being transported into all the successive compartments of the multilayer structures in exchange for sodium ions. The persistence of the higher field resonance after addition of cobalt ruled out the possibility that the appearance of this peak might be due to a leakage of phosphate.

Table 2 lists the effects of various ionophores and FCCP on the proton permeability of liposomes. No appreciable change in the internal pH was detectable after addition of FCCP as the electrogenic proton translocation brought about by this uncoupler soon generates a diffusion potential which prevents, in the absence of an exchangeable cation, any further proton influx. When valinomycin, which itself had no effect, was added together with FCCP, the pH gradient was completely destroyed within 1 h. A similar but faster effect was detected in the presence of nigericin, which catalyzes an electroneutral $K^+:H^+$ exchange. The addition of gramicidin allowed a slower but likewise complete proton equilibration across all bilayers, as shown in detail in the figure. A similar effect by gramicidin could be detected when sodium salts were used.

This result is relevant to the cellular action of gramicidin because it indicates direct action of this antibiotic on the membranes of subcellular structures.

Table 2. Effect of FCCP and various ionophores on proton permeability of liposomes. The liposomes loaded with 0.3 M K_2HPO_4 at pH 7 were washed twice and suspended in 1 ml of 0.3 M K_2SO_4 at pH 5. ^{31}P NMR-spectra were recorded at various times after the addition of the indicated drug. In parentheses are indicated the times at which proton equilibration was complete

Addition	pH of enclosed phosphate solution
1 µg FCCP	7*
2 µg valinomycin	7*
2 µg valinomycin + 1 µg FCCP	5 (60 min)
2 µg nigericin	5 (20 min)
200 µg gramicidin	5 (120 min)

*No change in the pH value could be detected for several h after the addition of either FCCP or valinomycin.

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Effects of disuse and nerve stump length on the development of fibrillation in denervated soleus muscle

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Summary. Both in normal (control) and in cordotomized (disused) rats, the soleus muscle was denervated either by cutting the sciatic nerve near the trochanter (proximal denervation) or by cutting the soleus nerve near the insertion into the muscle (distal denervation). In the control muscles, the development of fibrillation was not dependent on the level of nerve section. In disused muscles, the development of fibrillation was greater following distal denervation than following the proximal one.

Spinal cord section, performed a week before denervation, accelerates the onset of fibrillation in the soleus (slow) muscle of the rat, but reduces its development, which becomes similar to that observed in the anterior tibialis (fast) muscle[1-2].

The hypothesis was made[2] that the 'fast' pattern of fibrillation development in disused and denervated slow muscle represented indirect evidence of the muscular speeding following disuse. In the same paper it was also proposed, although on a purely theoretical basis, that the earlier onset of fibrillation would slow down the subsequent development because of the persisting release of a neurotrophic factor by the peripheral nerve stump. Indeed, by several authors it has been reported that the denervation changes are affected by the length of degenerating nerve fibres[3-6].

In the present work, the possible influence of the nerve stump on fibrillation development has been investigated in the disused-denervated preparation, by cutting, in cordotomized rats, the soleus nerve at 2 different levels, and then by comparing the spontaneous activity under the 2 experimental conditions.

Methods. All surgical procedures were performed in adult albino rats, 200-250 g in weight, under ether anaesthesia. The spinal cord was sectioned at the mid-thoracic level and 7 days later the sciatic nerve was cut bilaterally near the trochanter (proximal denervation), and unilaterally near the nerve insertion into the soleus muscle (distal denervation). The difference in nerve stump length achieved by cutting at the 2 levels was about 4 cm. Distal denervation was always associated with the proximal one, with the purpose of preventing the motor activity of the other muscles of the lower leg: this activity, in fact, would have introduced differences in the mechanical conditions of the distally denervated soleus muscle, in comparison with the contralateral muscle. In another group of animals, after spinal cord section and bilateral proximal denervation, a sham distal operation was performed in order to produce approximately the same amount of trauma caused by the

distal denervation. As a control, proximal or distal denervation were performed also in otherwise normal animals.

Recording of fibrillatory activity was made according to the previously described method[2]. The development of fibrillation was estimated by measuring the integrated electrical activity through a Beckman-Offner EMG integrator, at 24-h intervals, starting from the 2nd day after denervation.

Results. In the cordotomized animals, the development of fibrillation was greater in soleus muscle with distal denervation than in the contralateral muscle with proximal denervation, from 2nd to 5th day (figure 1, a). The difference was statistically significant at the 4th day ($p < 0.05$). In the present experiments the onset time of fibrillation was not selectively investigated, the first records having been taken 48 h after denervation, when fibrillation activity was already evident in all experimental conditions. However, the muscles with distal denervation showed on an average 48 h after denervation, a greater activity than the muscles with proximal denervation (figure 1, a). This might suggest an earlier onset of fibrillation in the first case with respect to the second.

In the animals with the sham distal operation, there were no significant changes in fibrillation development between the soleus muscles of the 2 limbs (figure 1, b). In the control animals, there were no differences in fibrillation activity according to the level of nerve section (figure 2).

Discussion. From the present work, it then results that, in the soleus muscle denervated a week after cordotomy, but not in the denervated control muscle, the nerve stump has a restraining influence on fibrillation development. This could partially account for the difference in fibrillation activity we had observed between disused-denervated and simply denervated muscles[2]. In this previous paper, we reported that, when disuse was caused by tenotomy or plaster cast immobilization of the limb, fibrillation began as precociously as after spinal cord section, but its development was not consistently different from that recorded in the control muscles: this suggests that other factors beside the nerve stump (e.g. the degree of muscular disuse[2]) are of importance in determining the pattern of fibrillation development in disused-denervated muscle.

There is a partial agreement between our results and the other data of the literature relating nerve stump length to the appearance of denervation phenomena[3-6]. In agreement with the previous works, our experiments show a negative influence of the nerve stump. Unlike the other authors, however, we were not able to detect any effect in the control denervated muscles; moreover, in disused-denervated muscles, we noted that the level of nerve section affects the development of denervation changes, and not merely the onset of the changes[5,6].

Our failure to detect any effect of the nerve stump in the control muscles might depend on our technique. Indeed, Salafsky et al.[5], who followed fibrillation development in distally and proximally denervated muscles, found a difference in the onset time of spontaneous activity by analyzing the frequency-voltage pattern of potentials, and not, as we did, an integrated electrical activity. It is possible that in the disuse pretreated muscles the effect of the nerve stump became more evident, and so detectable also by our technique, because of the more precocious onset of fibrillation.

The signs of nerve degeneration are progressing towards periphery at a certain speed[7,8]: it seems then reasonable to assume that the more precocious the onset of denervation phenomena, the greater will be the opportunity for the nerve fibres, not yet degenerated, to affect them.

On the other hand, a nerve trunk contains nerve fibres of different sizes, and it is known[7,9] that degeneration appears earlier in the large fibres than in the small ones. Conceivably, there must be a greater temporal dispersion of the effects of nerve degeneration in the case of a proximal than in the case of a distal denervation. The nature of such effects is still a matter of discussion. They are claimed to reflect the lack of a trophic influence of the nerve on the muscle[10-13], as well as the release of degeneration products by the nerve enhancing the effects of denervation[14,15]. In any case, we can assume that when the nerve is cut distally, there is a more synchronous effect of nerve degeneration on muscle, and so a facilitation of denervation changes, which in our experiments resulted in a greater initial fibrillation activity.

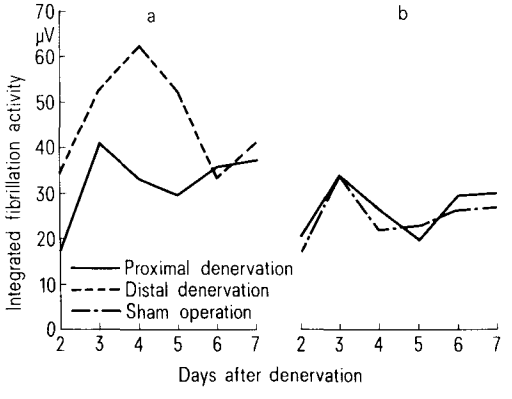

Fig. 1. Fibrillation development in soleus muscle of cordotomized animals, after proximal denervation, distal denervation and proximal denervation and sham distal operation (sham operation). Each point is the mean of values from at least 6 animals.

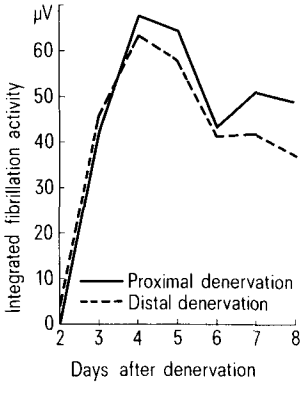

Fig. 2. Fibrillation development in soleus muscle of control animals, after proximal denervation and distal denervation. Each point is the mean of values from at least 9 animals.

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