RECOVERY IN PARTLY PARALYSED MUSCLES

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Until recent years it was widely held that damage to part of the nerve supply of a voluntary muscle had a permanent effect and that the surviving nerve fibres were unable to reinnervate muscle fibres deprived of their nerve supply. It was assumed that the only possibility of recovery of power in the muscle was by hypertrophy of muscle fibres whose nerve supply had escaped damage.

The problem has recently been approached by both physiological and histological methods. Van Harreveld (1945) showed that the contraction force of incompletely denervated muscles begins to improve about two weeks after operation and increases for six months. Edds (1950) and Hoffman (1950), in histological studies of rat muscle incompletely denervated by division of anterior primary rami, found evidence of new fibres retracing the empty neurilemmal tubes to the denervated motor end-plates. That a similar process occurs in skin partly deprived of its sensory nerve supply has been shown by Weddell, Guttman and Gutmann (1941).

It has not yet been generally accepted that surviving axons in incompletely denervated muscle can extend their existing field of innervation by producing new fibres. The problem is of great practical importance, for if this process takes place after an attack of poliomyelitis, and if ways could be found to promote it, a real advance in treatment would be at hand. The aim of this study has been to obtain further histological evidence of the extent and duration of this process: limb muscles of the rabbit have been examined for evidence of the development of new fibres for periods of up to three months after incomplete denervation.

MATERIALS AND METHODS

The extensor digitorum longus muscle of the rabbit was chosen because it is particularly suitable for incomplete denervation. The muscle is supplied by two branches of the peroneal nerve. Incomplete denervation of the muscle was effected by division of one or other of these branches. Precautions were taken to eliminate the possibility of regeneration along the divided nerve. Usually the upper branch, which is the smaller, was divided and the proximal stump crushed and implanted into a neighbouring muscle belly, as far from the distal stump as possible. Care was taken not to handle or damage the blood vessels which enter the muscles with the nerve, thus eliminating the possibility of ischaemic effects on regeneration (Allbrook and Aitken 1951). To divide one of the nerves entering the muscle, the muscle belly was lifted from its bed by a clip placed round the tendon of origin. It seemed possible that damage might be caused to the remaining nerve supply by thus lifting the muscle. In a number of animals mock operations were done, reproducing as exactly as possible the operative conditions without dividing the nerves.

The muscles were removed at periods from two days to three months after operation. They were fixed in a solution containing 10 per cent formalin and 2 per cent pyridine for a minimum period of ten days. Each specimen was cut into two blocks of approximately equal size. The upper and lower tendinous parts were rejected. Each block was cut into as many sections of 50 μ thickness as possible. All suitable sections were stained by a modified Bielschowsky silver impregnation technique (Aitken 1950). At least forty sections were prepared from each muscle examined.

OBSERVATIONS

When either of the nerves to the extensor digitorum longus was divided sections made two or three days later showed: 1) intact sprays of fibres approaching motor end-plates;

2) sprays with complete degeneration of all fibres; 3) sprays with one or few surviving fibres, the remainder degenerated; and 4) sprays with one or few degenerated fibres, the remainder intact.

When the upper nerve branch was divided the degenerating nerve fibres were mainly confined to the upper third of the muscle. In the remaining two-thirds the nerve fibres and end-plates were mainly intact. A few intact fibres and end-plates were found in the upper third, and similarly a few degenerate fibres in the lower two-thirds. Scattered throughout the muscle were sprays of fibres in which varying numbers of fibres had degenerated, as in

FIG. 1

FIG. 2

Figure 1—Schematic representation of the individual axons in the upper and lower nerve branches to the extensor digitorum longus each supplying groups of muscle fibres. Figure 2—To show an axon in the lower nerve branch supplying muscle fibres in widely separated parts of the muscle.

FIG. 3

FIG. 4

Figure 3—To show intact sprays of motor endings and sprays with complete degeneration of all fibres when the upper nerve branch is divided. Figure 4—The upper nerve branch has been divided; intact and degenerate fibres lie side by side. This is a direct consequence of the intermingling of individual axons within the muscle (cf. Fig. 7).

Figure 6, which shows a terminal spray with one surviving fibre and end-plate. There was no sharp boundary line between the parts of the muscle supplied by the two nerve branches.

These findings indicate that the intramuscular ramifications of the two nerve branches entering the muscle intermingle freely in such a way that it is possible for one parent fibre to innervate muscle fibres in widely different parts of the muscle (Figs. 1 to 4). The motor unit is therefore not confined to single groups of neighbouring muscle fibres, and the individual end-plates in a given zone of the muscle are frequently derived from different parent axons (Adrian 1925, Cooper 1929, van Harreveld 1946, Feindel, Hinshaw and Weddell 1952).

Although these findings have wide implications as regards the structure of the motor unit, the central fact as regards this investigation is that after incomplete denervation by this method there were areas in the muscle where intact and degenerate fibres lay side by

FIG. 5

Several terminal bundles of fibres and end-plates arising from an intramuscular nerve trunk as seen in the normal extensor digitorum longus.

FIG. 6

Three days after incomplete denervation. Terminal spray of fibres with one surviving fibre and end-plate. *n.ep*—normal end-plate; *d.n.f.*—degenerating nerve fibres.

FIG. 7

Three days after incomplete denervation. Empty neurilemmal tubes and motor end-plates are clearly seen. In areas such as this, where normal and degenerated fibres lie side by side, new fine fibres grow out and reinnervate the degenerated end-plates. *e.n.t.*—empty neurilemmal tube; *n.ep.*—normal end-plate; *e.ep.*—empty end-plate.

FIG. 8

Four days after incomplete denervation. New fine fibre reinnervating a denervated end-plate. Another denervated end-plate lies immediately adjacent. *ne.f.*—new fibre; *e.ep.*—empty end-plate.

FIG. 9

Four days after incomplete denervation. Reinnervation of motor end-plates by fine collateral fibre. The absence of "claws" in the reinnervated end-plate contrasts sharply with normal end-plate. *no.f.*—normal fibre; *n.ep.*—normal end-plate; *ne.f.*—new fibre; *r.ep.*—reinnervated end-plate.

FIG. 10

Seven days after incomplete denervation. Normal fibre and end-plate accompanied by new fine fibre reinnervating a denervated end-plate. Contrast between normal and reinnervated end-plate is striking. *n.ep.*—normal end-plate; *no.f.*—normal fibre; *ne.f.*—new fibre; *e.ep.*—empty end-plate.

side (Fig. 7). This was a frequent finding in muscles up to a week after incomplete denervation. Thereafter degenerate fibres were more difficult to find, though they were observed as late as thirty days after operation.

Growth of new fibres—Figure 5 shows a normal spray of fibres all intact, and Figure 6 a terminal spray with one surviving fibre and end-plate in a spray. It is in these areas where degenerate and intact nerve fibres lie in close proximity that new fine fibres appear and reinnervate the old end-plates (Figs. 8 to 16). This observation is in accord with those of other workers (Edds 1950). Very fine fibres appear as early as four days after incomplete denervation, and reinnervate denervated end-plates. The point of branching of the residual axons is usually difficult to determine, for they appear to come from far back in the nerve bundles. Nevertheless some preparations show the point of branching (Fig. 16). These fine fibres are not seen in the normal extensor digitorum longus. Terminal outgrowths sprouting from the

FIG. 11

Seven days after incomplete denervation. New fibre passing down swollen neurilemmal sheath to reinnervate end-plate. *ne.f*—new fibre; *r.ep*—reinnervated end-plate.

surviving end-plates and reinnervating neighbouring muscle fibres (Hoffman 1950) were not seen in these preparations. No signs of new fibre formation were seen in areas of the muscle containing only denervated nerve bundles. It has previously been recognised that the growth of new fibres occurs only in areas where degenerate and intact fibres are closely intermingled. The present investigation showed this important fact more clearly than those in which incomplete denervation is produced uniformly throughout the muscle by ramisection.

The following criteria were adopted in deciding which were new fibres and which were normal ones. 1) *Fibre size*: the contrast between the diameter of normal fibres approaching end-plates and new fibres appearing among them is marked. There is some variation of fibre size in normal muscles and therefore careful examination of the motor end-plate as well is important. 2) *Character of the motor end-plates*: there is a striking difference between the normal and the reinnervated motor end-plates. Gutmann and Young (1944), in a detailed

FIG. 12

Seven days after incomplete denervation. New fine fibre following old neurilemmal pathway to denervated end-plate. *ne.f.*—new fibre; *r.ep.*—reinnervated end-plate.

FIG. 13

Ten days after incomplete denervation. New fine fibre showing proximal thickening. *ne.f.*—new fibre; *r.ep.*—reinnervated end-plate.

FIG. 14

Ten days after incomplete denervation. Spray of fibres showing one normal fibre and end-plate, and two new fine fibres ending in motor end-plates. *n.ep*—normal end-plate; *r.ep*—reinnervated end-plate; *ne.f*—new fibre.

FIG. 15

Thirty days after incomplete denervation. New fine fibre branching off parent axon and entering empty end-plate. *ne.f*—new fibre; *r.ep*—reinnervated end-plate.

histological study of the myoneural junction in normal rabbit muscle, found the average number of terminal branches of the axon at the end-plate to be 5.8, with extremes of 2 and 11. These terminal branches, often referred to as "claws," are absent in the reinnervated end-plate (Fig. 9).

Using these criteria, it can be shown that production of new fibres is already evident four days after incomplete denervation of a muscle. During the succeeding weeks signs of reinnervation of the old end-plates are seen. It is important to decide whether all the new sprouts are formed in the days immediately after operation or whether more may be added later. When they are first formed the new fibres are very thin and presumably they increase in diameter later; the number of branches in the end-plates also increases. Unfortunately the rate at which these processes of maturation proceed is not known accurately. The number of fine fibres and simple end-plates seen is greatest during the first two weeks after operation,

FIG. 16

Forty days after incomplete denervation. Very fine fibre branching from parent axon and entering an empty end-plate. *ne.f*—new fibre; *r.ep*—reinnervated end-plate.

and seemingly declines thereafter. By the fourth week a few small fibres are still present, but none were seen later than the sixth week. These observations suggest that most if not all of the new sprouts are formed soon after the operation, and that they subsequently undergo growth and maturation.

DISCUSSION

Previous workers have examined muscles incompletely denervated by division of anterior primary rami. Their findings have been largely confirmed in this study in which incomplete denervation was accomplished by a peripheral method. This method results in a different pattern of denervation from that produced by ramisection, the chief difference being in the extent of the intermingling of intact and degenerate fibres, which occurs evenly throughout the whole muscle after ramisection. Sprouting of new fibres is therefore more extensive in muscles incompletely denervated by ramisection.

Growth of new fibres occurs only in areas where degenerated and intact fibres are closely

intermingled. So far this is the only observation that gives a clue to the stimulus required to promote the formation of new fibres. Now that it is established with certainty that collaterals can sprout from intact axons discovery of the stimulus required becomes of increasing importance. Since the growth of new fibres is delayed for three or four days after denervation, this may represent the time taken for the stimulus to develop. It is possible that striated muscle undergoes a rapid chemical change which provides the stimulus almost immediately, but that regeneration is prevented until the neurilemmal pathways are cleared of myelin. Degenerating myelin is cleared rapidly from the finer nerve bundles in partly denervated muscle. Examination of material three or four days after operation shows many empty neurilemmal tubes.

Work has been published suggesting the possibility that the stimulus is chemical. Van Harreveld (1947) studied the effects of an ether-extract of denervated muscle injected into the fascia surrounding the partly denervated sartorius muscle of the rabbit, and claimed that this caused more rapid recovery of the strength of contraction. Extract of normal muscle was ineffective. Hoffman (1950) injected extracts of denervated and normal rabbit muscle into incompletely denervated rat muscles. He found that both extracts produced blindly ending outgrowths from nerve fibres and end-plates.

Hoffman believed the stimulus to be the degenerating myelin present in the muscle after incomplete denervation, a view that is supported by the observation that regeneration occurs only when degenerated and intact fibres lie close together. He carried out a series of experiments two of which are of special interest. First, injection of ether-extract of white matter from ox brain caused a considerable increase in the number of outgrowths from intact axons, whereas extract of grey matter was ineffective. Secondly, ether-extract of yolk of egg was especially effective: in some regions of the muscles examined up to 50 per cent of the end-plates produced long blindly ending outgrowths. Moreover it is tempting to correlate the observation that degenerating myelin is rapidly cleared from the muscle with the finding that growth of new fibres is probably at its greatest soon after incomplete denervation.

So far there has been a large measure of agreement between independent workers using the rat and rabbit for their experimental work. When attempts are made to correlate this work with the work on primates and on poliomyelitis in man difficulties appear. Edds and Small (1951), working on the monkey, found that collateral regeneration of residual intramuscular axons occurred only to a limited degree after slight denervation and did not take place at all after extensive denervation. The concept of the nervous system as a static structure has undergone a radical change in recent years, and this growth of new fibres within partly denervated muscle is an excellent illustration of the lability of nervous tissue. It may be, however, that in higher animals this lability is diminished. Only further work can provide the answer to this.

Human muscle affected by poliomyelitis can continue to show an increase of power up to one year or more after the onset of the disease. This suggests that if sprouting of collaterals from intact axons is responsible for recovery, it must occur for at least a year after incomplete denervation. This does not accord with the observations in the rat and rabbit. Van Harreveld (1947) claimed that the power of contraction in the sartorius and quadriceps muscles of the rabbit continued to increase up to six months after incomplete denervation. This might be explained in two ways: sprouting of intact axons and hypertrophy of muscle fibres. Van Harreveld concluded that the first mechanism was the most important, but measurement of the "mean fibre surface" of the muscle fibres showed it to be greater on the side operated upon than on the other side, and he therefore considered hypertrophy of muscle fibres to be a definite but subsidiary factor in the recovery of the muscle. Weiss and Edds (1946) measured the greatest fibre size (diameter) in partly denervated muscles to decide whether hypertrophy played a part in recovery, but they failed to show any increase in size as compared with normal controls.

In assessing the possible importance of these mechanisms in the recovery of human muscles after poliomyelitis it must be remembered that the situation is complicated by the treatment given, for there is a possibility that regular exercises cause hypertrophy of the remaining muscle fibres in a paretic muscle.

The elucidation of the whole problem must await the discovery of the true stimulus to branching of intramuscular axons and whether this stimulus is capable of producing its effect in the partly paralysed primate muscle.

SUMMARY

1. The extensor digitorum longus of the rabbit was partly denervated by section of one of its two nerve branches and examined histologically for evidence of sprouting of new fibres.
2. Sections from material fixed two and three days after operation showed terminal bundles in which varying numbers of axons and motor end-plates have degenerated. This supports the concept that the motor unit is not confined to single groups of neighbouring muscle fibres, but innervates fibres scattered throughout the muscle.
3. New fine fibres branching from intact intramuscular axons to reinnervate denervated muscle fibres were observed as early as four days after operation.
4. Such new fibres were most numerous in the early weeks after operation and their numbers then declined. Two months after operation no small fibres or simple end-plates were seen.
5. No new fibres were seen in areas of the muscle containing only denervated nerve fibres. The new fibres were formed only under the stimulus of proximity to the degenerating ones.
6. The relationship of these findings to the mechanism of recovery of human muscle affected by poliomyelitis is discussed.

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