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8. The normal prothrombin time for calves in our laboratory is 16.5 ± 3.3 seconds (mean ± standard deviation, $N = 57$).
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Alteration in Connections Between Muscle and Anterior Horn Motoneurons After Peripheral Nerve Repair

Abstract. *The connections between the spinal cord and lower leg muscles of the rat are significantly altered by repair of the intervening sciatic nerve. Muscles supplied by the peroneal branch of the sciatic are innervated by fewer motoneurons after sciatic repair. Many of these neurons originally innervated the peroneal muscles, and others formerly served the antagonistic tibial muscles. Perikarya in the size range of alpha motoneurons regained peripheral connections with greater frequency than those in the gamma range. There are thus postoperative defects in the extent and specificity of alpha reinnervation as well as in the degree of gamma control.*

The results of peripheral nerve repair in humans are often disappointing. Fine coordination is impaired, and individual muscles no longer act independently of one another. Reinnervation of muscle by inappropriate motoneurons may be a cause of poor postoperative function (*1*), but has not been clearly demonstrated. We have shown that after repair of the rat sciatic nerve, the peroneal muscles are reinnervated by appropriate motoneurons as well as by many that previously served their antagonists. We have also found that few motoneurons in the gamma size class regain peripheral connections. There are thus anatomical defects in both the specificity of muscle reinnervation and the extent of gamma control after peripheral nerve repair; these defects may result in the deterioration of function commonly experienced.

Experiments were performed on five 250-g female albino rats. In two normal animals horseradish peroxidase (HRP) was injected into the peroneal or the tibial muscle compartments to determine the relative locations of their motoneuron pools. In three additional rats the right sciatic nerve was severed in midthigh, and epineurial repair was performed with 10-0 nylon sutures under magnification (×3 to ×8). After 3 months, HRP was injected into both peroneal compartments of these animals. Each muscle group was injected with 20 μl of 20 percent HRP (Sigma VI) in 5-μl portions under anesthesia (Chloropent, 3 ml per kilogram of body weight). Nerves supplying adjacent muscles were severed through a more proximal incision to limit the central transport of HRP to the chosen pathway (*2*). After 48 hours the animals were reanesthetized and perfused with fixative according to procedure II of Rosene and Mesulam (*3*). The lumbosacral cords were dissected out, cut in 40-μm cross sections, and reacted with H_2O_2 and tetramethyl benzidine (*4*). The sections were serially mounted, counterstained with neutral red, and examined to determine the location, number, and size of labeled cells present in each section. Cell profiles that appeared in two adjacent sections were counted only once. Neuronal diameters were estimated by the method of Burke *et al.* (*2*).

In normal rats, injecting the peroneal or tibial muscle compartments of the lower leg resulted in the labeling of discrete pools of motoneurons in the anterior horn of the spinal cord. Others have demonstrated similar compartmentalization (*2*, *5*). The location of the peroneal motoneuron pool was defined in the coronal plane at different cord levels. Neurons labeled by peroneal muscle injection after nerve repair were scored as "in" or "out" of the normal peroneal pool location by comparing their position with that of the normal pool on the opposite, control side of the same animal.

The six control peroneal pools contained an average of 395 cells (range, 368 to 434) (*6*). In one animal, bilateral peroneal compartment injection labeled 368 cells on the right and 424 on the left. There was thus a variation in pool size of 13 percent from side to side and 15 percent overall. In normal peroneal pools, most cells were concentrated in the fourth lumbar (L_4) segment, with an abrupt proximal termination and gradual attenuation throughout L_5 (Fig. 1). In one animal, the normal tibial pool contained 866 cells extending from L_4 to L_6 and was most prominent in its caudal extreme.

The three postoperative peroneal pools contained an average of 273 cells (range, 245 to 291). The mean postoperative pool was thus 69 percent the size of its normal counterpart, a variation far greater than the 15 percent variability in normal pool size. The anatomical distribution of motoneurons innervating the peroneal muscles was also changed postoperatively. The peak concentration of labeled cells, which normally occurred at the L_4 level, shifted to the L_5 and even L_6 levels (Fig. 1). In addition, 29 to 47 percent of the cells labeled by peroneal muscle injection were within the area normally occupied on coronal section by the tibial pool. Similar overlap was very unusual (0.5 to 1 percent of labeled cells) in the unoperated animals. Furthermore, the size distribu-

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tion of labeled cells was altered after nerve repair. Normal peroneal pools contained two cell populations, one with large perikarya of diameters 30 to 50 μm and a second with smaller perikarya, most of which were 18 to 21 μm in diameter. In normal pools, 16 to 22 percent of labeled cells were within the smaller size class, whereas only 2 to 3 percent of cells labeled postoperatively were this small.

Peripheral nerve repair thus alters the relationship between muscles and their innervating neurons. Many anterior horn motoneurons do not reestablish peripheral connections. The anatomical distribution of those whose axons do regenerate and subsequently innervate the peroneal muscles is strikingly different from that in the normal animal. Peak concentrations of peroneal motoneurons normally occur at the L_4 level, but there was a caudal shift to the L_5 and even L_6 root levels after nerve repair. In fact, 29 to 47 percent of cells labeled postoperatively were outside the limits of the normal peroneal pool (Fig. 2) and lay in areas previously occupied by tibial motoneurons. Perikarya that formerly innervated the tibial compartment thus reinnervated peroneal muscles. Such misdirected regeneration may result from imperfect alignment of proximal and distal axons at the nerve juncture, so that regenerating tibial axons enter peroneal axon sheaths in the distal stump. The use of higher magnification and the alignment of individual fascicles within the nerve (perineurial repair) may result in the formation of more appropriate connections than are seen here (*7*).

The finding that many tibial axons reinnervate the rat peroneal muscles is consistent with earlier research that showed an absence of neurotropism in the peripheral reinnervation of adult mammals. Weiss and Hoag (*8*) demonstrated that motor axons, regardless of their previous connections, behaved as equals when reinnervating muscle. Their observations were confirmed by Bernstein and Guth (*9*) in a different experimental model. Miledi and Stefani (*10*) have shown that reinnervation of fast and slow twitch muscle fibers is equally nonspecific, and Kimura *et al.* (*11*) have clinically demonstrated aberrant regeneration of the injured facial nerve. Inappropriate reinnervation may even occur in newborn rats after injury to the brachial plexus (*12*). In contrast, the current evidence for specificity of motor regeneration has been provided by experiments on lower vertebrates (*13*) and probably does not apply to mammalian systems.

Our results further suggest that few gamma motoneurons regenerate postoperatively. Burke *et al.* found a bimodal distribution of the diameters of anterior horn cells (range, 18 to 78 μm) after HRP injection of the cat gastrocnemius and soleus muscles (*2*). Of these cells, 25 to 30 percent formed a well-defined group with mean diameter 25 to 30 μm and were thought to be gamma motoneurons. We have found a similar bimodal size distribution of HRP-labeled rat motoneurons, but within the diameter range

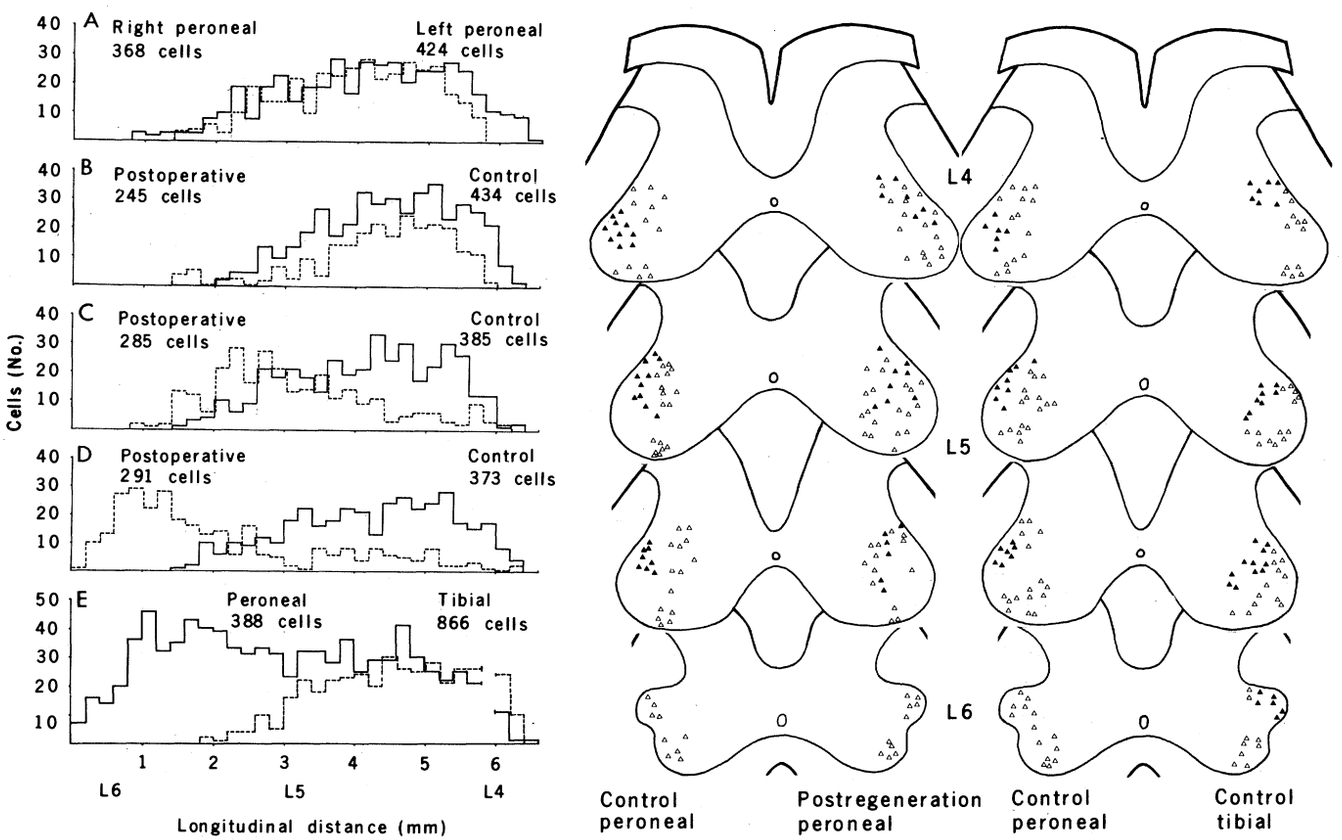

Fig. 1 (left). Longitudinal distribution of labeled cells along the rostro-caudal extent of the spinal cord. Each bar represents the labeled cells contained in five consecutive 40-μm sections. Caudal is to the left, rostral to the right, and root levels are only approximate because of lateral asymmetry. (A) Bilateral peroneal muscle injections in a control animal. (E) Tibial and peroneal muscle injection on opposite sides of the same control animal. (The gap at 6 mm represents sections lost in processing.) (B to D) The varying longitudinal distribution of neurons labeled by peroneal muscle injection after sciatic-nerve repair. In these animals the normal peroneal pool is labeled on the unoperated contralateral side (*18*). Fig. 2 (right). Individual representative spinal cord sections in the coronal plane from two animals showing the relative locations of normal tibial and peroneal pools (right) and the location of cells labeled by postoperative peroneal muscle injection (left). Closed triangles represent labeled motoneurons, and open triangles represent the remaining unlabeled motoneurons. The margins of the gray matter, including ventral and dorsal horns, are indicated by thin lines, and the margins of the cord itself by thick lines. Cells labeled by peroneal muscle injection after sciatic repair occupied the normal domains of both peroneal and tibial neurons, though they did not extend to the S_1 level in this case.

of 10 to 50 μm. Sixteen to 22 percent of these cells formed the presumed gamma group, with mean diameter 19 μm. Postoperatively, however, only 2 to 3 percent of labeled cells were this small. This discrepancy is unlikely to result from posttraumatic swelling of the anterior horn cell, since the disappearance of small cells was not accompanied by increase in maximum cell size (*14*). Brown and Butler (*15*) and Thulin (*16*) have provided electrophysiologic evidence for the return of gamma function in the cat after the nerve to the tenissimus muscle was crushed or a 1-cm segment of posterior tibial nerve was resected. However, Takano was unable to demonstrate gamma reinnervation of muscle spindles 6 months after local freezing of the cat sciatic nerve (*17*). In Brown and Butler's and in Thulin's experiments, nerve was disrupted near its termination; in Takano's and ours, however, the injury was midway between spinal cord and muscle. The failure of gamma reinnervation after sciatic injury may thus be attributed to either (i) the inability of gamma motoneurons to regenerate over relatively long distances or (ii) the allowance of insufficient time for regeneration to occur.

The importance of gamma regeneration has not been established. Takano demonstrated a return of normal gait in cats after freezing the sciatic nerve, even though gamma reinnervation could not be demonstrated (*17*). Conversely, Thulin showed that, after posterior tibial nerve injury, push-off in gait did not occur with adequate gastroc-soleus strength until gamma reinnervation was reestablished (*16*). More research is needed to define the patterns and functional consequences of gamma reinnervation after various types of injury.

We have demonstrated alterations in the quantity, position, and size distribution of the anterior horn cells serving a muscle group after repair of the innervating multifascicular nerve. Mark has summarized the deficits following nerve severance and repair in higher vertebrates as loss of coordinated movement with capacity for only graded mass contraction (*1*). He suggests that undirected growth of axon sprouts has led many axons to the wrong muscle, so that a pool of motoneurons that previously served one muscle controls motor units scattered among several. Demands for contraction of the original muscle result in weak contraction of the whole group. We have demonstrated the anatomical changes Mark hypothesized and provided evidence for alterations in the gamma control mechanism. However, the functional consequences of these changes will depend on the quality of sensory regeneration and the degree of central adaptation to anterior horn disorganization. Combined anatomic and physiologic investigation will be necessary to assess the contribution of each to the end results of nerve repair.

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Bladder-Surface Glycosaminoglycans: An Efficient Mechanism of Environmental Adaptation

Abstract. *The transitional epithelium of the urinary bladder secretes and binds to its surface a glycosaminoglycan that inhibits the adherence of bacteria. Synthetic sulfonated glycosaminoglycans instilled intraluminally into bladders whose natural mucin layer has been removed are as effective as the natural mucin in preventing bacterial adherence. It also appears that adherence of calcium and protein is reduced in the presence of both the natural mucin layer and the synthetic sulfonated glycosaminoglycan sodium pentosanpolysulfate, suggesting that the antiadherence activity of both natural and synthetic surface glycosaminoglycans in the bladder extends to the molecular and ionic levels.*

The urinary bladder endosurface faces a relatively hostile environment, one that contains high levels of calcium, potential carcinogens, and at times pathogenic microorganisms. Our previous studies showed that the transitional cell epithelium produces and maintains at its surface a glycosaminoglycan whose presence may explain why the bladder surface is so resistant to these insults (*1-9*). The presence of glycosaminoglycan is associated with a marked impairment of the ability of bacteria to adhere to the surface. Furthermore, it is possible to remove the natural glycosaminoglycan and reproduce its antiadherence effect in vivo with several synthetic glycosaminoglycans (*3, 4, 9*). These findings suggested a new mode of antibacterial therapy in which a natural immune mechanism is augmented. We conducted the experiments reported here in order to determine whether the antiadherence effect of natural and synthetic glycosaminoglycans extends to the molecular and ionic levels, a phenomenon that would open the possibility of many new therapeutic uses for the synthetic compounds.

The assay for measuring bacterial adherence to the bladder mucosa of the rabbit in vivo was described in detail by Parsons and Mulholland (*5*). We used the same assay in the present studies, except that ^{45}Ca or ^{14}C-labeled protein was substituted for bacteria.

Male New Zealand White rabbits (2 to 3 kg) were given, via penile catheter, ^{45}Ca (0.1 μCi; New England Nuclear) or ^{14}C-labeled protein (0.1 μCi; Amersham), each suspended in 0.5 ml of physiological saline solution (PSS). Group 1 (control) rabbits, whose bladder mucin

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