THE EFFECT OF DENERVATION ON THE REGENERATION OF SKELETAL MUSCLE AFTER INJURY

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In 1865 Waldeyer pointed out that regeneration of skeletal muscle can occur after injury. Since then, others have shown that muscle regeneration may follow various types of damage including trauma (Weber 1867, Neumann 1868), ischaemia (Clark and Blomfield 1943), and toxic degeneration (Hoffman 1867, Forbus 1926). More recently the details of the histological processes involved have been described by Clark (1946).

Although it has been established that some degree of muscle repair can take place in the complete absence of innervation, as in the case of transplanted muscle tissue (Jores 1909, Clark 1946), the present investigation was undertaken to compare the histological processes and end-result of regeneration of completely denervated muscle with the corresponding findings in innervated muscle.

It is of some clinical importance to know the response of denervated muscle to injury. In some types of peripheral nerve injury, and in other lesions affecting the lower motor neuron, muscle may be denervated for long periods. Can it be expected that the denervated muscle will show an unusual response to trauma? The delicacy with which denervated muscle has been treated in the past seems to be based on a vague assumption that it is either fragile or likely to respond abnormally to damage. The present experiments were carried out to determine how far that assumption is justified.

METHOD

The rat was chosen as a convenient experimental animal. To allow comparison between different lesions, a standard muscle crush, by the technique of Clark, was employed. Under anaesthesia, the whole breadth of the muscle was crushed transversely with smooth-bladed artery forceps, producing a band of damage three millimetres wide. Histological study shows that this injury produces complete necrosis and fragmentation of muscle fibres, but that retraction of the adjacent undamaged muscle is prevented by the connective tissue stroma that remains in the crushed area. The gastrocnemius muscle was used in preference to the gracilis chosen by Clark because the small bulk of the gracilis made its identification difficult after prolonged denervation. In each experimental animal the gastrocnemius of one limb was denervated by division of the sciatic nerve in the thigh. One centimetre of nerve was excised to prevent, so far as possible, regeneration during the experiment. Three weeks later the gastrocnemius in each limb was crushed in its lowest third. Animals were killed at intervals from two hours to thirty-two weeks after injury, and the crushed muscles were examined histologically. In each animal the normally innervated muscle provided a control for comparison with the denervated one. In all, eighty-six injured muscles were examined.

The muscles were fixed in Helly's fluid, and sections were stained with haematoxylin and eosin, and by the tartrazine and lissamine fast red technique (Lendrum 1947). A few of the normally innervated muscles were fixed in 10 per cent formol-saline so that sections could also be stained to show nerve fibres and myelin sheaths.

INJURY OF NORMAL MUSCLE

Regeneration of normal muscle—Necrosis of muscle fibres and the development of granulation tissue are the earliest findings, although as early as the fourth day after injury
Fig. 1
Histological appearance of normal rat muscle (gastrocnemius) (x 220).

Fig. 2
Histological appearance of gastrocnemius muscle three weeks after section of sciatic nerve (x 220).
FIG. 3
Normal muscle two days after crush. In the crushed area sarcolemmal tubes are occupied by necrotic muscle fibres and numerous phagocytic cells are present (× 230).

FIG. 4
Denervated muscle two days after crush. A similar field to Figure 3, but showing almost complete phagocytosis of the necrotic muscle fibres (× 230).

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regenerating muscle fibres grow into the area of damage. When the area of damage is small, as in the present experiments, the regenerating fibres can completely repair the gap, fibrous granulation tissue disappearing in from two to five weeks. After this time the site of damage is indicated only by the irregular arrangement of the muscle fibres crossing the former gap. The following is a more detailed account of the progress of muscle repair as it was seen in the specimens of normal muscle.

**Immediate response**—Sections of muscles removed two hours after the crush show complete interruption of all muscle fibres, the two parts being united only by connective tissue. Haemorrhage has occurred in the crushed region, and muscle fibres here are necrotic. No other significant abnormality is found.

Two days after the injury the zone of necrosis is clearly demarcated. Active phagocytosis of the necrotic fibres is well advanced, and these have become separated from the adjacent normal muscle (Fig. 3). No regeneration of muscle fibres is seen. The central part of the crushed area is occupied by necrotic muscle fibres and blood-clot, and polymorphonuclear leucocytes are conspicuous between the muscle fibres and around small blood vessels. Mononuclear inflammatory cells, including lymphocytes and macrophages, are also present. At the periphery of the crushed area other changes are found. Some empty sarcolemmal tubes are seen, where the muscle fibres have been phagocytosed by polymorphs and macrophages. At the margin of the crush vascular granulation tissue is developing, and there is some proliferation of fibroblasts between the muscle fibres, with numerous mitoses among the nuclei of these cells.

**Four days**—By the fourth day, specific muscle regeneration has become conspicuous. The muscle fibres at the margin of the area of damage send out slender strap-like protrusions across it (Fig. 5). These new fibres are more basophilic than normal muscle; they show longitudinal myofibrils but no cross-striation at this stage. Their numerous nuclei are frequently arranged close together in a longitudinal string. Mitoses are not seen in the regenerating muscle fibres, although they are present in the fibroblastic tissue between them.

The necrotic muscle fibres have now almost all been removed. Numerous inflammatory cells are present, and there is conspicuous fibroblastic proliferation, not only in the crushed area, but between intact muscle fibres at the margin. The central part of the crush consists of vascular granulation tissue.

It has now become apparent that the degree of damage is not the same at all points in the crushed area. In some regions the crushed sarcolemmal tubes remain more or less in continuity, while in others they are completely destroyed by the injury. The behaviour of regenerating muscle fibres appears to be governed by the state of the sarcolemmal tubes. Where a tube is present the fibre is guided across the crushed area along its original path, and if the tube extends without interruption across the zone of damage the regenerating fibres may have bridged this gap completely by four days. In other areas, where sarcolemmal tubes have been destroyed, the regenerating fibres grow irregularly into fibrous granulation tissue, and have covered considerably less distance than those growing within sarcolemmal tubes.

**One week**—At one week there is further outgrowth of new strap-like fibres, and more of them have bridged the crushed area. At this stage regenerated fibres may show cross-striation as well as longitudinal fibrillation, and show some increase in diameter. Some of the new fibres, particularly at the margins of the crush, appear surprisingly mature. Fibroblastic proliferation is conspicuous and in areas where sarcolemmal tubes are absent may be so extensive as to obscure scattered slender muscle fibres (Fig. 7), especially when the plane of section does not allow their continuity with normal muscle fibres to be traced. The new muscle fibres growing into granulation tissue possess, as yet, no sarcolemmal sheaths, and lie free in the tissue spaces; they frequently show bulbous, club-like terminal enlargements, which are most noticeable in areas where the fibres appear to meet most resistance from the
FIG. 7
Normal muscle one week after crush. Regenerating muscle fibres extending into granulation tissue, and showing multi-nucleated terminal enlargements (× 210).

FIG. 8
Denervated muscle, one week after crush. Regenerating fibres extending into granulation tissue (× 210).
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FIG. 9
Normal muscle two weeks after crush. Numerous strap-like fibres extend into the crushed area, and proliferating fibroblasts are present between them (× 190).

FIG. 10
Denervated muscle, two weeks after crush. Again regenerating muscle fibres and proliferating fibroblasts are seen (× 190).
FIG. 11
Normal muscle three weeks after crush (x 190).

FIG. 12
Denervated muscle three weeks after crush. In this, as in Figure 11, the area of the crush is occupied by an interlacing network of regenerated fibres (x 190).
FIG. 13
Normal muscle eight weeks after crush. An irregular network of muscle fibres is all that marks the site of the crush. Most of the fibres are of normal diameter, and show normal cross-striation; fibroblastic tissue is much diminished, but a few fibroblastic nuclei can still be identified between the muscle fibres (x 220).

FIG. 14
Denervated muscle, eight weeks after crush. The regenerated fibres are slender, but they show clear cross-striation (x 220).
granulation tissue into which they are advancing (Fig. 7). In one specimen, for instance, these bulbous terminations were very numerous where a tendinous intersection lay across the path of the regenerating fibres. Their distribution is consistent with Clark’s suggestion that these enlargements are produced by obstructions to the outflow of muscle cytoplasm. At one week the muscle shows a less intense inflammatory reaction than at four days. Two to five weeks—During this period the effectiveness of repair increases (Figs. 9 and 11). More and more fibres bridge the gap, and they take on an increasingly mature appearance. Their cross-striation is now uniformly developed, and most of them have lost the basophilic staining of the earlier stages. In a few areas, however, slender fibres, identical with those present at an earlier stage of regeneration, are still seen, but these diminish in number as the weeks pass. Fibroblastic granulation tissue, so conspicuous at one week, also decreases until, by five weeks, usually only a few small areas are present.

Where sarcolemmal tubes remained intact, the structural pattern of the new fibres is normal, but where they were destroyed the fibres are irregularly woven together (Fig. 11). New sarcolemmal tubes are found around regenerated fibres, and appear to be formed simply by condensation of fibroblasts and collagen fibres on the surface of the muscle fibres. Later stages (six to thirty-two weeks)—After about six weeks the site of the crush has usually become inconspicuous, both to the naked eye and on histological examination (Fig. 13). It is sometimes impossible to detect the slightest abnormality in the previously damaged area, even though in some cases it had been marked by placing a thread at the time of injury. More often the area is identified by some irregular interlacing of the otherwise parallel muscle fibres. An occasional regenerated fibre may still have more than the normal number of nuclei, and in one six-week specimen a few slender regenerating fibres were still present. Occasionally small areas of residual fibrous tissue may be found.

INJURY OF DENERVATED MUSCLE

Histology of denervated muscle—After section of the sciatic nerve, the gastrocnemius muscle shows progressive atrophy (Figs. 1 and 2). The diameter of individual muscle fibres is greatly reduced, and sarcolemmal nuclei—whether by active proliferation, or by mere shrinkage of fibres—become more conspicuous than in normal muscle. Longitudinal myofibrils and cross-striation persist. Individual fibres show some variation in the extent and rate of atrophy, but by three weeks the loss in volume of the denervated muscle is considerable; most fibres show pronounced histological changes, and Wallerian degeneration of the nerves is complete. For these reasons, three weeks was chosen as the period of denervation at which to crush the muscle and to investigate repair. After this period the denervated muscle shows continued wasting and atrophic change. Eventually fibrosis develops and few muscle fibres remain.

Regeneration of denervated muscle—The general process of muscle repair as seen in sections of denervated muscle after crushing injury is the same as already described in the normal control preparations. As the following descriptions show, only minor differences can be found when the two groups are compared.

Immediate response—Apart from the presence of denervation atrophy, the picture in the hours following injury—interruption and necrosis of muscle fibres, and haemorrhage in the crushed region—is like that in normal muscle.

After two days, phagocytosis of muscle fibres is taking place (Fig. 4). It appears to be more complete than in the normal muscle, presumably because of the smaller bulk of the necrotic fibres. Empty sarcolemmal tubes are present, and there is some formation of fibroblastic granulation tissue, but no evidence of regeneration of muscle fibres is found.

Four days—As with the normal muscle, the denervated specimens show active growth of slender regenerating fibres into the damaged area by four days. The denervated fibres from which these arise are more slender than normal fibres, but the outgrowths have the same
FIG. 5
Normal muscle four days after crush. Newly developed strap-like fibres streaming into the crushed area. Here regeneration is occurring in an area of intact sarcolemmal tubes (×210).

FIG. 6
Denervated muscle four days after crush. A field from the margin of the crush, showing the continuity between the narrow regenerated fibres and the thicker undamaged fibres. Here regeneration is occurring in an area of intact sarcolemmal tubes (×210).
histological appearance as those developing in normal muscle. In some areas the new fibres grow along sarcolemmal tubes (Fig. 6) and, as was noted with normal muscle, it is in these areas that their rate of growth is greatest. Phagocytosis of necrotic fibres is still active and, as at the two-day stage, it is more complete in the denervated muscle than in the normal.

One week—At this stage, many of the newly developed fibres of the denervated muscle have bridged the crushed area. The extent of their outgrowth depends, as does that of regenerating fibres in normal muscle, on the extent of disorganisation of sarcolemmal tubes (Fig. 8). Occasional new fibres show cross-striation, and when the path of the growing fibres is blocked by fibrous tissue the same bulbous club-like terminal enlargements that are found in normal muscle are produced. As in the normal controls, fibroblastic proliferation is conspicuous at this stage.

Two to five weeks—More and more of the crushed area becomes occupied by newly developed fibres, and fibroblastic tissue becomes less conspicuous. The course exactly resembles repair in the normal muscle (Fig. 10), except that the maturing fibres achieve only the thickness of the surrounding denervated muscle fibres.

Later stages (six to thirty-two weeks)—After six to eight weeks it is often difficult or impossible to recognise the region of the crush because of extreme atrophy of muscle fibres. As part of the denervation changes, the entire muscle may show some degree of fibrosis by the end of this period. If, however, the site of the crush can be recognised, it is distinguished, as in the controls, by irregularly interlacing muscle fibres (Fig. 12). In one thirty-two-week specimen the site was clearly indicated by this feature.

DISCUSSION

During the course of these experiments we studied a paper by Kirby, who we found had demonstrated in 1892 the regeneration of muscle fibres under conditions of complete denervation. Using rabbits, Kirby produced ischaemic necrosis of part of the gastrocnemius by ligating it for three hours, and investigated the regeneration of muscle fibres into the necrotic area. He found that previous severance of the sciatic nerve did not retard regeneration. We bring forward our own findings, however, because they allow a more detailed comparison of the cellular processes of muscle repair in normal and denervated muscle. Moreover, in spite of the interest in muscle regeneration that has been aroused by the recent papers of Clark and Blomfield, few people are aware of Kirby’s earlier work in this field.

The experiments described in the present paper show that, after a simple crushing injury, the fibres of a totally denervated muscle regenerate in the same manner as those of normal muscle. Moreover, the final effectiveness of the muscle repair is the same in the denervated tissue as in the normal, and the intermediate stages of repair are exactly comparable in the two groups.

The active repair of denervated fibres plays its part even in a crush injury of normal muscle, because in such lesions nerve stains revealed extensive nerve damage in the neighbourhood of the crush. Many of the regenerating muscle fibres must have been without their nerve supply.

Both in normal and denervated muscle, our findings are consistent with the view that the regeneration of muscle fibres takes place from the ends of damaged fibres adjacent to the crushed area. No evidence of metaplastic formation of muscle fibres from connective tissue cells was seen.

Again, both in normal and in denervated muscle, the direction and the rate of regeneration of individual muscle fibres depend alike on the presence or absence of intact sarcolemmal tubes. In their presence, a normal pattern of muscle fibres is quickly re-established, and regenerating fibres grow as much as three millimetres in two days or less (that is, between the second and fourth days). Where sarcolemmal tubes are destroyed, and the regenerating
fibres encounter vascular granulation tissue, an irregular pattern of interlacing muscle fibres results, and the effective rate of repair is much less. In these experiments the superficial part of the crushed muscle, over a distance rather greater than the crush itself, often showed the less severe type of injury with preservation of sarcolemmal tubes and in these areas rapid and effective regeneration had taken place. In the early stages of the work it was not clear whether these slender fibres on the surface of the innervated muscle specimens near the crush represented regenerated or denervated fibres. But the fact that they were conspicuous within one week of injury, when denervation atrophy could not have accounted for fibres of such small diameter, as well as their presence in the previously denervated specimens, established that they were regenerated fibres. Moreover, the presence of regenerating fibres, growing rapidly within sarcolemmal tubes as early as four days after injury, explained the method of their formation.

Both in the normal and denervated muscle, the fibroblastic tissue that develops during the first week after the crush soon disappears. At a later stage, some fibrous tissue may develop in the denervated muscle as part of its progressive atrophy, but the "scar" in the normal muscle consists only of regenerated muscle fibres.

In these experiments, as in those of most other writers on the subject, mitoses of muscle nuclei have not been observed, although the regenerating fibres contain large numbers of nuclei which are often strung end to end along their length. In contrast, the connective tissue surrounding the regenerating muscle fibres contains many mitoses in the days following the crush. Surprisingly enough, the regenerated fibres, both in normal and in denervated muscle, develop conspicuous cross-striation at an early stage in their maturation.

SUMMARY

1. The repair of a simple crush injury was studied in rats, in both normally innervated and completely denervated muscle. In each case the histological findings at periods from two hours to thirty-two weeks are described.
2. The denervated muscle showed active and effective repair.
3. A comparison with the findings in normally innervated muscle establishes that the cellular processes of repair do not depend on connections with the central nervous system.

REFERENCES


