**THE ADAPTIVE RESPONSE OF SKELETAL MUSCLE: WHAT IS THE EVIDENCE?**

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ABSTRACT

Adult skeletal muscle is capable of adapting its properties in response to changing functional demands. This now sounds like a statement of the obvious, and many people assume it has always been this way. Yet a mere forty years ago the picture was entirely different. In this review and personal memoir I outline the scientific context in which the theory was generated, the objections to it from entrenched opinion, and the way those objections were progressively met. The material should be of some historical interest, but more importantly it collects together the full range of evidence on which the current paradigm is based.

**Key words:** stimulation, hypothesis, adaptive, transformation, plasticity, trophic, history

BACKGROUND

Plasticity.Unlike most highly differentiated tissues skeletal muscle has a significant capacity for further change even after the adult state has been attained. The property was discovered by Buller, Eccles & Eccles in the course of experiments in which the motor nerves supplying predominantly fast (Type 1) and predominantly slow (Type 2) muscles were interchanged.1 These authors, and others who adopted the same experimental model, found that each cross-reinnervated muscle tended to acquire the original physiological and biochemical characteristics of the other, even in adult animals (Fig. 1a).

**FIGURE 1 NEAR HERE**

The changes were unexpected and surprising, because they were observed in a mature organism, in cells that had already undergone differentiation. Behaviour such as this is frequently referred to as ‘plasticity’. The explanation offered was that the muscles had responded to the influence of chemical trophic factors transported to them along the motor nerves.1,2 The existence of a perpetual proximal-to-distal axonal flow, as demonstrated in the classical experiments of Paul Weiss,3 provided a plausible mechanism for conveying such substances to the muscles.

The chemotrophic hypothesis was well received and widely accepted. There was, however, an alternative to this explanation.

Impulse activity.Organisms use their fast and slow muscles in different ways. Slow muscles tend to be employed in the maintenance of posture, fast muscles for rapid, forceful contractions. There are corresponding differences in the impulse traffic delivered to them by their motor nerves: slow muscles typically undergo sustained low-frequency impulse activity (also known as tonic activity), whereas fast muscles respond to intermittent bursts of high-frequency activity (also known as phasic activity).4,5 Could these differences be responsible for the effect of exchanging the motor nerves (Fig. 1b)?

Vrbová investigated what would happen if the impulse traffic reaching the slow soleus muscle was abolished completely, something that could be achieved by transecting the spinal cord and dividing the muscle tendon. Under these conditions the muscle became faster-contracting. Although this observation was suggestive of an influence of impulse activity on muscle properties it was criticized on the grounds that the muscle was rendered severely atrophic by this procedure, and the results were therefore influenced by contraction of adjacent fast muscles.6 Proponents of the chemotrophic explanation remained unmoved.

I was introduced to this topic by Ricardo Miledi during a Master’s course in Physiology at University College London. However, I moved to the University of Birmingham in 1964 to undertake a PhD on the biotelemetric recording of muscle force in freely moving primates. I was developing the electronic instrumentation for this project when I stumbled across a circuit that was too unstable for its original design goal of generating single pulses, but could be persuaded to generate a low-frequency train of single pulses. What attracted my attention was the current drain, which was extremely low: the smallest electronic devices available at that time – hearing aids – lasted for only 36 hours; this circuit had the potential for lasting weeks or months on similar batteries. I immediately recognized the potential of such a device, which could be used for the converse of silencing a slow muscle, namely, imposing a postural pattern of activity on a fast muscle. Dr Vrbová had recently joined the Department of Anatomy and I suggested the experiment to her. She had two objections. First, these muscles would not be electrically silent: we would be superimposing impulse activity of the slow muscle type over the top of existing fast-muscle activity. Second, the animals in her experiments had been paraplegic, and therefore incapable of much movement; it would be difficult to maintain stimulation in an intact, conscious animal in any conventional way. To the first objection I responded that a continuous low-frequency pattern of activity should surely be more influential than intermittent, and possibly infrequent, bursts. To the second, I pointed out that my circuit could form the basis of a totally implantable stimulator, and this would make it possible to conduct the experiment in a freely moving animal. Interestingly, Buller, Eccles, and Eccles had, in their original paper, entertained the idea of imitating activity with electrical stimulation.1 They concluded ‘It would be very difficult technically to arrange artificial stimulation of several weeks duration so that there was a prolonged transposition of these characteristic frequencies of action, independently of nerve cross-union.’ Fortunately I had overlooked that paragraph, so, undeterred, I spent the next few months developing the stimulator.7

The results were unequivocal. Stimulating the motor nerve to a rabbit fast muscle for several weeks at 10 Hz – a tonic pattern similar to that normally found in the nerves to rabbit slow muscles – induced markedly slower contractile characteristics, even though the muscle itself had not been disturbed in any other way (Fig. 1c).8

Recognizing the importance of this observation, my supervisor, Eric Ashton, allowed me to pursue the topic for my PhD. When it came to writing the discussion I found I was dissatisfied with all the existing hypotheses. Vrbová had come closest when she attributed the influence of activity to the different firing frequencies of motor neurones supplying fast and slow muscles.9 From my survey of the background literature, however, it seemed that an adaptive mechanism would provide a more logical and economical explanation for all the observed phenomena. In such a model the emphasis would be on the aggregate amount of impulses delivered to a muscle rather than their frequency. Thus, sustained high levels of use would induce slow, fatigue-resistant properties; low or intermittent levels of use would allow the fibres to retain, or revert to, a native fast, fatigue-susceptible state. In this way a muscle could optimize its properties to suit the type of work normally demanded of it.10-13

Although experiments with the implantable stimulator had yielded striking results, published work continued to focus on the chemotrophic interpretation. Understandably, the scientific community was not ready to abandon the chemotrophic hypothesis, which carried, after all, the imprimatur of a Nobel prizewinner rather than that of a newly qualified PhD. Instead various attempts were made to reconcile the effects of electrical stimulation with the established view. We will now look at these alternative explanations and how they were addressed.

CHALLENGES TO THE ADAPTIVE HYPOTHESIS

Hypothesis: the effect of stimulation is muscle-specific. In the initial stimulation experiments changes in contractile speed had been demonstrated in rabbit tibialis anterior (TA) muscles, which are predominantly fast. There was a risk that these observations would be dismissed as muscle-specific. We pre-empted such objections by conducting similar experiments in the flexor digitorum longus (FDL) muscles of cats. These underwent essentially the same sort of change.8 Since that 1969 publication, adaptive transformation has been shown in TA, extensor digitorum longus, peroneus longus, latissimus dorsi, rectus femoris, rectus abdominis, gracilis, serratus anterior, and pectoralis major. Clearly the effect is not muscle-specific.

Hypothesis: the effects of stimulation are species-specific. Buller and his colleagues conducted their experiments in cats, whereas the effects of stimulation had been demonstrated in rabbits. But in the subsequent four decades the results have been reproduced in rat,14-16 cat,8,17,18 dog,19-23 goat,24,25 sheep,26-28 pig,29 and man.30,31. These species differ only in their sensitivity to increased activity. There is therefore no basis for proposing an inherent difference between mammalian species in the response to a change in use, and the basic mechanisms of adaptation appear to be the same.

Some authors repeatedly asserted that rat muscles had a more restricted adaptive capacity.32-36 It would be extraordinary if rat was the sole exception to what appears to be a universal response, and in fact it is not. Smaller mammals have faster muscles and normally activate them at higher frequencies, so they simply need a more demanding regime of activity to elicit the same degree of change.14,16

Hypothesis: the effects of stimulation are unrelated to cross-reinnervation. By the early 1970s there were many studies of the effects of cross-reinnervation on fast and slow muscles, and proponents of that model were inclined to regard the effect of chronic stimulation as a separate phenomenon, one that was incidental, and distinct from that of cross-reinnervation.

I had conceived of a way of testing this notion in 1970, but first I needed to improve the electronic and physical design of the stimulators and electrodes, and the surgical technique for implanting them. With these changes I could maintain stimulation reliably for 24 h/day over many months. The animals, which were of course unrestricted in their movements, behaved normally and increased in body weight, discounting the suggestion from some quarters that the procedure was stressful. (This was later supported by measurements of cortisone, the stress corticosteroid in rabbits, which declined, if anything, during the period of stimulation.37)

In 1972, a period spent in Elwood Henneman’s laboratory at Harvard Medical School gave me the opportunity of collaborating with Frank Sréter, John Gergely, and Flaviu Romanul. Our initial goal was to look for possible changes in the newly discovered myosin light chains. The result was striking: fast muscles that had been stimulated at 10 Hz for 4 weeks contained light chains of both fast and slow muscle types;38 after stimulation for 10 weeks slow light chains alone were present.39 These qualitative changes provided the first evidence that fully differentiated muscles could switch from the expression of one set of genes to another.

Encouraged by the way the work in Boston was going I returned to Birmingham in 1972 determined to embark on the project I’d long had in mind. In the first part of the experiment I stimulated the anterior tibial muscles in two rabbits continuously for 5 months (Fig. 1c).

The second part of the experiment involved two groups of rabbits. In one group I dissected out the branch of the common peroneal nerve that normally supplies the fast TA muscle, and anastomosed it to the nerve supplying the slow soleus muscle. This was a classical cross-reinnervation procedure, although to minimize the pull on the nerves I performed the cross in one direction only. The other group of animals underwent the same procedure, but in these animals a stimulator delivered impulses at 10 Hz to the cross-reinnervated soleus muscle in an approximation of its original physiological activity (Fig. 1d).

The results, which could now be based on a battery of physiological and biochemical measures, were clear cut. A long-term change in activity without a change in innervation produced effects greater than cross-reinnervation in every respect.11 Cross-reinnervation alone reproduced the results published previously by others. But under conditions in which cross-reinnervation was not accompanied by a change in activity, because stimulation had been used to maintain the original level of activity, no change in characteristics could be observed.11 Such a detailed complementarity of effect would be hard to explain other than in terms of an identical underlying mechanism.

This experiment, more than any other, was responsible for a shift in the mind-set of many – although not all – workers who had previously supported the chemotrophic theory.

Hypothesis: the effects of stimulation are mediated by activity-dependent secretion. The combined cross-reinnervation and stimulation experiment could not eliminate one possible defence of the chemotrophic theory. It could be argued that electrical stimulation of the nerve sent impulses back to the spinal cord and caused the motor neurones there to synthesize or release a ‘slow-nerve’ type of trophic substance. Indeed, this was an argument that H. Gonzalez-Serratos had taxed me with when I presented the initial results of chronic stimulation at the 1967 Oxford meeting of the Physiological Society.

There are two routes whereby peripheral stimulation could lead to increased activity in the motor neurone pool. The direct route is by conduction of the impulse back along the motor axons, so-called antidromic stimulation. The indirect route arises because motor nerves contain afferent as well as efferent fibres, which complete the stretch reflex arc. The latter, at least, could be eliminated as a mechanism. In 1973 Al-Amood and his colleagues stimulated ventral roots at 10 Hz in cats in which the dorsal roots had been cut, and measured the physiological changes in the FDL muscle.40 The results confirmed and extended the original observations on intact animals.8

Eliminating the antidromic route was more problematic, because interrupting the motor nerve (denervation) has a deleterious effect on the muscle, as is well known from both animal studies and clinical experience. Moreover the time course and extent of the consequent changes is strongly species-specific.41-43 For example, the extensive necrosis and regeneration seen in rat muscles within months of denervation is not apparent in rabbit muscles denervated for up to one year, nor in human muscles several years after a denervating injury. All such muscles nevertheless show severe atrophy and ultrastructural disorder. We should not, therefore, assume that a valid comparison can be made between the results of stimulating denervated and innervated muscles. This is particularly true for the rat, in which regenerative phenomena are a component of the response to denervation.41 All the same, experiments of this type have been carried out.

Technically, stimulating under these conditions is not easy. The electrical charge required to activate a denervated muscle is 100 – 500 times greater than that needed to activate an innervated muscle. In the 1970s this was beyond the scope of implantable stimulators. Some workers addressed the problem by using an external stimulator connected by flexible wires to the animal. This enabled them to observe a degree of type transformation in denervated rat muscles.44-46

Thirty years later Helmut Kern interested me in the therapeutic potential of stimulation for promoting the recovery of human muscles denervated by traumatic injury and we began a productive collaboration.47 Rabbit muscles appeared to be similar to human muscles in their response to denervation, and with the collaboration of bioengineering colleagues in Vienna, we were able to achieve stimulation of denervated muscles in rabbits with an implantable stimulator.48 The experiments were carried out on fast muscles with established denervation of 10 or 39 weeks’ duration, the idea being to model the clinical situation more closely. In contrast to stimulation of innervated muscles we were unable to produce a complete transformation from fast to slow, even after 12 weeks of stimulation. Instead the muscle acquired an intermediate pattern typical of the fast-oxidative 2A type without, however, an improvement in fatigue resistance.49 This condition may be akin to the prolonged intermediate stage described in the rat by Gundersen.46

The experiments in both rats and rabbits suggested that, over an extended time course, a limited degree of muscle type transformation could take place in the absence of the nerve. In my view, however, it would be incautious to interpret these results as definitive proof or disproof of the existence of ‘fast-nerve’ or ‘slow-nerve’ trophic factors.

A more convincing test would be to isolate the motor neurones from the effects of antidromic stimulation by implementing a long-term conduction block in the intact nerve. The block would be applied proximal to the stimulating electrodes, and additional electrodes would be implanted to monitor its efficacy. Although feasible, such an experiment would be technically difficult.

Hypothesis: the effect of stimulation is the result of internal cross-reinnervation. The chemotrophic hypothesis continued to be strongly defended during the 1970s. In the course of a plenary lecture at the 1972 World Neuroscience Congress, Lloyd Guth advanced two interesting theories to explain the effects of long-term stimulation without the need to depart from the original paradigm. One of these theories proposed an internal cross-reinnervation, in which the axons innervating fast motor units degenerated under conditions of stimulation and were replaced by the axons innervating slow motor units.

The rabbit TA muscle actually contains a very small proportion of slow fibres (1%–5%), so such a mechanism calls for extensive branching of small-diameter, slow motor axons, which appears inherently unlikely. Unlikely does not mean impossible, however, so we set about testing this hypothesis in two ways. Chris Morris and I used vital staining to demonstrate the axon branches and endplates in stimulated rabbit TA muscles. We found a normal pattern of terminal arborization and no increase in collateral sprouting.50 The extensive branching required by the proposition was not present.

The second test was a study of the reversibility of the effects of stimulation. Even if the radical increase in activity brought about by stimulation did result in extensive degeneration, branching, and redistribution of intramuscular axons it seemed unlikely that the mere cessation of stimulation would result in a reversal of these major structural changes. It would therefore be predicted that muscle composition would remain undisturbed after the removal of stimulation, with a predominance of slow fibres and evidence of type grouping. It did not. Our experimental work between 1975 and 1989 showed that cessation of stimulation resulted, over a period, in the complete recovery of the original physiological, histochemical, biochemical, and ultrastructural characteristics of the fast muscle.50-53

The internal reinnervation hypothesis could therefore be rejected.

Hypothesis: the effect of stimulation is the result of selective hypertrophy of slow muscle fibres. The second theory put forward by Guth for the effects of stimulation was that it brought about atrophy or degeneration of fast muscle fibres and compensatory hypertrophy of slow muscle fibres. This proposal was more easily disproved. Because rabbit TA muscles contain no more than 5% of slow fibres, such a mechanism would have to produce an abundance of very small or degenerated fast muscle fibres together with slow muscle fibres of enormous size. Histochemical sections showed no such appearance.13,19,51,52,54-57

In 1990 we were collaborating with Larry Stephenson on the use of transformed skeletal muscle grafts in cardiac assist. This gave us the opportunity of conducting a detailed investigation of fibre size in canine latissimus dorsi muscles (LDM) that had been subjected to chronic stimulation for one year. The control unstimulated LDMs contained a mixed population of slow and fast fibres in an approximate ratio of 30:70. The stimulated LDMs contained exclusively fibres of the slow muscle type. The fibre size distribution in the stimulated muscles remained Gaussian, and there was, if anything, a slight reduction in mean fibre size.23

When these observations are combined with the complete recovery of mixed type distribution following cessation of stimulation, this hypothesis too becomes untenable.

Hypothesis: the effect of stimulation is the result of generalized degeneration and regeneration. Thus far explanations based on the chemotrophic hypothesis had not been upheld because they made predictions that could be disproved experimentally. There remained, however, the question as to whether the stimulation-induced transformation took place within existing muscle fibres. The alternative possibility, brought to prominence by Maier and his colleagues in 1986, was a partial or complete degeneration of the muscle, leaving behind a population of satellite cells from which new slow muscle fibres were formed.58 This hypothesis was based on evidence of damage, amounting to as much as 25% of the muscle and affecting mainly fast fibres, when the muscle was subjected to 10 Hz stimulation for 12 h a day.58-60

Two questions need to be posed in relation to this issue:

1. Is damage of this extent an inescapable accompaniment of electrical stimulation?

2. Can type transformation be demonstrated in the absence of such damage?

In relation to the first question, studies in my own laboratory had revealed no histological evidence of widespread degeneration; indeed, rabbit muscles, which are more or less completely transformed to the slow type after 6 weeks of continuous stimulation at 10 Hz, were histologically normal at all intermediate stages. It was, however, important to seek a more definitive answer, because damage had been reported to be present at the very stage when regulatory events underlying gene re-expression, and the resulting mRNA synthesis, are known to occur. These phenomena are the subject of considerable research interest (see below) and if extensive damage were confirmed it would alter entirely the way observations in this period should be interpreted. In 1992 we decided to put the issue on a thorough quantitative basis, using statistically valid sampling protocols and multivariate analysis to take into account variation within the cross-section and length of the muscle as well as between muscles and subjects. We found that the volume percentage of degenerating fibres was 3.4 ± 3.8% (mean ± SD) for continuous stimulation, and 1.0 ± 1.0% for intermittent stimulation.55-57 There are possible explanations for the much higher incidence of stimulation-induced damage observed by Maier and colleagues, and these have been discussed elsewhere.61 However, the first question had received a clear answer: significant damage is *not* a necessary feature of chronic electrical stimulation.

Can type transformation be demonstrated in the absence of significant damage? As the TA muscle in the rabbit contains no more than 5% of Type 1 fibres, fast-to-slow type transformation based on degeneration would have to involve at least 95% of the fibres in this muscle. In our hands damage affected on average 3–4% of these fibres, so it cannot account for the observed transformation. Regenerating muscle fibres, on the other hand, would be expected to exhibit recapitulation of developmental isoforms of myosin, yet during stimulation-induced transformation neither the neonatal62 nor the embryonic myosin isoform19 were present. (Not surprisingly, both isoforms were associated with the damage reported by Maier and associates.60)

The most persuasive evidence that damage is not a prerequisite for type transformation is that it can be demonstrated directly within existing fibres. Partially transformed fibres showed myofibrillar ATPase staining that was intermediate in staining intensity.54 Immunohistochemistry with type-specific antimyosins revealed a mixture of myosin isoforms in partially transformed fibres.63 Gel electrophoresis performed on single fibres from transforming muscle revealed the simultaneous presence of myosin light chain isoforms of both the fast and the slow type.64 The decline of fast, and increase of slow, myosin heavy chains (MHCs) was visualized in individual fibres by immunogold staining with silver enhancement.61 Immunogold electron microscopy also showed that during transformation fast and slow MHCs were present at the sarcomere level in the same muscles.65 The answer to the second question is therefore that type transformation can indeed be demonstrated in the absence of damage.

The conclusion from these studies is that the transformation of type induced by chronic electrical stimulation is a distinct phenomenon, and any contribution from damage is incidental, not obligatory. Damage should nevertheless be avoided because of the ambiguity it introduces into experimental results.

Hypothesis: the effects of stimulation are the result of selective proliferation. A related explanation for type transformation is one involving proliferation within the slow muscle fibre population.

There is no observable increase in the total number of muscle fibres in stimulated muscles.13,66 This means that proliferation of slow fibres would have to be matched by degeneration of fast fibres. Since the rabbit TA is at least 95% fast this hypothesis fares no better than the wholesale degeneration hypothesis, which has already been dismissed as a possible mechanism.

It would also be difficult to maintain this hypothesis in the face of events following cessation of stimulation, during which there is a complete recovery of the mosaic histological appearance of the muscle.52 Reversal of the postulated changes would require large-scale proliferation of fast fibres and a matching degeneration of slow fibres, processes that would certainly not be expected under these entirely physiological conditions.

The fact that myosin transitions can be shown to take place within fibres, as described above, shows that an explanation based on selective proliferation is not only highly improbable but completely unnecessary.

Hypothesis: stimulation is not physiologically relevant. In the 1970s some people no doubt felt that results obtained with chronic stimulation could be safely ignored, because it is unrelated to the way in which muscle contractions are normally elicited. At the organ level this is clearly true: stimulation activates the entire muscle, bypassing the usual orderly recruitment of motor units.67 At a cellular level, however, individual muscle fibres have no way of distinguishing between impulses that are generated in the spinal cord and those evoked by electrical stimulation of the motor nerve. The fibres should therefore respond to both influences in the same way. Could this be demonstrated?

In July 1977, I was invited to take part in a Symposium in Szeged entitled ‘Functional Specificity of Muscle Fibres’, where I spoke about the apparent hierarchy of stability in the properties of skeletal muscle and how it could be understood as a series of thresholds for change.53,61,68 In subsequent small-group discussions Dr Jan Henriksson and I were both struck by the essential congruity between the effects of endurance exercise and those of chronic stimulation. Muscle properties that changed in response to exercise – fatigue resistance and metabolic profile, for example – were the same as those that changed at an early stage of stimulation. Properties that appeared to be more stable under exercise conditions – such as the proportion of Type 1 and Type 2 fibres – were those that changed only with more prolonged stimulation. These observations were entirely consistent with the notion of thresholds I had presented earlier. We explored these ideas in a review that drew together the effects of stimulation and the effects of exercise in both animals and man.13 We concluded that the adaptive changes seen in endurance training were less complete only because exercise imposed a less extreme change in activity on a more restricted pool of motor units, and not because the underlying processes were fundamentally different.

This conclusion was reinforced by changes observed when impulse activity was chronically intensified by increased postural loading. The soleus muscles of rats subjected to twice normal gravity in an animal centrifuge showed a shift in the proportion of slow oxidative fibers from 82% to 100%.69,70 One month after the body weight of mice was increased by placing lead blocks under the skin of the lower back, the soleus muscles showed a marked increase in the proportion of both slow MHC and the slow type of sarcoplasmic reticulum calcium pump (SERCA2a).71 Five weeks after tenotomy of the synergistic gastrocnemius muscle in rats, the plantaris and soleus muscles showed a pronounced shift from fast towards slow MHCs.72 Rat plantaris muscles overloaded by removal of both the gastrocnemius and the soleus muscles showed a significant shift from fast towards slow MHCs after only 14 days.73

These various non-electrical interventions all result in changes that are qualitatively similar, although not as fully developed, as those elicited by chronic electrical stimulation. They point to the inherent ability of skeletal muscle to respond to an increase in functional demand, however engendered.

Such a concept is obviously relevant to sports physiology, but it also has a bearing on the interpretation of pathological conditions in which changes in biochemical profile could reflect a recent history of overuse or disuse.74,75

Hypothesis: muscle type transformation requires a specific pattern of impulse activity. By 1980 the notion that changes in impulse activity brought about a transformation of muscle type was firmly established. The adaptive hypothesis placed the emphasis on the amount of impulse activity, but there remained the possibility that pattern also played a part. Subsequent work showed that stimulation regimes that delivered the same number of impulses produced the same fast-to-slow transformation, regardless of pattern or frequency.18,76-81

The reciprocal transformation from a slow, fatigue-resistant towards a fast, fatigue-susceptible state, can be produced simply by cessation of impulse activity. Such a trend has long been familiar to clinicians in the field of spinal-cord injury 82, and it has been convincingly demonstrated experimentally. After just 14 days of microgravity in spaceflight rat soleus muscles showed a shift from slow toward fast MHCs.83 Hind limb suspension produced similar changes in the non-weight-bearing soleus muscles.83 After up to 90 days of spinal cord isolation, which renders muscles electrically silent without interrupting the motor nerve, slow isoforms of MHC were replaced almost completely by the corresponding fast isoforms at both the mRNA and protein levels.84,85 Similar, although less complete, changes were seen in cat soleus, which is normally almost homogenously slow.86 If a mere reduction in impulse activity can produce these changes, what is the role of phasic high-frequency activity? Such patterns are better than continuous low-frequency patterns when it comes to preserving force and mass, because overall protein synthesis is enhanced by strong muscle contractions.87-92 The primary effect of brief high-frequency bursts applied to an otherwise silent muscle is thus to boost protein synthesis non-specifically, enhancing – but not initiating – the shift towards fast muscle characteristics. (See Ref. 61 for a more detailed discussion.)

These complementary results support the view, central to the adaptive hypothesis, that an increase in aggregate impulse activity induces a fast-to-slow transformation, whereas a decrease in activity allows the muscle to revert to a fast default state.

With what we now know about the role of impulse activity we may return to the original cross-reinnervation experiments of 1960.1 We see that the adaptive hypothesis provides a simple and straightforward explanation of the changes seen in cross-reinnervated muscles (illustrated schematically in Fig. 1b). What would the result of that experiment have been if all impulse activity had been abolished by section of the spinal cord and the lumbosacral dorsal roots? The authors actually sought an answer to this very question and included the results in the paper.1 Under these conditions a fast muscle reinnervated by a nerve that had previously supplied a slow muscle remained fast. This experiment is the inverse of the one illustrated in Fig. 1d and the result is precisely what the adaptive hypothesis would have predicted. The authors interpreted it differently only because they were starting with an imperfect understanding of the effects of increased and decreased use on the muscles. Subsequent experiments, with a broader range of outcome measures, yielded a similar result, although again they were interpreted somewhat differently.93,94

APPLICATIONS

Once the adaptive nature of muscle has been recognized it becomes possible to pursue its significance in both basic research and clinical applications.

Basic research.Structural and functional differences between the fibre types of skeletal muscle are underpinned by differences in protein profile. These extend to families of proteins involved in: metabolism, including enzymes encoded in nuclei and mitochondria; membranes, including proteins involved in internal transport and storage of calcium; the regulation of contraction; and the contractile apparatus itself. The redifferentiation of skeletal muscle in response to a change in activity is therefore an attractive model in which to investigate the regulation and coordination of expression of the corresponding genes. A definitive pathway for the phenomena has yet to emerge, although a number of candidate pathways have been described.95,96 It seems unlikely, however, that adaptive changes are the product of a single pathway. For example, chronic stimulation produces a monophasic decline in enzymes of glycolysis97,98 but the response of mitochondrial volume and enzymes of oxidative metabolism is biphasic.98,99 In the latter case, we showed that the secondary, declining phase coincides with the transition from fast to slow myosin heavy chains,62 and fails to occur under stimulating conditions that do not produce that transition.100,101 This is strongly suggestive of a linkage between metabolic changes and myosin isoforms. Other signalling pathways may be responsible for coordinating expression of enzymes encoded by nuclear and mitochondrial DNA.102-104 Changes in metabolites105,106 point to interactions that may be adaptive but may or may not take place at the gene level. We can no doubt anticipate further progress in this complex and rapidly moving field.

Clinical applications*.* Electrical stimulation of muscles in human subjects paralysed by stroke or spinal cord injury is being used to restore the forces and coordinated movements needed for posture and movement. Because such stimulation is unphysiological there is a risk of premature onset of fatigue. Functional stimulation should therefore be preceded by a less challenging program of stimulation which can be progressively escalated to adapt the muscles, rendering them more fatigue-resistant. This will allow periods of grasping and manipulating (upper limb) and standing and walking (lower limb) to be extended safely.107-109 In cases of ventilatory insufficiency, stimulation may be used to activate the diaphragm or abdominal muscles to improve tidal volume. Again, to avoid fatigue during continuous breathing activity the muscles should first be adapted by prior stimulation.110-113 With suitable prestimulation an artificial sphincter can be created to treat patients with fecal incontinence114,115 or to provide better management of voiding in patients fitted with a stoma.116-122 Suitably stimulated skeletal muscles can even be called upon to perform sustained cardiac work, a regime that greatly exceeds the functional demands habitually placed on them. This enables a suitably configured pedicled graft of skeletal muscle to augment cardiac function in cases of endstage heart failure (for reviews see 61,123,124).

In each case the adaptive nature of skeletal muscle provides a workable basis for developing these new clinical modalities.

CONCLUSIONS

Little more than forty years ago it was widely accepted that the neural influence on muscle fibre types was mediated by chemotrophic substances transported along the motor nerves. These days it is equally strongly believed that fibre types reflect the predominant patterns of activity imposed on a muscle by its motor nerve, and that they respond adaptively to a sustained increase or decrease in activity. I have been fortunate in seeing this dramatic change of paradigm take place within my scientific lifetime. Although I argued strenuously for the hypothesis at conferences, in seminars, and in discussion forums, it happened mainly because my group and others were prepared to consider all the other explanations for the phenomena and to design experiments that subjected them to critical evaluation. These experiments have been reviewed here.

 That the new paradigm was accepted is also a tribute to people like Arthur Buller and Lloyd Guth, strong exponents of the chemotrophic hypothesis, who were nevertheless open-minded enough to recognize the explanatory power and experimental robustness of the adaptive hypothesis. They became my friends and colleagues, as did Elwood Henneman, who was quick to perceive the developmental significance of adaptive change.

Karl Popper pointed out that a theory can not be proved, only disproved. However a good hypothesis generates questions that can be subjected to experimental test. Chemotrophism failed to do this because, although trophic substances undoubtedly exist, none has ever been found that modifies fast or slow muscle characteristics. Electrical stimulation of skeletal muscles, on the other hand, has proved to be highly reproducible, and has spawned an extensive literature.36,61 Currently, the notion that muscle responds adaptively to use not only presents the best working hypothesis for explaining the phenomena of muscle plasticity; it also provides a platform from which to address problems in both basic research and clinical applications.

ABBREVIATIONS

FDL Flexor digitorum longus

LDM Latissimus dorsi muscle

MHC Myosin heavy chain

TA Tibialis anterior

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**FIGURE LEGENDS**

**Figure 1.** Schematic diagram illustrating the experimental models described in the text. (a) cross-reinnervation interpreted as a chemotrophic process; (b) cross-reinnervation interpreted as an adaptive process; (c) chronic stimulation; (d) cross-reinnervation combined with chronic stimulation. F, fast muscle characteristics; S, slow muscle characteristics; ‘F’, nerve that normally supplies a fast muscle; ‘S’, nerve that normally supplies a slow muscle.