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PHYSIOLOGICAL AND STRUCTURAL CHANGES IN THE CAT'S SOLEUS MUSCLE DUE TO IMMOBILIZATION AT DIFFERENT LENGTHS BY PLASTER CASTS*

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SUMMARY

1. Passive length–tension curves were established for cat soleus muscles that had been immobilized in different positions. Muscles that had been immobilized in the lengthened position showed no difference in their length–tension properties to those of normal muscles. However, those immobilized in the shortened position showed a considerable decrease in extensibility.

2. Muscle fibre length, sarcomere length and the total number of sarcomeres along single teased fibres were also determined for muscles immobilized in different positions. Soleus muscles immobilized in the lengthened position were found to have 20% more sarcomeres in series than normal muscles whilst those immobilized in the shortened position had 40% less than normal muscles.

3. When the plaster casts were removed from muscles that had been immobilized in the shortened position, the length–tension curves and sarcomere number returned to normal within 4 weeks. Muscles that were immobilized in a shortened position and then immobilized in a second position were found to rapidly adjust to the second position with respect to their passive length–tension properties and sarcomere number.

4. A change in the number of sarcomere in series seems to be the way in which the sarcomere length of the muscle is adjusted to its new functional length. The change in the length–tension properties which accompanies a decrease in sarcomere number appears to be the mechanism which prevents the muscle from being overstretched.

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INTRODUCTION

Striated muscle is known to be a very adaptable tissue. Its ability to adapt in length was first demonstrated by the experiments of Marey (1887) in which he transplanted the distal end of the triceps surae tendon to a point farther down the calcaneum. In these experiments the muscle adapted to its new functional length within a few weeks. More recently Alder, Crawford & Edwards (1958) immobilized the rabbit tibialis anterior muscle in its shortened position by mechanically fixing the ankle in dorsi-flexion. This resulted in a diminished muscle length and a change in the length–tension curve of the muscle in that the isometric tetanic tensions, equivalent in degree to those of the control muscle of the normal leg, were exerted at a shorter belly length and through a reduced range of movement of the foot. Similar results have been reported for cat muscle by Tardieu, Tardieu, Gagnard & Tabary (1969), who also found that the length at which the muscle begins to develop contractile tension is increased if the muscle is immobilized in the lengthened position.

The aim of the present work was to investigate further the way in which adult muscle adapts to a change in functional length due to immobilization and its physiological implications. Goldspink (1968) and Williams & Goldspink (1971) have shown that the increase in fibre length during normal growth is associated with a large increase in the number of sarcomeres along the length of the fibres. As the actin and myosin filaments are of a constant length, the adaptation of adult muscles to a different functional length must presumably involve the production or removal of a certain number of sarcomeres in series in order to maintain the correct sarcomere length in relation to the whole muscle length. Therefore the intention was to study how immobilization of the muscle at different lengths affects the number of sarcomeres in series along the fibres. It was felt that it was also important to measure any change in the mechanical properties of the muscles, especially their passive length–tension properties.

METHODS

Experimental animals. The twenty-seven adult cats used in this study were divided into five experimental groups. The first group of six cats were normal untreated animals and their muscles served as the controls for the experimental groups. The second group of five animals had one of their hind limbs immobilized in complete dorsi-flexion by plaster cast so that the soleus muscle was maintained in the lengthened position. The muscles were physiologically and histologically examined after 4 weeks of immobilization. A third group of six cats were subjected to immobilization of one of their hind limbs 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. A fourth group of four cats had one of their hind

limbs immobilized in full plantar-flexion. However, in this case the casts were removed after 4 weeks and the muscles allowed to recover for a further 4 weeks after which the animals were killed and the muscles examined. In the last group (fifth group), one of the limbs of each of six cats was plastered in the full plantar-flexion position. After 4 weeks the casts were removed and the limbs were replastered in an intermediate position between plantar- and dorsi-flexion. The physiology and histology of the muscles were studied after 4 weeks of immobilization in the second position.

Determination of the passive length–tension curves. The animals were induced into a state of deep anaesthesia by an injection of pentobarbitone. After sectioning the sciatic nerve each animal was placed on an operating table ventral side down with its hind limbs arranged in a symmetrical manner. The limbs were firmly affixed to the table and the soleus muscle was exposed by removing the overlying skin and reflecting the gastrocnemius muscle to one side. A mark was then made on the muscle which permitted the measurement of muscle length and this could then be related to the different angles of the tibia-calcaneum. The calcaneum was then sectioned and the achilles tendon was attached to a strain gauge coupled to a pen-recording system. The strain gauge was mounted on a micrometer screw so that the muscle length could be accurately adjusted. The tension for the different degrees of stretch of the muscle was plotted as the passive tension at the various ankle positions. Measurements were made on several animals for each group. In all they were carried out on fourteen of the total of twenty-seven cats used in this study.

Determination of the number of sarcomeres in series. After the length–tension curve had been established the number of sarcomeres was determined by the method of Close (1964). Muscles were fixed *in situ* at a determined length using glutaraldehyde fixative (2·5 % solution containing 5 % glucose in a 0·062 M phosphate buffer at pH 7·2) after tying them to a specially made metal spur.

In the case of the other muscles for which the length–tension curve was not obtained, the limbs were pinned out on wooden board and the soleus was exposed and fixed in the same manner. In general, the muscles (experimental and control) were fixed in the same articular angle position for both limbs of each animal. However, some difficulty was encountered in the case of the muscles immobilized in the shortened position because a relatively slight degree of stretching resulted in the tearing of the muscle. The muscles on the normal side of these animals had to be stretched to some extent because it is impracticable to determine sarcomere number on slack muscle fibres. Hence in the third group the articular angle of the ankle was different for the experimental and control muscles.

After the muscles had been tied to the metal spur they were fixed by first pipetting fixative over them for 20 min during which time the animals were killed by intracardiac injection of pentobarbitone. Following this the whole limbs were removed from the animals and placed in fixative for a further 12 hr. The muscles were then dissected out and washed in phosphate buffer at pH 7·2. Small bundles of fibres were then separated under a stereomicroscope using a pair of fine needles. Some bundles were then placed in 70 % glycerol for several days while other bundles were placed in 30 % (w/v) HNO_3 for 2–3 days before being washed and placed in 70 % glycerol for several days. The acid treatment was carried out in order to hydrolyse the connective tissue and thus facilitate the teasing apart of the individual fibres. Individual muscle fibres from the glycerol-treated bundles were then mounted on microscope slides using glycerol jelly. Photomicrographs were taken on high contrast sheet film at a magnification of $\times 100$ or $\times 200$ of different regions of the fibres under a Wild phase contrast microscope. The number of sarcomeres was then determined by direct counting for different regions along the length of the fibre.

Each sample length counted was about 1 mm long and contained 300–400 sarcomeres. The average sarcomere length for the fibre was then calculated. Following this, the entire fibre was photographed at a lower magnification (× 8) and the total length of the fibre was measured from this using a curvimeter. The total number of sarcomeres was then obtained by dividing the fibre length by the average length of the sarcomere.

RESULTS

Physiological results. Some of the length–tension curves obtained for normal and immobilized cat soleus muscles are shown in Figs. 1 and 2. The curves for muscles from the right and left side of the normal animals (group 1) were almost invariably superimposed. In the second group of

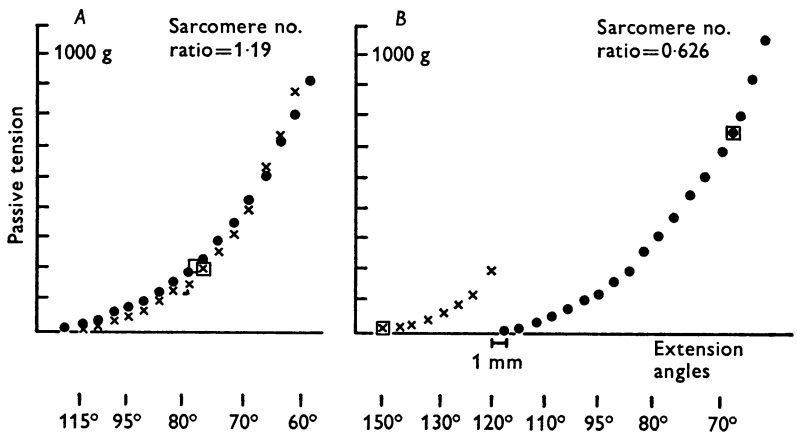

Fig. 1. Plots of passive tension against extension for muscles that have been immobilized in the lengthened position (*A*) and in the shortened position (*B*). The measured angles of the ankle during extension of the muscle are indicated on the abscissa together with a scale representing the change in muscle length. In each plot the length–tension relationship for the immobilized muscle (×) is given with that of its contralateral control muscle (●) and the point at which fixative solution was applied to the muscles is indicated by the squares. The sarcomere number ratio is given above each plot.

animals in which the muscle from one side had been immobilized in the lengthened position there was also no significant difference between the length–tension curves (Fig. 1*A*). However, in the third group in which soleus was immobilized in the shortened position the length–tension curves for the immobilized sides and the non-immobilized sides were very different (Fig. 1*B*). In this case the passive tension due to stretch commenced at a much greater angle (shorter muscle length) than for the contralateral muscle. Indeed the immobilized muscle could not be stretched to any great length before it ripped, hence the passive tension

curve shown in Fig. 1 *B* is rather short. In fact the immobilized muscles could not be extended through the normal length range of the control muscles.

In the fourth group which had been allowed to recover after immobilization of the soleus in plantar flexion, the length–tension curves for the experimental and control muscles did not differ much and the first part of the curves were usually superimposed. Considerable differences were, however, recorded in the fifth group of animals in which the limb was

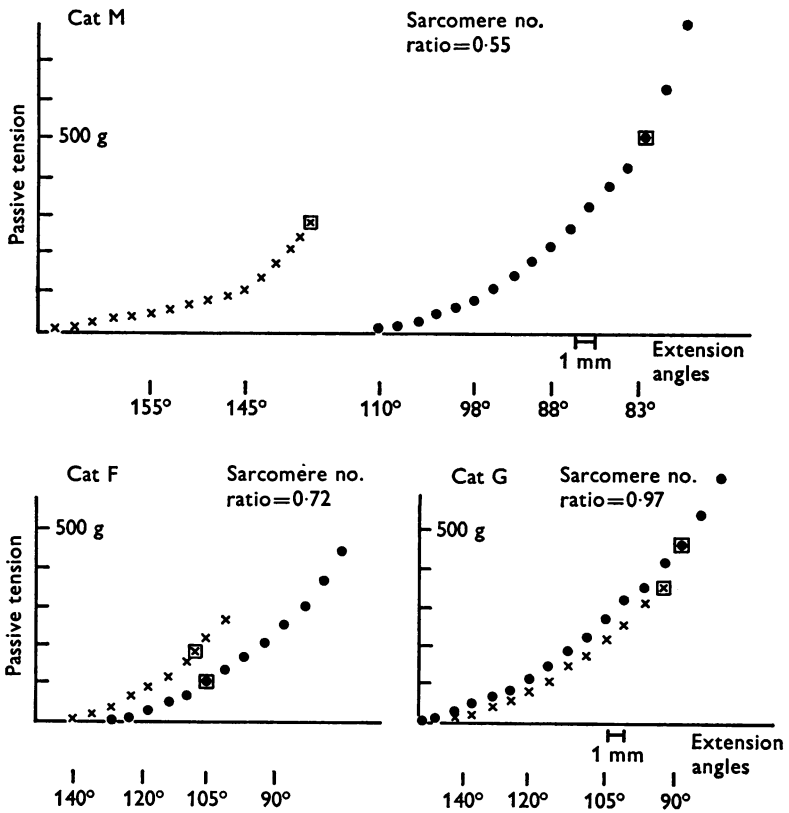

Fig. 2. Plots of passive tension against extension for muscles that have been immobilized in different positions by two successive plaster casts. The angle of the second immobilization of the ankle was as follows: cat M, 120°; cat F, 90°; cat G, 85° (see Table 5). The measured angles of the ankle during extension of the muscle are indicated on the abscissa together with a scale representing the change in muscle length. In each plot the length–tension relationship for the immobilized muscle (×) is given with that of its contralateral control muscle (●) and the point at which the fixative solution was applied to the muscles is indicated by the squares. The sarcomere number ratio is given above each plot.

replastered in an intermediate position. As the variation between animals was quite considerable, the results from three separate animals are present (Fig. 2). As will be seen from cat F which had its hind limb plastered the second time at an articular angle of 90° the curve was intermediate between those for muscles immobilized in the shortened position (Fig. 1 *B*) and that of its control muscle. Cat G which had its limb replastered at an

TABLE 1. Group control

Cat	Angle of fixation (°)	Post-fixation treatment	Sarcomere Length (μm)	Sarcomere Ratio	Fibre Length (μm)	Fibre Ratio	Sarcomeres No.	Sarcomeres Ratio
1	60	HNO_3	2·624 2·670	0·9857	38,000 38,800	0·9793	14,435 14,529	0·9935
2	80	HNO_3	2·193 2·252	0·974	31,100 32,800	0·9543	14,178 14,544	0·9748
3	77	HNO_3	2·444 2·379	1·0276	35,500 34,880	1·0178	14,525 14,662	0·9906
	77	Glycerol	2·635 2·655	0·9928	37,500 37,750	0·9934	14,395 14,230	1·011
4	30	HNO_3	3·102 3·022	1·0265	46,740 46,610	1·0027	15,067 15,426	0·9767
	30	Glycerol	3·331 3·330	1·0004	47,750 48,870	0·9770	14,334 14,678	0·9766
5	30	HNO_3	2·943 2·942	1·0004	35·250 35,000	1·0007	11,978 11,898	1·0067
	30	Glycerol	3·114 3·157	0·9864	37,800 36,000	1·05	12,139 11,404	1·0645
6	80	HNO_3	2·440 2·473	0·9866	29,780 31,862	0·9347	12,205 12,884	0·9473
	80	Glycerol	2·611 2·610	1·0004	31,562 32,025	0·9855	12,088 12,270	0·9852
			Mean = 0·998 S.D. = 0·0173		Mean = 0·9895 S.D. = 0·0322		Mean = 0·9927 S.D. = 0·031	

angle of 85° exhibited a passive length–tension curve which was almost identical to that of its control. Cat M which had its limb plastered the second time at an angle of 120° exhibited a length–tension curve which was very different from its control muscle and was in fact quite similar to the ones for those muscles immobilized in the shortened position.

Histological results. The results of the fibre length, sarcomere length and sarcomere number measurements are shown in Tables 1–5. In addition to giving the actual values the results are also expressed as a ratio of the value for the control muscle to that of the experimental muscle. In the case of the

control group (Table 1) in which the muscles from both sides of the animal were normal, the denominator side was selected at random. The results for the control group show that there is very little variation between the pairs of muscles taken from normal, untreated animals.

The data for the immobilization of the soleus in the lengthened position are shown in Table 2. The means of the ratios between the normal and the immobilized muscles were 0·885 (S.D. $\pm$ 0·02) for sarcomere length, 1·05 (S.D. = 0·043) for fibre length and 1·19 (S.D. $\pm$ 0·06) for the total number of sarcomeres in series. Thus the muscle immobilized in the lengthened position showed a decrease in sarcomere length but an increase in fibre length and an even greater increase in the number of sarcomeres in series (19 % increase). All the means were significantly different from those shown for the normal muscles in Table 1 ($P < 0{\cdot}001$).

The results of the experiment in which the soleus muscle was immobilized in the shortened position are shown in Table 3. As mentioned above in the Methods section it was not usually possible to fix the control muscles at the same articular angle as the experimental muscles because this made sarcomere counting difficult. Therefore the ratios for fibre length and sarcomere length are only given for the one case in which fixation at the same articular angle was attempted successfully. In spite of this experimental difficulty, it is possible to see from the data given in Table 3 that although the degree of stretch of the immobilized muscles is less (larger articular angle), the average sarcomere length is equal to or even longer than in the control muscles. This is seen to be associated with a very marked reduction in the number of sarcomeres in series in the immobilized muscles. The mean ratio for sarcomere number for the whole group of muscles immobilized in the shortened position was 0·593 (S.D. = 0·104), in other words there were 40 % fewer sarcomeres in the immobilized side than in the control muscles. Statistical comparison of the means of the control group and groups II and III using the analysis of variance method ($F^2_{22} = 146$) indicated that the differences in sarcomere number are highly significant.

The results of the fourth group of animals which were subjected to transient immobilization of the muscle in its shortened position are shown in Table 4. The mean of the ratios for the estimated sarcomere number is 0·965 (S.D. = 0·032). A statistical comparison of this mean with the one for the control group indicated that there was no significant difference. However, there was a significant difference between the muscle in group III which had been fixed after immobilization in the shortened position ($P < 0{\cdot}01$), which demonstrates that the recovery of full sarcomere complement is quite rapid following the removal of the plaster cast.

The results of the experiment in which animals were subjected to

TABLE 2. Immobilized lengthened soleus

Cat	Plaster cast	Angle of fixation (°)	Post-fixation treatment	Sarcomere Length (μm)	Sarcomere Ratio	Fibre Length (μm)	Fibre Ratio	Sarcomere No.	Sarcomere Ratio
O	Plastered 33 days	70	HNO_3	2·191	0·896	34,412	1·0745	15,705	1·199
	Control	70	HNO_3	2·445		32,025		13,098	
S	Plastered 26 days	60	HNO_3	2·453	0·855	40,400	1·0125	16,470	1·184
	Control	60	HNO_3	2·869		39,900		13,906	
Y	Plastered 36 days	58	HNO_3	1·970	0·903	32,500	1·0236	16,498	1·134
	Control	58	HNO_3	2·182		31,750		14,548	
	Plastered 36 days	58	Glycerol	2·275	0·857	37,500	1·1278	16,481	1·315
	Control	58	Glycerol	2·652		33,250		12,538	
A	Plastered 28 days	70	HNO_3	2·310	0·904	39,270	1·0850	16,992	1·199
	Control	70	HNO_3	2·555		36,200		14,165	
	Plastered 28 days	70	Glycerol	2·329	0·889	40,397	1·0325	17,343	1·162
	Control	70	Glycerol	2·620		39,125		14,930	
AI	Plastered 21 days	30	HNO_3	2·970	0·894	45,667	1·0186	15,376	1·1393
	Control	30	HNO_3	3·322		44,833		13,496	
				Mean = 0·8854		Mean = 1·0535		Mean = 1·1903	
				S.D. = 0·02		S.D. = 0·043		S.D. = 0·061	

TABLE 3. Immobilized shortened soleus

Cat	Plaster cast	Angle of fixation (°)	Post-fixation treatment	Sarcomere Length (μm)	Sarcomere Ratio	Fibre Length (μm)	Fibre Ratio	Sarcomeres No.	Sarcomeres Ratio
J	Plastered 34 days	155	HNO_3	2·223	—	23,750	—	10,682	0·626
	Control	70	HNO_3	2·475		42,250		17,073	
Z	Plastered 21 days	140	HNO_3	2·867	1·665	17,700	0·777	6,174	0·467
	Control	140	HNO_3	1·722		22,770		13,223	
W	Plastered 34 days	140	HNO_3	2·872	—	25,120	—	8,746	0·607
	Control	73	HNO_3	2·493		35,940		14,414	
X	Plastered 43 days	145	Glycerol	3·134	—	22,790	—	7,273	0·495
	Control	60	Glycerol	2·959		43,500		14,701	
	Plastered 43 days	145	HNO_3	3·078	—	22,640	—	7,355	0·594
	Control	60	HNO_3	2·838		35,120		12,374	
AB	Plastered 28 days	135	Glycerol	3·429	—	22,665	—	6,609	0·575
	Control	40	Glycerol	3·385		38,895		11,492	
	Plastered 28 days	135	HNO_3	3·268	—	22,940	—	7,019	0·549
	Control	40	HNO_3	3·043		38,905		12,785	
AH	Plastered 21 days	90	HNO_3	3·565	—	43,522	—	12,208	0·8311
	Control	30	HNO_3	3·304		48,522		14,686	
								Mean =	0·593
								S.D. =	0·104

TABLE 4. Shortened soleus transient immobilization

Cat	Plastered (days)	Moving freely (days)	Angle of fixation (°)	Post-fixation treatment	Sarcomere Length (μm)	Sarcomere Ratio	Fibre length (μm)	Sarcomeres No.	Sarcomeres Ratio
A	29	25	85	HNO_3	2·584 2·292	1·127	36,000 34,000	13,934 14,832	0·9395
C	33	28	80	HNO_3	2·636 2·46	1·07	32,640 32,350	12,381 13,142	0·9422
H	34	42	88	HNO_3	2·404 2·159	1·1135	28,906 25,562	12,024 11,839	1·016
U	41	28	100 70	HNO_3 HNO_3	2·306 2·584	—	33,210 38,230	14,405 14,797	0·9735
			100 70	Glycerol Glycerol	2·433 2·73	—	34,270 40,365	14,087 14,779	0·9532
								Mean = 0·9649 S.D. = 0·032	

TABLE 5. Successive plaster casts at different angles

Cat	Plastered shortened (days)	Second plaster cast (days)		Angle of fixation (°)	Post-fixation treatment	Sarcomere Length (μm)	Sarcomere Ratio	Fibre Length (μm)	Fibre Ratio	Sarcomeres No.	Sarcomeres Ratio
E	29	26	110°	110	HNO_3	2·39 1·985	1·204	23,426 22,667	1·0335	9,752 11,578	0·8423
F	29	33	90°	105	HNO_3	2·95 2·30	1·2826	25,800 29,500	0·8746	9,829 13,586	0·7235
G	30	25	85°	90	HNO_3	2·4425 2·326	1·05	32,750 32,160	1·0184	13,408 13,822	0·97
K	27	32	100°	120	Glycerol	2·257 2·048	1·102	29,587 31,588	0·9367	13,109 15,424	0·8499
				120	HNO_3	2·172 2·033	1·0684	29,000 33,669	0·8613	13,347 16,561	0·8059
L	27	39	100°	100	HNO_3	2·49441 2·46185	1·0132	25,781 36,719	0·7021	10,336 14,915	0·6930
M	40	28	120°	128 85	HNO_3	2·6941 2·715	—	22,969 41,797	—	8,526 15,395	0·5538
										Mean = 0·7660 S.D. = 0·0143	

immobilization of one limb at two different angles are shown in Table 5. The results in this case vary according to the last angle at which they were immobilized. In the case of the muscle with the second immobilization angle at 120° (muscle in shortened position) there was a considerable reduction of sarcomeres in series (ratio = 0·55). However, when the second angle was 85° this gave a ratio of 0·97, indicating that the sarcomere number was almost completely normal. Statistical comparison of the means for sarcomere number for group V indicated a significant difference with group III in which the muscles were permanently immobilized at the shortened length.

Relationship of sarcomere number and the passive length–tension curve measurements. In the case of the control group of cats the length–tension curve for the muscles from the left and right sides were superimposed and the sarcomere number ratio was essentially unity. In the group of animals in which the muscle was immobilized in the lengthened position the situation was the same as far as the length–tension properties were concerned; however, the sarcomere number ratio was different as the immobilized muscles possess more sarcomeres in series (ratio 1·19). In the third group (Fig. 1*B*) in which muscles were immobilized in the shortened position there was a wide separation in the length–tension curves and this was associated with a decreased sarcomere number. For the fourth group in which the plaster casts had been removed from the limbs after immobilization in the shortened position, the sarcomere number and the length–tension curves were the same in the experimental and control animals indicating that the muscles had completely recovered from the effects of immobilization.

In the fifth group of animals the diversity of the experimental conditions did not permit the comparison of the sarcomere number values and the length–tension curves for the whole group. However, it is seen (Fig. 2) that those pairs of muscles which showed the greater separation of the length–tension curves are those in which the ratio of sarcomere numbers was lowest.

DISCUSSION

The results presented here demonstrate that striated muscle is a very adaptable tissue and in particular they show that sarcomere number, fibre length and sarcomere length are adjusted to the functional length of the whole muscle. The physiological significance of this finding is apparent when one considers that the maximum contractile tension and the maximum rate of shortening of the muscle are obtained at the sarcomere length at which there is maximum interaction of the myosin cross-bridges with the actin filaments. Muscle is apparently able to adjust its

fibre length and sarcomere length by producing more sarcomeres or by removing sarcomeres. In other words, it is able to adjust its sarcomere number to give the maximum functional overlap of the myosin cross-bridges and actin filaments according to its functional length. In the experiments reported here the functional length of the soleus muscles was altered by immobilization of the hind limb in different positions.

When the limb was immobilized with the muscle at its maximum length, the muscle fibres were found to have produced 19 % more sarcomeres in series. On the other hand, when the limb was immobilized with the muscle in its shortened position the muscle fibres were found to have lost 40 % of the sarcomeres in series. These marked changes in sarcomere number were found to take place in a relatively short period of time. Also the experiments in which the plaster cast was removed showed that the muscle as well as adjusting to the new functional length, could rapidly readjust to its original length once the cast was removed.

Associated with the changes in fibre length and the number and length of the sarcomeres there was a reduced extensibility of the muscles immobilized in the shortened position. This may be due partly to the shortening of the muscle fibres, but can more probably be attributed to an increase in the connective tissue in the belly of the muscle. A greater abundance of connective tissue was observed in the muscle belly of immobilized muscles when dissecting out the single fibres for the histological measurements. The physiological significance of the change in length–tension properties is not clear. However, it seems that one of the main functions of the connective tissue in the muscle belly is to prevent the muscle fibres from being overstretched. This is particularly important in the shortened muscle as stretching even through the normal range of movement would result in the sarcomeres being pulled out to the point at which there is no interdigitation of the myosin and actin filaments thus causing permanent damage to the muscle. Changes in the elastic properties of the muscle immobilized in the lengthened position appear not to be necessary because the adaptation is in the reverse direction and there is no greater chance of the muscle being overstretched than in the case of the normal muscle. The change in the elastic properties of the muscle may be therefore regarded as a protective type of adaptation which accompanies a reduction in the number of sarcomeres along its fibres.

The cellular and molecular events involved in adaptation of the fibre and the connective tissue elements to the functional length of the muscle are not known; however they do provide an extremely interesting subject for further study and it is especially important that this type of adaptation should be more fully understood because of its relevance to muscle physiology, physiotherapy, physiopathology and surgery.

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