitude was found to decrease rapidly with time (Figure 5). When placed under 1 MPa, the CNAP amplitude was reduced to 10 percent of the baseline value in 30 minutes and to zero (i.e., exhibiting a complete conduction block) in 45 minutes. After removal of the stress, the amplitude recovered rapidly to the baseline value within 15 minutes. When placed under 1.75 MPa stress, the CNAP amplitude decreased with time at a slightly higher rate, and complete conduction block occurred at 30 minutes. When the stress was removed, a much slower recovery was seen. At the end of the 1-hour recovery, only 20 percent of the baseline values was attained, which was significantly different from both the SHAM ($P < 0.05$) and the 1-MPa group ($P < 0.05$).

Discussion

Characterization of the biomechanical properties of peripheral nerves is essential for the understanding of the effects of stretch on the conduction properties of neural tissue. Trauma to the extremities (e.g., high-

energy traction to the brachial plexus) and clinical situations (e.g., limb lengthening, treatment of long bone fractures, and nerve repair under tension) often cause stretch injuries to the nerve (Sunderland 1978, Lundborg 1988, Galardi et al. 1990), and result in disability in forms of motor and sensory impairment, as well as pain (Seddon 1972, Sunderland 1978, Lundborg 1988). It is generally assumed that, within certain limits, the nerve can adapt to stretch with transient functional changes. However, data on properties of peripheral nerves, such as the amount of tensile stress or strain that will cause structural damages, are limited and inconsistent. An early investigations indicated that the "breaking point" of stretched nerve ranged from 18 to 165 kg for the sciatic nerve and 20 to 50 kg for the median nerve (Takimoto 1917). Later, studies of the popliteal nerve showed that elongation of 11 percent or more caused severe, extensive damage (Hight and Sanders 1943). Markedly different results, however, were reported by Denny-Brown and Doherty (1945), who claimed that peripheral nerve segments could be extended 100 percent without structural damage, whereas Hoen and Brackett (1956) indicated that 25 to 50 percent elongation without damage was possible. Liu et al. (1948), in yet another study, concluded that peripheral nerve could be damaged if stretched beyond 4 percent. Later investigations reported that the tensile strength varied from 0.5 to 3.1 kg/mm² (or 4.9 to 30.4 MPa), the strain at the "elastic limit" ranged from 8 percent to 20 percent, and the maximum strain (at failure) was approximately 30 percent (Sunderland and Bradley 1961). These variations of peripheral nerve mechanical properties are believed to have been caused by difficulties in testing of flexible nerve tissues.

In addition, peripheral nerves also exhibit time-dependent viscoelastic behavior (Hartung and Arnold 1973, Kendall et al. 1979), including creep (i.e., gradual elongation of the nerve with time when the tissue is placed under a fixed tension) and stress relaxation (i.e., relaxation of tension in the nerve following stretch to a fixed elongation.) These viscoelastic phenomena are important properties, in that they help limit sudden, excessive elongation upon loading, or relieve the high degree of tension developed in the nerve following stretch; yet, these properties are not well-described for peripheral nerves.

Various histologic changes of peripheral nerves, subsequent to stretch including elongation of fascicles, compression of nerve fibers, changes in connective tissues, and rupture of blood vessels have been reported. Functional disturbances in peripheral nerves following stretching have also been reported by many investigators (Mitchell 1872, Carlson 1910, Takimoto 1917, Hight and Sanders 1943, Bullock 1945, Denny-

Brown and Doherty 1945, Adey 1951, Turner 1951, Hoen and Brackett 1956, Sunderland 1981). However, basic understanding of the relationship between mechanical stress or strain and nerve function is still lacking. In fact, although mechanical stretching has long been known to cause functional changes in peripheral nerves, the data regarding the point at which these changes occur are scanty and conflicting. Mitchell (1872), for example, reported that peripheral nerve may be stretched up to 25 percent strain without interruption of conduction, whereas Denny-Brown and Doherty (1945) reported 100 percent, and Hight and Sanders (1943) reported 11 percent. These disparate data are caused, in part, by the insufficient and inconsistent information available regarding the biomechanical properties of peripheral nerves.

The mechanical properties of peripheral nerves are important, not only for the protection of the nerve fascicles, but also for the integrity of neurophysiologic function. The perineurium of the nerve fascicles has been found to be the principal load-carrying connective-tissue component of peripheral nerve, providing much of its resilience characteristics. It has also been shown to have important function of maintaining the physiochemical environment of the endoneurial space by acting as a diffusion barrier against several macromolecular substances (Waggner et al. 1965, Olsson and Reese 1969, Oldfors 1981). Physical damage or changes in the diffusion properties of perineurium caused by stretching could lead to pronounced acute, or long-term, impairment of nerve function (Lundborg 1975, 1988, Rydevik and Lundborg 1977). The precise effects of elongation on the diffusion properties of perineurium, and on the function of the nerve, are not clearly known. In a study of intraneural blood flow in the rabbit tibial nerve during gradual stretching (Lundborg and Rydevik 1973), it was shown that impairment of venular blood flow was induced when a nerve was stretched 8 percent beyond in vivo length. Complete intraneural ischemia was induced at 15 percent beyond in vivo length. As found in our in vitro studies, structural injury to the perineurial sheath occurs at 27 percent beyond the in situ strain. Thus, a sequence of changes takes place when a peripheral nerve is stretched beyond its in situ dimensions. In fact, these results indicate that the conduction loss found at low strain levels is primarily due to the mechanical deformation. These data clearly demonstrate the sensitivity of peripheral nerves to mechanical stretching and elevated tension in the nerve. Severe alteration of conduction properties may result from even a small amount of strain of 6 percent. On the other hand, because the peripheral nerve is under minimal in situ stress, increased stress level, even of an amount less than 10 percent of the ultimate

strength of the nerve, can cause drastic changes in the conduction characteristics of the nerve; and a slightly higher stress, such as 15 percent of the ultimate strength, can induce irreversible functional deficit. These findings indicate the importance of avoiding elevated stress in the nerve and provide insight into the clinical understanding of stretch injuries of peripheral nerves.

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