J. Physiol. (1974), **236**, *pp.* 733–742
With 2 text-figures
Printed in Great Britain

EFFECT OF DENERVATION ON THE ADAPTATION OF SARCOMERE NUMBER AND MUSCLE EXTENSIBILITY TO THE FUNCTIONAL LENGTH OF THE MUSCLE*

BY G. GOLDSPINK, C. TABARY, J. C. TABARY, C. TARDIEU AND G. TARDIEU

From the Hôpital Raymond Poincaré, 92380 Garches, France and the Muscle Research Laboratory, Department of Zoology, University of Hull

(*Received 13 September 1973*)

SUMMARY

1. The effects of denervation on the response of the cat soleus muscle to immobilization at different lengths by plaster casts has been investigated for a period of 4 weeks.

2. The passive length–tension properties of the denervated immobilized muscles were not significantly different from those of non-denervated muscles. Muscles immobilized in the shortened position showed a marked decrease in extensibility whether they were denervated or not. In all the other cases the length–tension curves were not significantly different from those of normal muscles.

3. The denervated soleus muscle immobilized in the lengthened position was found to produce 25% more sarcomeres in series, whilst those immobilized in the shortened position lost 35%. This adaptation was essentially the same as in muscles that had been immobilized but not denervated.

4. Denervation was found to have no effect on the recovery of muscles that had been subjected to 4 weeks immobilization in the shortened position. In these muscles the sarcomere increased back to the normal level within 4 weeks after removal of the plaster cast.

5. The adjustment of sarcomere number to the functional length of the muscles does not therefore seem to be directly under neuronal control. It appears to be a myogenic response to the amount of passive tension the muscle is subjected to.

* All experiments described in this paper were carried out in France.

INTRODUCTION

The results previously presented (Tabary, Tabary, Tardieu, Tardieu & Goldspink, 1972) demonstrated that striated muscle is a very adaptable tissue and, in particular, they show that the sarcomere number is adjusted to the functional length of the muscle. When the hind limb of a cat was immobilized with the soleus muscle at its maximum length, the muscle fibres were found to have produced 19 % more sarcomeres in series. When the soleus muscle was immobilized with the muscle in its shortened position, the muscle fibres were found to have lost 40 % of sarcomeres in series. These changes take place in a short period of time (4 weeks) and were found to be quite reversible. Associated with the changes in the number of sarcomeres, there was also a marked modification in the extensibility of the muscle when it was immobilized in the shortened position.

The physiological regulation of sarcomere production in series is not known; however, there are several possible working hypotheses. For instance, a change of length which will induce a change in the passive tension will be detected by the receptors of the muscle and this may result in modifications to the muscle structure mediated by some sort of neural influence entering the muscle via the reflex pathways. On the other hand the control might be mainly myogenic. In other words, it may be a direct effect of passive tension on the muscle fibres and in particular on the myofibrils. Combined clinical and ultrastructural studies (Tabary, Goldspink, Tardieu, Tabary, Lombard, Tardieu & Chigot, 1971) have shown that in children with cerebral lesion, the regulation of muscle length and sarcomere number is often disturbed. This suggests that the nervous system is in some way either directly or indirectly involved.

It was therefore of interest to see if the regulation of the sarcomere number is modified by denervating the muscles before immobilization in both the lengthened and the shortened position. In addition to measuring changes of sarcomere number it was felt that it was also important to measure the mechanical properties of the muscles, especially their passive length properties.

METHODS

Denervation procedure. Two kinds of denervation were performed.

The resection of the nerve to the soleus and to the lateral head of gastrocnemius was performed by removing a 8 mm length of nerve. The advantage of this procedure was that the innervation of the majority of the hind limb muscles was preserved. The disadvantage is that there is a slight chance of the soleus muscle becoming re-innervated because the muscle is not very far from the proximal end of the resected nerve. This procedure was carried out on half the denervated animals. In the other half the denervation was carried out by removing a 10–12 mm length of either the sciatic nerve or the medial popliteal part of the nerve. This later procedure had the advantage that for certain all the nerves to the soleus were cut and reinnervation of the soleus was not possible. In this case, however, the denervation involved more muscles and consequently this affected the gait of the animal more drastically.

Experimental animals. Thirty-six adult cats were divided into three experimental groups, each of them being subdivided into two sub-groups according to the type of denervation used. The first group acted as the control group. In this case 12 cats were denervated but not immobilized. Six of them had, on one side, a resection of the nerve to the soleus and to the lateral head of gastrocnemius. The other six animals had, on one side, a resection of the medial popliteal (four cats) or sciatic (two cats) part of the nerve. Four weeks after the denervation, the denervated and the normal contralateral muscles were physiologically and histologically examined. The second group of twelve cats was subjected to the same type of denervations, but each hind limb of the operated side was immediately immobilized by a plaster cast in complete dorsiflexion so that the soleus muscle was maintained in the lengthened position. The muscles were physiologically and histologically examined after 4 weeks of immobilization. In the third group a further twelve cats were denervated and the hind limbs immobilized in complete plantar flexion so that the soleus muscle was in its most shortened position. The muscles were again examined after 4 weeks of immobilization.

In addition, a fourth group of three cats was used to look at the reversibility of the adaptation in denervated muscle. These had one hind limb immobilized in full plantar flexion. In this case the casts were removed after 4 weeks and the nerve to the soleus resected only at this time. After a further 4 weeks the animals were killed and the muscles physiologically and histologically examined.

Determination of the passive length-tension curves. The animals were induced into a state of deep anaesthesia by an injection of pentobarbitone. Before the soleus muscle was exposed, the sciatic nerve was sectioned at a high level in the thigh. The denervation procedure was then tested by supramaximally stimulating the end distal to the cut after the muscle had been exposed. In no case did the stimulation result in a contraction of the soleus. The limbs were then symmetrically affixed to the table and the passive length–tension characteristics measured as previously described (Tabary *et al.* 1972). The passive tension was related to the angle of the tibia-calcaneum and then to muscle length. Passive length–tension measurement was made on several animals from each group.

Determination of the number of sarcomeres in series. The number of sarcomeres was determined by the method of Close (1964), with certain minor modifications.

Muscles were fixed *in situ* at a determined length using glutaraldehyde fixative (2·5 %) after tying them to a metal spur to prevent any further length changes. The whole limbs were then placed in fixative for 24 hr. In the case of the muscles for which the length–tension curve was not obtained, the soleus was exposed as in the first case; the limb was pinned out on wooden board and the soleus fixed at a known length. In the first and second groups, the muscles were all fixed at the same articular angle in dorsiflexion, as it is very difficult to determine sarcomere number on slack muscle fibres. In the third group (muscle immobilized in the shortened position) the angle had to be different for the experimental and control side because stretching the muscles which had been immobilized in the shortened position resulted in tearing of the tissue.

The muscles were dissected out and washed in phosphate buffer at pH 7·2 for 24 hr. Small bundles of fibres were separated and placed in 50 % glycerol for 10 days or more. Individual fibres were then separated under a stereomicroscope, making sure that the two tendinous ends were visible. Each fibre was spread on a microscope slide and its length was measured at low magnification (× 60). It was then mounted in glycerol jelly with the coverslip supported by glass spacers and the number of sarcomeres counted for about twenty regions along the length of the fibre (magnification: × 750). Each sample length counted contained about 50 sarcomeres. The average sarcomere length for the fibre was calculated and the total number of sarcomeres was then obtained by dividing the fibre length by the average length of the sarcomere.

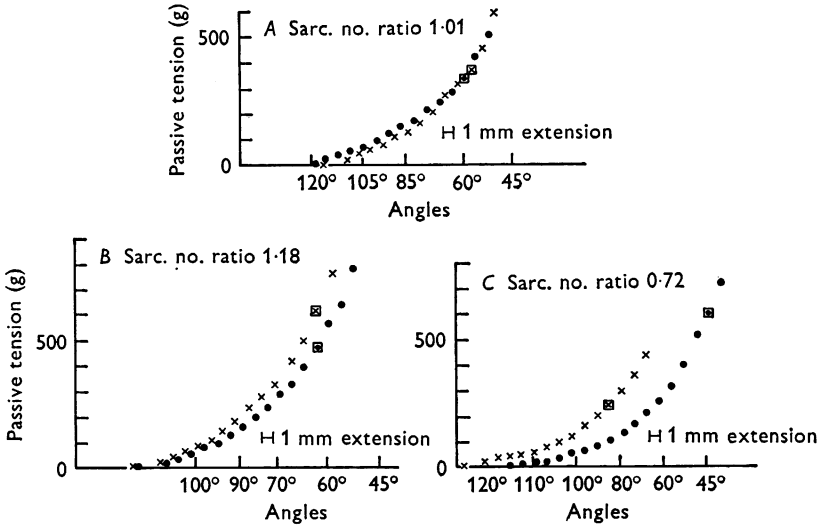

Fig. 1. Plots of passive tension against extension for denervated soleus muscles (×) and their contralateral control muscle (●). In plot *A* the denervated muscle was not immobilized. In plot *B* it was immobilized in the lengthened position and in *C* it was immobilized in the shortened position. The measured angles of the ankle during extension of the muscle are indicated on the abscissa together with the scale representing the change in muscle length. In each plot the point at which fixative solutions were applied to the muscle is indicated by the squares. The sarcomere number ratio is given with each plot.

RESULTS

1. *Physiological results*

Some of the length–tension curves obtained for denervated cat soleus muscles are shown in Fig. 1. Curves for denervated and control sides of cats which had not been subjected to immobilization were found to superimpose almost exactly (Fig. 1*A*). There was also no significant difference between the length–tension curves of muscles which had been denervated and immobilized in the lengthened position and control muscles, irrespective of the denervation procedure used (Fig. 1*B*).

In the third group, in which the denervated soleus was immobilized in the shortened position, the curves for the immobilized sides and the non-immobilized sides were very different. This was true for muscle denervation by resection of the soleus nerve, or resection of the sciatic nerve. For example, the curves showed that for an angle of the ankle of 80° the passive tension of the control muscle is about 150 g, whilst that of the experimental muscle is about 300 g (Fig. 1*C*).

2. *Sarcomere number results*

Effect of denervation on the number of sarcomeres. The results for mean sarcomere length, fibre length and sarcomere number measurement following denervation without immobilization are shown in Table 1.

TABLE 1. The detailed results for the non-immobilized soleus muscles which have been denervated by resection of the nerve to the soleus

			Sarcomere length		Fibre length		Sarcomeres	
Animal	Muscle	Angle of fixation	Length μm	Ratio	Length μm	Ratio	Number	Ratio
K	Denervated	20°	3·311	0·981	38,000	0·907	11,571	0·932
	Control	20°	3·375		41,887		12,408	
P	Denervated	20°	3·333	0·972	48,083	1·040	14,426	1·070
	Control	20°	3·430		46,250		13,484	
AF	Denervated	88°	2·201	0·925	36,000	0·975	16,357	1·054
	Control	88°	2·379		36,920		15,517	
BG	Denervated	20°	3·419	0·985	47,200	1·035	13,805	1·050
	Control	20°	3·470		45,600		13,141	
BM	Denervated	60°	2·771	1·006	35,400	0·992	12,767	0·986
	Control	60°	2·754		35,666		12,951	
BP	Denervated	60°	2·552	0·960	37,900	1·033	15,209	1·100
	Control	60°	2·655		36,700		13,828	
			Mean =	0·969	Mean =	1·008	Mean =	1·025
			S.E. =	0·010	S.E. =	0·020	S.E. =	0·014

In addition to giving the actual values, the results are also expressed as a ratio of the value for the experimental muscle to that of the control muscle. Only detailed results for the six cats on which resection of the nerve to soleus and lateral head of gastrocnemius was carried out are given in Table 1. The muscles denervated by resection of the sciatic nerve gave essentially the same result and therefore are not presented except in a summarized form in Table 2. The mean for the ratios for the number of sarcomeres is 1·025 (S.E. ± 0·014). This mean is not significantly different from unity. That is to say, by 4 weeks, the number of sarcomeres was not modified by denervation.

In Table 2 the means for the muscle denervated by both procedures are given and compared with the means for denervated muscles which have been immobilized in the lengthened position and the shortened position. Also given are the results for muscles immobilized for 4 weeks before the plaster cast was removed and then allowed a further 4 weeks to recover before the animals were killed.

TABLE 2. Summarized results for denervated non-immobilized muscles, denervated muscles immobilized in both the lengthened and shortened positions and denervated muscles which have been subject to transient immobilization

	A Denervated. Non-immobilized		*B* Denervated. Immobilized lengthened		*C* Denervated. Immobilized shortened		*D* Denerv. Transient immobil.
Soleus	N to Sol	Med Pop/Sc.	N to Sol	Med Pop/Sc.	N to Sol	Med Pop/Sc.	N to Sol
Sarc. length	0·969 (±0·010)	0·963 (±0·014)	0·849*** (±0·014)	0·829** (±0·031)	—	—	1·005
Fibre length	1·008 (±0·020)	0·982 (±0·010)	1·057 (±0·022)	1·030 (±0·033)	—	—	0·999
Sarc. no.	1·025 (±0·014)	1·051 (±0·033)	1·255*** (±0·023)	1·253** (±0·046)	0·645*** (±0·063)	0·750** (±0·065)	0·996

Results are given as means of the ratios of the experimental to the control muscles together with S.E. They are presented for both methods of denervation, i.e. resection of the nerve to the soleus (N to Sol) and resection of the medial popliteal and sciatic nerve (Med Pop/Sc.). The significance levels are indicated:

** Significance at 0·01 level } for calculated difference between *B* and *A*,
*** Significance at 0·001 level } and *C* and *A* means.

Effect of denervation on the adaptation of sarcomere number due to immobilization. The data for the denervated soleus immobilized in the lengthened position, given in Table 2, show that there was a significant decrease in sarcomere length, no significant increase in the fibre length and consequently a significant increase in the number of sarcomeres in series. The increase in sarcomere number was in the region of 25%. There is no significant difference between the subgroups of muscles denervated by the two different procedures.

The results of the experiment in which the soleus muscle was immobilized in the shortened position are summarized in Table 2. However, as mentioned in the Methods section, it was not possible to fix the control and the experimental muscles at the same length. In this case therefore it was only possible to compare the data for the number of sarcomeres in series as the fibre length and sarcomere length were inevitably different from that of the other muscles. The mean ratios of the sarcomere number, however, are seen to have decreased in both subgroups. There is also a significant difference between the sarcomere number ratio of these and the non-immobilized denervated soleus muscles. The decrease in sarcomere number is of the order of 25–35 %. The difference between the two subgroups of denervation is not significant.

The sarcomere ratio results observed for the transient immobilization of the denervated soleus muscles showed that these muscles had fully recovered their normal sarcomere number in the 4 weeks after the plaster cast had been removed. This was in spite of the fact that they had been denervated and previously immobilized in the shortened position.

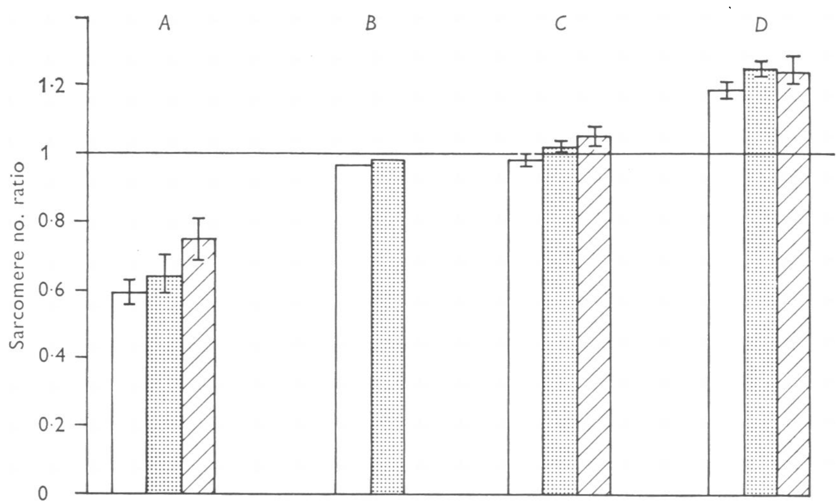

Fig. 2. Number of sarcomeres in a single fibre for non-denervated soleus muscles (hollow blocks), soleus muscles denervated by resection of the nerve to soleus (stippled blocks) and soleus muscles denervated by resection of medial popliteal or sciatic nerve (hatched blocks). The blocks refer to the mean of the ratio of the sarcomere number for the experimental muscle to the contralateral muscle. The bars indicate the S.E. of the means. The various experimental situations are immobilized in the shortened position (*A*), immobilized in the same position and then allowed to recover (*B*), not immobilized (*C*), immobilized in the lengthened position (*D*).

3. *Relationship of sarcomere number and the passive length–tension measurement*

In the first group (control group) the length–tension curves were superimposed for both normal and denervated muscles, when plotted on the same axis, and the sarcomere number ratios were not significantly different. In the group of animals in which the muscle was immobilized in the lengthened position there was no significant difference between the length–tension curves for the normal and immobilized muscles, in spite of an increased sarcomere number ratio. In the group of animals in which the muscle was immobilized in the shortened position, there was quite a wide separation in the length–tension curves and this was associated with a decreased sarcomere number ratio. We must point out, however, that in one of the animals there was a wide separation of the passive length–tension curves but the sarcomere ratio was surprisingly little diminished (0·95). This suggests that any association between the sarcomere number changes and the passive length–tension changes is not a direct one.

4. *Comparison between results of denervated and non-denervated muscles*

The results of the present series of experiments with denervated soleus muscle were compared with the data for muscles which had been immobilized but not denervated (Tabary *et al.* 1972). The physiological results were similar for the denervated and non-denervated muscle. The length–tension curve was modified only when the muscle was immobilized in the shortened position, whether the muscle was denervated or not.

The sarcomere number results were also very similar in both non-denervated and muscles denervated by both procedures. These are summarized in Fig. 2. It will be noted that in the four different experimental situations, the modifications of the sarcomere number ratios were the same for denervated and normal muscle. Factorial analyses confirm that there is no significant difference between non-denervated and denervated muscles (the F value being 2·37, $P > 0·05$). In other words, adaptation of sarcomere number to the change in muscle length caused by immobilization was unaffected by denervation. Also the recovery after transient immobilization in those muscles which had been denervated was complete after 4 weeks, just as it was in the non-denervated muscle.

DISCUSSION

It is well known that a denervated soleus becomes atrophic. Its weight may drop by about 50 % of the control after 4 weeks and this is usually associated with a reduction in fibre diameter of more than 50 %. However, the reduced fibres still possess a certain degree of regularity and the striations are apparently unchanged. Pellegrino & Franzini (1963) in an electron microscope study showed that in the soleus of the rat 4 weeks after denervation, there were only scattered and rare areas of degeneration. The arrangement of myofibrils was preserved. Consequently it was possible in this study to look for the effect of denervation on the number of sarcomeres along single teased fibres from this muscle.

The results presented here demonstrate that denervation does not suppress the adjustment of the sarcomere number to the functional length of the muscle. The fibres of the soleus muscle were found to add on about 25 % more sarcomeres in series when it was immobilized in the lengthened position and to lose about 30 % of sarcomeres in series when it was immobilized in the shortened position. These changes occurred whether the muscle was denervated or not. Also denervation had no effect on the recovery of the muscle following immobilization or on the decrease in extensibility of the muscle when immobilized in the shortened position. This adaptation is therefore presumably not mediated through a reflex pathway.

An important myogenic factor that appears to be involved is the direct effect of increasing or decreasing the resting tension on skeletal muscle. This mechanism was suggested by Schiaffino & Hanzlikovǻa (1970) to explain the rapid and striking hypertrophy of the soleus muscle following tenotomy of the gastrocnemius muscle. However, one of the interesting aspects of this study is that the denervated muscle, which is normally undergoing quite rapid atrophy, that is to say losing myofilaments in parallel, is capable of producing myofilaments in new sarcomeres in series if the functional length of the muscle is increased. This finding gives some insight into the influence of the innervation on myofibrillar protein synthesis. From these results it seems that the atrophy of denervated muscle cannot be explained as a direct effect of the absence of the nerve supply suppressing protein synthesis. Rather it appears to be a result of the change in the activity pattern of the muscle. Certainly myogenic factors such as passive resting tension seem to have an overriding effect.

Another interesting aspect is the decrease in the extensibility of the muscle immobilized in the shortened position. This occurs whether the muscle is denervated or not; work now in progress indicates that it may be due to an increase of connective tissue.

The study of myogenic and neurogenic factors involved in controlling muscle growth and adaptation is being studied further with particular attention being paid to the influence of stretch and of the pattern of activity on muscle protein synthesis.

This research was supported by a Faculté de Medecine de Paris Ouest grant.

Madame Tardieu is Maitre de Recherche, INSERM, Paris, Grant number 712 1568. Dr Goldspink was in receipt of a research grant from the Spastics Society of Great Britain. The authors wish to acknowledge the assistance given by Doctors Liliane Gagnard and Micheline Lombard.

REFERENCES

CLOSE, R. (1964). Dynamic properties of fast and slow skeletal muscles of the rat during development. *J. Physiol* **173**, 74–95.

PELLEGRINO, C. & FRANZINI, C. (1963). An electron microscope study of denervation atrophy in red and white skeletal muscle fibres. *J. cell Biol.* **17**, 327–349.

SCHIAFFINO, S. & HANZLIKOVÁ, V. (1970). On the mechanism of compensatory hypertrophy in skeletal muscles. *Experientia* **26**, 152–153.

TABARY, J. C., GOLDSPINK, G., TARDIEU, C., TABARY, C., LOMBARD, M., TARDIEU, G. & CHIGOT, P. (1971). Nature de la rétraction musculaire des I.M.C. Mesure de l'allongement des sarcomères d'un muscle étiré. *Revue Chir. orthop. répar. Appar. moteur* **57**, 463–470.

TABARY, J. C., TABARY, C., TARDIEU, C., TARDIEU, G. & GOLDSPINK, G. (1972). Physiological and structural changes in the cat's soleus muscle due to immobilization at different lengths by plaster casts. *J. Physiol.* **224**, 231–244.