

## EXPERIMENTAL STUDY ON INNERVATION OF THE SKELETAL MUSCLE

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(Received December 10, 1953)

The main objects of this series of experiments are to study innervation of the skeletal muscle, especially the muscle spindle, and to observe certain correlations between the nervous system and the skeletal muscle under various conditions, such as denervation, relaxation and ischaemic contracture.

### METHOD

The gastrocnemius muscle of dog and guinea-pig were chosen as a convenient experimental muscle. Various procedures were undertaken, such as removal of the posterior root ganglia in dog, division of the sciatic nerve and tendon and forcible binding of the hind limb by plaster cast in guinea-pig.

Animals were killed at intervals from twenty-hours to fifty days after the above procedures. The muscles were macroscopically examined, weighed and then fixed in 10 per cent formol or 70 per cent alcohol, and sections were stained with haematoxylin and eosin, by Weigert's and Ramon y Cajal's techniques.

### EXPERIMENT

#### *1) Anatomical Structure of the Normal Gastrocnemius Muscle of Guinea-pig and its Innervation.*

The normal gastrocnemius muscle of guinea-pig is approximately 1.5 centimeters long, spindle shaped muscle fibre bundle embraced with the thin fascia.

The nerve fibres of four different types are found, such as heavily myelinated, moderately myelinated, slenderly myelinated and unmyelinated nerve fibre.

A) In ordinary striated muscle fibres, a large number of moderately myelinated nerve fibres and a few unmyelinated nerve fibres are found. The former end up in the typical terminal network in the muscle fibre and the latter may be thought to terminate in the muscle fibre as simple end-plate.

B) The muscle spindle is a complex of the nerve fibres and striated muscle fibres which lie within the fleshy substance of the muscle.

This is a narrow, elongated structure slightly thickened in the middle, arranged lengthwise with the bundles of ordinary muscle fibres.

Each spindle consists of several long slender striated muscle fibres generally

called "Weismann's or intrafusal fibre". This is enveloped by a connective tissue capsule containing tissue fluid.

Each spindle is supplied by the nerve fibres of three different types; a) heavily myelinated, b) moderately myelinated and c) unmyelinated nerve fibre (Figs. 3 and 4).

a) The heavily myelinated nerve fibres wind themselves around the Weismann's fibres and are in close contact with the sarcolemma, forming spirals. In dog, removal of the lumbar posterior root ganglia related to the sciatic nerve resulted in staggering gait and hypotonicity of the hind limb. In such a case, it was histologically demonstrated that the heavily myelinated nerve fibres distributed in the muscle spindle were ruptured in to segments, whereas moderately myelinated nerve fibres remained intact (Figs, 3,4 and 5).

Therefore, it is thought, as already proved by Hines and Tower, 1928, these heavily myelinated nerve fibres are afferent.

b) The spindle is supplied by the moderately myelinated nerve fibres which terminate in the axial muscle fibres with typical motor plates and remain intact even after removal of the appropriate posterior root ganglia (Figs. 5 and 8).

c) The unmyelinated nerve fibres are found, which run along the Weismann's fibres (Fig. 7).

C) Around the blood vessels, the unmyelinated and slenderly myelinated nerve fibres considered to be autonomic are found.

## 2) *Division of the Sciatic Nerve of Guinea-pig.*

The sciatic nerve of one limb was cut and 5 millimeters of it was excised to prevent, so far as possible, regeneration during the experiment.

After operation flaccid paralysis of the muscle occurred and no resistance against passive movement of the ankle was offered. Approximately fifteen days after operation a small ulcer in heel region developed, no tendency to healing being found. These appearances continued until later period.

The muscle wasted progressively and by the fiftieth day after operation, the weight of the muscle was reduced to 33 to 35 per cent of the control (Fig. 1, a).

### Histological Findings:

#### a) Muscle Fibre

On fifth post-operative day, the individual muscle fibres, including the Weismann's fibres, showed great decrease in diameter and the sarcolemmal nuclei — whether by active proliferation or by mere shrinkage of fibres—were conspicuous.

However, the longitudinal myofibrils and cross-striation persisted. These pathological changes rapidly progressed.

Fifty days post-operative, adipose or fibrous tissue was seen to have replaced most of the muscle fibres.

#### b) Nerve Fibre

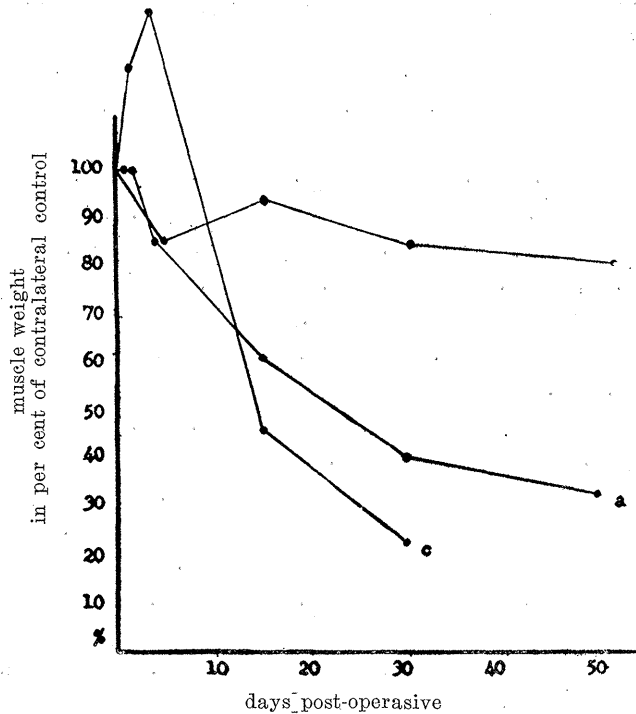


Fig. 1

a.....division of sciatic nerve  
 b.....division of tendon  
 c.....combined procedures

After operation the degeneration of the heavily and moderately myelinated axis cylinder rapidly progressed, and by the thirtieth day, the myelinated nerve fibres had almost completely disappeared. However, many unmyelinated nerve fibres remained intact.

In two cases, even at the thirtieth and fiftieth days, one or two moderately myelinated nerve fibres remained in area of the degenerated muscle fibres and, just adjacent to it, several sound muscle fibres were seen (Fig. 6).

On the contrary, in this experiment, although the unmyelinated nerve fibres remained unaffected, atrophy of the ordinary and Weismann's muscle fibres was marked (Fig 7).

#### Discussion

This experiment proves that whereas the moderately myelinated nerve fibre holds an important role in atrophy of the denervated muscle, the unmyelinated fibre shows no such relationship.

Moreover, many clinical and experimental facts suggest that this moderately myelinated nerve fibre passes through the anterior spinal root.

3) *Section of the Tendon of the Gastrocnemius Muscle of Guinea-pig.*

The gastrocnemius muscle of one limb was relaxed by excision of 3 millimeters of its tendon.

After operation talipes calcaneus developed and no resistance against passive movement of the ankle was offered, Fifteen days post-operative, the tendon was found fibrously adherent to the surrounding tissue.

The muscle appeared to be slightly edematous and atrophic.

By thirty days post-operative, union of divided tendon ends plus lengthening of the tendon and a slight muscular atrophy were found. With the lapse of time, this muscular atrophy gradually advanced, much slower, however, than in denervated muscle (Fig. 1, b).

Histological Findings:

a) Muscle Fibre

In five days post-operative, individual muscle fibres were wavy shaped, separate from each other and increased in diameter, and the sarcolemmal nuclei conspicuous. With the progress of time, proliferation of the sarcolemmal nuclei gradually became more marked, much slower, however, than in denervated muscle.

b) Nerve Fibre

Fifteen days post-operative, the axis cylinders showed loss of smoothness in shape and course, and round or elliptic swelling or diminished affinity to silver of the motor endings became noticeable (Fig. 8).

Thereafter the change ceased to progress. Advanced change such as rupture of the axis cylinder was not seen, and the myelin sheath always remained intact (Fig. 9).

#### Discussion

Kirita demonstrated that, in the human gastrocnemius muscle, analogous morphological changes in the muscle and nerve fibres were caused by inactivity of the muscle.

These facts prove that performance of physiological function of the muscle is essential to maintainance of its normal metabolism.

It is probable that inactivity of the muscle may cause stasis of lymph stream followed by disturbance of metabolism, resulting in muscle atrophy and morphological changes in the nerve fibres.

However, it may considered that this muscular atrophy is caused by loss of nutritive impulse passing through reflex arc.

This problem was studied in the next experiment, in which combined divisions of the sciatic nerve and the tendon were undertaken.

4) *Combined Divisions of the Sciatic Nerve and the Tendon of the Gastrocnemius Muscle of Guinea-pig.*

Divisions of the sciatic nerve and the tendon of the gastrocnemius muscle of one limb were undertaken at same time. After operation muscular atrophy progressed more rapidly and more markedly than in the two previous experiments.

By the thirtieth post-operative day, the weight of the muscle was already reduced to 22 per cent of the contralateral control (Fig. 1, c).

#### Histological Findings:

Five days after operation, the muscle fibres showed edematous swelling and marked proliferation of the sarcolemmal nuclei. After that the diameter of the individual muscle fibres, including the Weismann's fibres, began to decrease and, in parallel with it, the proliferation of the sarcolemmal nuclei rapidly progressed. In thirty days, muscular atrophy was the most pronounced of all in the three experiments.

#### Discussion

If disuse muscular atrophy were due to loss of nutritive impulse which controls metabolism of the muscle, the degree of atrophy caused by these combined procedures would be equal to that caused by division of the sciatic nerve alone.

As above mentioned, however, the combined procedures resulted in much more marked muscular atrophy. This fact prove that the so-called disuse muscular atrophy may be the result of a metabolic disturbance of the muscle due to its inactivity, the nervous system not participating in the process.

#### 5) *Forcible Binding of the Hind Limb of Guinea-pig by Plaster Cast.*

A clipped hind limb of guinea-pig was forcibly bound by plaster of paris.

Immediately after that, edema and cyanosis of the foot began to appear and rapidly progressed. Fifteen hours later the plaster was removed. The limb showed complete, flaccid motor paralysis, no resistance being elicited by passive movement of the ankle.

Moreover, edema of the foot spread to the whole limb and rapidly increased in degree for about twenty-four hours. After that it began gradually to decrease and has almost completely disappeared in the course of two to five days.

Motor paralysis of the limb, in the vast majority of animals, recovered in proportion to the diminution of edema. Animals were killed at intervals from twenty-four hours to fifty days after removal of plaster.

During the first and second days, edematous swelling of the muscle accompanied with several haemorrhages were found. This edema of the muscle disappeared in the course of several days.

The weight of the muscle was increased for two days after the removal of the plaster cast, probably due to edema; subsequently, during several days it was rapidly reduced in parallel with diminution of edema. Finally, it began gradually to increase again after recovering from the motor paralysis (Fig. 2).

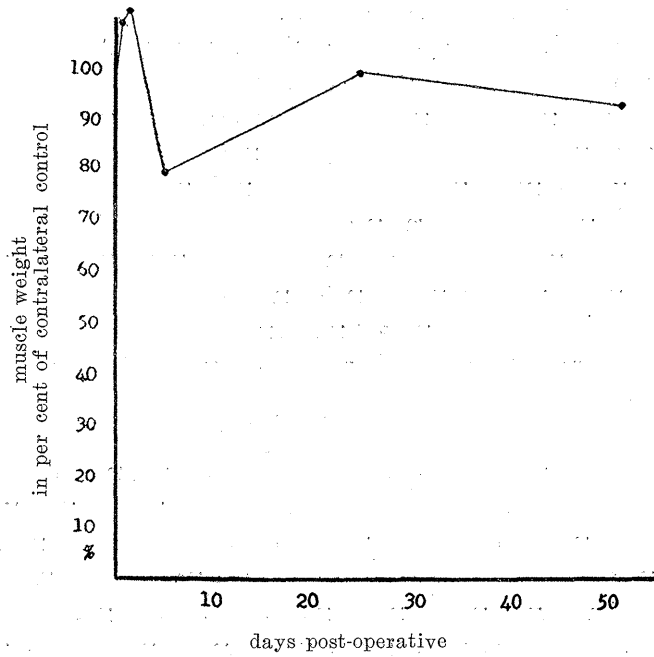


Fig. 2

Histological Findings:

a) Muscle Fibre

Two days after removal of plaster cast, the interfibrillar spaces of the muscle were markedly widened and the fibrils markedly increased in diameter, due to edema. In some sections, haemorrhages were seen here and there.

By the fifth day, the diameter of the muscle fibres became already reduced and the sarcolemmal nuclei conspicuous. By twenty-four days, however, the muscle fibres regained their normal appearance.

b) Nerve Fibre

Immediately after the removal of plaster cast degeneration of nerve fibres of all types, including the unmyelinated nerve fibres, was noticed and this pathological change was severer in the area of haemorrhage than others.

This degeneration of nerve fibres continued for five days, after which regeneration began to take, progressing with increasing rapidity with time.

By fifty days, a large number of tree-like nerve fibres were found, being especially conspicuous in the muscle spindle (Fig. 10).

Discussion

These experiments suggest that the muscle degeneration and regeneration, as shown in histological findings of the muscle, progressed in parallel with those of the nervous system.

Regarding pathogenesis of the so-called ischaemic contracture, no definite conclusion was reached, although a great deal of studies were made.

Neither of the femoral artery nor division of the sciatic nerve alone, as mentioned elsewhere, was not sufficient to produce ischaemic contracture.

In the experiment of division of the sciatic nerve, it is especially worth noting that many unmyelinated nerve fibres remained intact.

On the other hand, forcible binding of the limb by plaster cast resulted in a typical ischaemic contracture accompanied by degeneration of the nerve fibres of all varieties, including the unmyelinated nerve fibres.

These facts suggest that the unmyelinated nerve fibre may play an important role in the production of the so-called ischaemic contracture.

#### SUMMARY

1) The skeletal muscle is supplied by four varieties of nerve fibres.

In the striated muscle fibre, the moderately myelinated and unmyelinated nerve fibres are found. The former constitute the typical motor network in the muscle fibre. In the muscle spindle, the heavily myelinated (afferent), moderately myelinated (efferent) and unmyelinated nerve fibre are found.

Coursing the blood vessels the unmyelinated and slenderly myelinated nerve fibres are found.

2) The moderately myelinated nerve fibre may play an important part in producing atrophy of the denervated muscle, and the unmyelinated nerve fibre may have no close relation with it.

3) Disuse atrophy of the muscle may be caused by metabolic disturbance of the muscle due to its inactivity, and although slight morphological changes in the nerve fibres are present, they do not take part in producing this muscular atrophy.

4) It may be thought that the unmyelinated nerve fibre play an important role in producing so-called ischaemic contracture.

## EXPLANATION OF PLATE I

- Eig. 3. The heavily myelinated and moderately myelinated nerve fibres in the normal muscle spindle, in guinea-pig. Ramon y Cajal's staining.
- Eig. 4. The heavily myelinated nerve fibres wind themselves around the Weismann's fibres, forming spirals, in guinea-pig. Weigert's staining.
- Eig. 5. Degeneration of the heavily myelinated nerve fibre after removal of the related nerve root ganglia, in dog. Weigert's staining.
- Eig. 6. The moderately myelinated nerve fibre and muscle fibres which remained intact thirty days after section of the sciatic nerve, in guinea-pig. Weigert's staining.



PLATE I



Fig. 4



Fig. 6

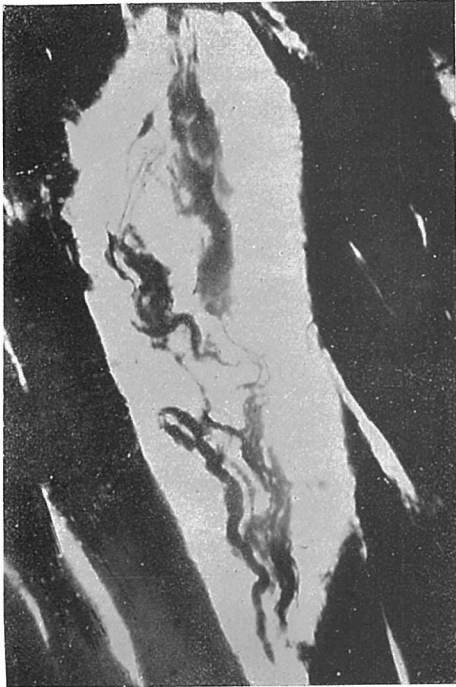


Fig. 3



Fig. 5

## EXPLANATION OF PLATE II

- Fig. 7. Severe degeneration of the ordinary muscle fibres and the Weismann's fibres fifty days after section of the sciatic nerve, in guinea-pig, and although the all myelinated nerve fibres have already disappeared, the unmyelinated nerve fibres coursing along the Weismann's fibres are seen. Ramon y Cajal's staining.
- Fig. 8. The motor end-plates in the Weismann's fibres which show loss of smoothness in shape and course fifteen days after section of the tendon of gastrocnemius muscle, in guinea-pig. Ramon y Cajal's staining.
- Fig. 9. The normal myelin sheath fifty days after section of the tendon of gastrocnemius muscle, in guinea-pig. Weigert's staining.
- Fig. 10. The excessively regenerated nerve fibres in the muscle spindle fifty days after forcible binding of the limb of guinea-pig by plaster cast for fifteen hours. Ramon y Cajal's staining.

PLATE II

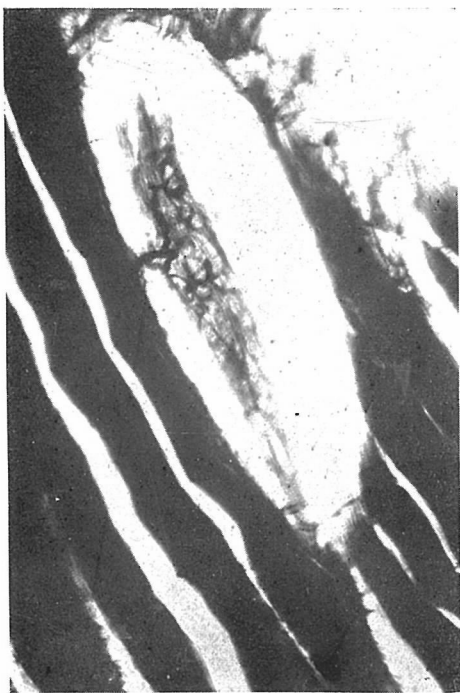


Fig. 8

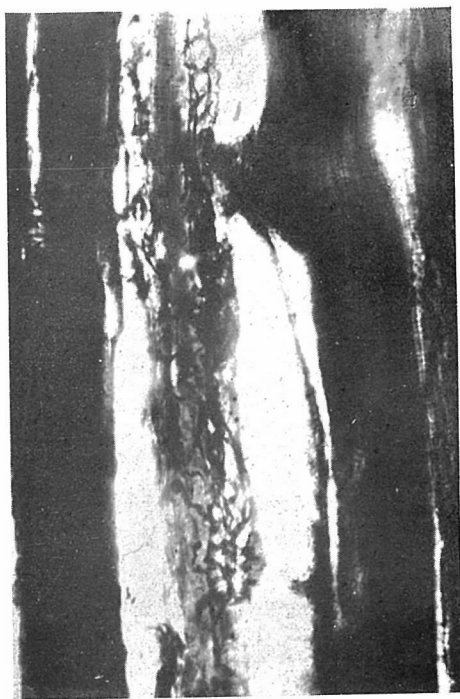


Fig. 10

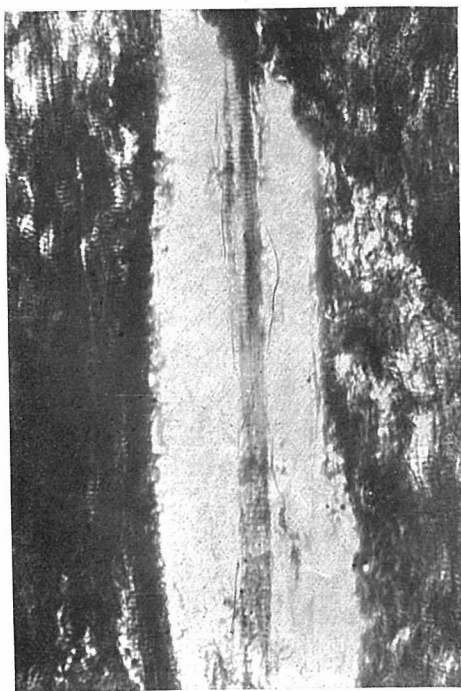


Fig. 7



Fig. 9