

## Paradigm shifts: how electrical stimulation opened up new avenues in science and medicine

Stanley Salmons

*Emeritus Professor, University of Liverpool, Liverpool, United Kingdom.*

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### Abstract

The discovery that skeletal muscle can respond adaptively to use, even to the extent of re-expressing its genome, overturned two paradigms and led to new insights into gene regulation and a variety of clinical applications.

**Key Words:** muscle, fast, slow, stimulation, implantable device, adaptation, gene expression, paradigm.

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What is a paradigm? A formal definition could be: ‘A generally accepted set of assumptions and concepts that constitute a way of viewing reality in an intellectual discipline’. Put more simply, it’s a theory that seems to explain everything.

By their very nature, paradigms are firmly entrenched, and overturning them is no easy matter. Historically, the alternative theory has often been accepted only after the death of its discoverer. I therefore consider myself fortunate in having had a hand in disproving two paradigms within my scientific lifetime. These form the subject of this article.

The first of these paradigms is the notion of ‘terminal differentiation’. This states that once cells have differentiated fully into their adult state they are incapable of expressing their genome further. In the 1960s, I quizzed two professors about this, both of them members of the Royal Society. Both asserted that this was an indisputable fact.

The second paradigm is the notion of chemotropic influence. This will take more explanation, and I will first show how I was introduced to it.

After I had graduated in Physics at Imperial College London, and pursued a postgraduate degree in Electronics and Communication, I decided that my real interests lay in the life sciences. I therefore enrolled, with three others, in the two-year Master’s course in Physiology at University College London. During the second year we were assigned to the wonderful Ricardo Miledi as tutor, who set us the task of writing essays on topics that were at the cutting edge of physiology. The subject of one of these was a paper by A.J. Buller, J.C. Eccles, and R.M. Eccles.<sup>1</sup> Let’s place this in context.

As long ago as 1874, Ranvier described ‘white’ and ‘red’ muscles in animals and noticed that the ‘white’ muscles contracted and relaxed more quickly than the ‘red’ muscles. Using these contractile speeds we can refer to these broad

types of skeletal muscle as ‘fast’ and ‘slow’. Nearly a century later it was shown that the nerves supplying fast muscles carry brief, high-frequency bursts of impulses, whereas those supplying slow muscles conduct prolonged low-frequency trains.<sup>2,3</sup>

These different patterns interested Jack Eccles, who was already well-known for his contributions to neurophysiology (he shared the Nobel Prize in Physiology or Medicine in 1963). At that time Arthur Buller was working in his lab; he had become Professor and Head of the Department of Physiology at Bristol when I first met him. As he explained it to me, Eccles wanted to see if the type of muscle influenced the firing patterns of the respective motor neurons. For these experiments they cut and cross-anastomosed the motor nerves, so that each type of muscle would be reinnervated by the other’s nerve. The effects of this cross-reinnervation were studied some weeks later.

They failed to demonstrate any difference in the firing patterns of the neurons. But during one of these laborious experiments – at about three o’clock in the morning – Eccles suggested that they had a look at the contractile speed of the muscles instead. To their surprise a remarkable change had occurred; the fast muscle had become slower-contracting and the slow muscle had become faster-contracting. This finding was the subject of the paper that Miledi gave us to study. After considering possible explanations the authors suggested that the muscles had responded to the influence of ‘quickening’ and ‘slowing’ chemical trophic factors transported to them along their motor nerves. There was no direct evidence for such factors – no more than there had been for other postulated substances from the past, the basis of previously discredited paradigms such as aether, caloric fluid, and phlogiston. However, in fairness it was a perfectly reasonable proposition. Paul Weiss had demonstrated axonal flow,<sup>4</sup> so there was a candidate mechanism

for the transport of such substances, and this was reinforced by the discovery of neurotrophins such as Nerve Growth Factor that had other functions.<sup>5</sup>

At all events, the convincing arguments in the paper, and the standing of its principal author, led to its widespread acceptance. We had a new paradigm for the differentiation of skeletal muscle into fast and slow types.

This, however, would not form the subject of my own research. At least, not yet.

### University of Birmingham

I got married soon after my graduation from University College London, and as my wife was studying medicine at the University of Birmingham Medical School, I looked for research positions there. I accepted a research fellowship in the Department of Anatomy. The aim of the project was to study muscle activity in freely-moving primates, using radiotelemetry. This was ideally suited to my background in physics and electronics as well as physiology. The original objective was to record electromyographic signals (EMG) but I judged that muscle force should also be measured, as the two are not closely correlated under all conditions. For this I developed the buckle transducer, which allowed force to be measured without cutting the tendon.<sup>6,7</sup> The principle is illustrated in Figure 1. Tension in the tendon is recorded by strain gauges incorporated into the frame or the cross-piece.

The associated circuit design was challenging. Primates are adept at removing devices attached to them. Such devices would also require connections to be made through the skin, with the accompanying risk of infection. To avoid these problems, it would be essential to implant the device. But if we were to observe the normal locomotive behaviour of the animals, data would need to be transmitted over several metres, and the implant would have to work for several weeks. The only way to manage this with a small, battery-operated device was to use a pulse technique. I decided to record muscle tension by modulating pulse width, while the integrated EMG would modulate the pulse frequency. This had the advantage of ensuring that the most detailed information was collected at times of maximum activity.

This was the 1960s, before the era of integrated circuits. It was possible to buy a single gate (the devices in your smartphone have many millions of gates) but the current drain of

such a circuit was about 10 mA, which was prohibitive. Fortunately, this was the time of the Space Race, and miniature discrete components had become available. These typically included transistors of the size of a match head and resistors 4 mm long and 1 mm in diameter. I designed much of the circuitry around these components. However I remained dissatisfied with the overall current drain, and I began looking for a better circuit that could be triggered to generate a single pulse. A change in semiconductor technology provided the solution.

Briefly, junction transistors consisted of a sandwich of semiconductor layers into which n-type and p-type doping substances had been diffused. The semiconductor used at that time was germanium, and the transistors had a ‘pnp’ configuration. When silicon transistors first appeared they had an ‘npn’ configuration. I was interested to find a circuit that combined transistors of both types and this presented new possibilities to me. I constructed a circuit of this kind that was designed to deliver a single pulse.

The circuit misbehaved. Instead of generating a single pulse it generated an unending sequence of pulses.

Because of the importance of minimising current drain I always measured it by having a multimeter in the circuit, and I noticed that the needle of the meter was not moving. I turned up the sensitivity. At the maximum sensitivity the needle moved, indicating a current drain of only 21  $\mu$ A. I stared at it.

***Lesson 1: If an experiment yields an unanticipated result, pay close attention: this could be your big break!***

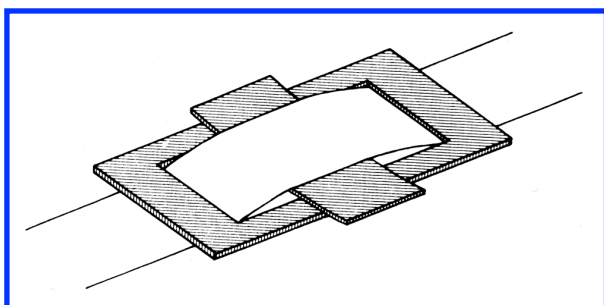
The writer and scientist Isaac Asimov put it well: The most exciting phrase to hear in science, the one that heralds new discoveries, is not ‘Eureka!’ but ‘That’s funny.’

The significance struck me like a bolt of lightning. As a generator of single pulses the circuit was worthless, but it would make a fantastic stimulator!

I knew exactly what experiment I wanted to do with this circuit. In fact Buller must have had something similar in mind when he wrote: “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.”

My newly discovered circuit would make this difficult task feasible! I was a member of The Biological Engineering Society and the International Society on Biotelemetry, and took electronics journals and newspapers, so I was conversant with the techniques I’d need to use to miniaturise the device. I had also conducted animal experiments during my Physiology degree. There was just one problem: although I was surgically skilled I had never conducted aseptic procedures, those in which the animals recovered from anaesthesia. There was, however, someone in the Department who had.

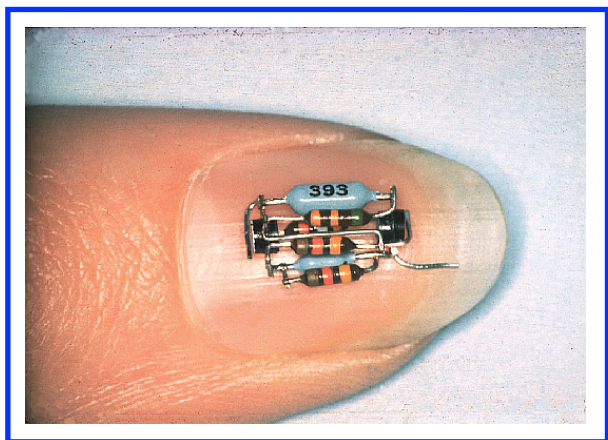
Dr Gerta Vrbova had joined the Department of Anatomy not long before. I had read her papers, in which she investigated what would happen if the impulse traffic reaching the slow soleus muscle were abolished, which was achieved by transecting the spinal cord and dividing the muscle ten-



**Figure 1.** Principle of the buckle transducer.

don. Under these conditions, the muscle became faster contracting. The observation was suggestive of an influence of impulse activity on muscle properties, but it was criticized on the grounds that the muscle was rendered severely atrophic by this procedure, and the results could therefore be influenced by contraction of adjacent fast muscles. Proponents of the chemotrophic explanation remained unmoved. The experiment I wanted to do was the opposite of silencing a slow muscle; I wanted to impose a postural type of low-frequency pattern on a fast muscle. I remember putting the suggestion to her, not in her room but leaning on one of the wide windowsills of the corridor that led to the main lecture theatre. She had two objections. First, these muscles would not be electrically silent, so we would be superimposing impulse activity of the slow-muscle type over the top of existing fast muscle activity. To this I responded that the effect of a continuous low-frequency pattern of activity should surely overcome that of intermittent, and possibly infrequent, bursts. Her second objection was that 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. In answer to this I told her that I believed I could construct a totally implantable stimulator, and this would make it possible to conduct the experiment in a freely moving animal. She shrugged and said we could try it.

It took six months of development. I reduced the current drain from the initial 21  $\mu\text{A}$  to just 6  $\mu\text{A}$ . I would base the design on existing mercury (later zinc-air) batteries of the type used in hearing aids. But whereas in hearing aids they would last for just 36 hours, in my device they would last for several months. Initially I soldered miniature components to a flexible printed circuit, on which I had etched the required pattern electrochemically. This could then be rolled up, the components interdigitating. Later I purchased a miniature spot-welder, and assembled the circuit in three dimensions (Figure 2). John Powell, our skilled departmental workshop technician, turned a hollow bullet-shaped device out of medical quality stainless steel, 30mm long by 8 mm diameter. It was in two halves, and I embedded the circuit in resin in one half and placed the batteries in the other.



**Figure 2.** Implantable stimulator circuit.

When the halves were clipped together the stimulator began to work. For the leads I obtained pacemaker wire, which I insulated with thin-walled silicone rubber tubing. I swelled these with xylene, threaded the wire through them, then allowed them to contract again. The electrodes were medical quality stainless steel, welded to the ends. These were placed near the common peroneal nerve of a rabbit to stimulate muscles in the front compartment of one hind limb, and delivered pulses of 0.5 msec duration at a frequency of 10 Hz.

The first experiment served only to show that the rabbit would not tolerate a device placed subcutaneously. In order to place it in the peritoneal cavity I encapsulated it in silicone rubber into which I incorporated a piece of Dacron mesh. The mesh was included when we sewed up the peritoneal wall, thus preventing the device from migrating and strangulating the intestine with the leads. The remaining issue was a tendency for the muscle contractions, which could be observed and palpated externally, to weaken or even cease entirely the moment the skin was closed. This required one or the other electrode to be moved. It was a problem I solved later.

The device enabled the long-term effects of stimulation to be studied in a freely-moving animal unencumbered by leads. We demonstrated it at the meeting of the Physiological Society held at the National Institute for Medical Research, Mill Hill, London (now the Crick Institute in Central London). In those days it was permitted to exhibit animals and we had taken a cage with a large white rabbit that had the stimulator implanted. The device itself was also displayed. It attracted a lot of attention.

When we conducted the terminal experiment on the first rabbit there was never any doubt about the result. After only a few weeks of stimulation the fast tibialis anterior and extensor digitorum muscles clearly contracted and relaxed more slowly. Thus encouraged we embarked on a series.

In the course of this series I made some changes to the experimental procedure. In the measurements of twitch contraction I was concerned about the resting tension of the muscle, since overstretching can itself extend the duration of contraction and, especially, relaxation. I felt it was not enough to test this by prodding the tendon with a finger. I therefore repeated the measurements with the muscle lengthened and shortened by 2 mm from the expected optimum. The contractions were recorded on 35 mm film, and as I was doing all the analysis, I could select only measurements obtained with the optimum settings. I also felt it was important to measure the rate of rise of tetanic tension, because under these conditions the muscle was fully activated.

All this time I was still working on my original PhD project, which had some techniques in common. Nevertheless, the stimulation experiments were clearly a better vehicle for a PhD thesis. The Introduction was just one page, but I wrote an extensive Discussion, based on a survey of all the literature that involved changes in muscle use. It struck me that wherever changes were observed in the contractile speed of a muscle it could be explained in terms of a change in the impulse activity reaching it. The thesis concluded:

‘A slow time course of relaxation is appropriate to the maintenance of a smooth contraction for prolonged periods. Furthermore a given tension can be maintained with less frequent activation than would be required of a fast muscle. As a result, the rate of utilisation of chemical energy by a postural muscle is low enough to be supported by a predominantly oxidative metabolism. Slowness, it seems, enables a postural muscle to be continuously active by eliminating the need for short-term energy reserves and the recovery periods required to replenish them. I would therefore suggest that the speed:activity relationship represents an adaptive response which enables a skeletal muscle to perform the type of activity demanded of it.’<sup>8</sup>

My PhD was awarded in 1968.

I shall always be grateful to the memory of my supervisor, Professor Eric Ashton, for allowing me the freedom to pursue this new and productive research avenue. It was fortuitous for me, however, in the sense that our main collaborator, Dr Charles Oxnard, had left for a senior post in Australia, and not long afterwards the primate colony was disbanded, ending any possibility of conducting the experiments he’d intended. Meanwhile I was appointed to a Stothert Research Fellowship of the Royal Society, and Eric Ashton became a valued colleague rather than a supervisor. When it came to writing up the work for publication Vrbova suggested that we combine her earlier experiments with the present ones. I agreed, but it did present problems.

***Lesson 2: Regardless of the seniority of any others involved, never allow a paper on which you are an author to be published unless you have had the opportunity to read, and if necessary revise, the content.***

There’s a corollary to that, of course, if you are the principal author:

***Lesson 3: Always give every coauthor on your paper, however junior, the chance to read, and if necessary revise, the content. A person can fairly be expected to be called upon to defend a paper on which they are a coauthor, and they can hardly do so if they’ve never seen it.***

Those principles were as important for me then as they are now. The 1959 paper was eventually published with all my alterations.<sup>9</sup>

### Harvard Medical School, Boston

Shortly after this I attended the 8<sup>th</sup> International Conference on Medical and Biological Engineering, at Chicago. While there I made contacts that led to the offer of an NSF Fellowship with Professor Elwood Henneman, at Harvard Medical School.

Working with Henneman was one of the most formative

experiences of my career and he became a role model for me. My colleague Peter Clamann and I would invariably meet him for a sandwich lunch in his room, where we would discuss, among other things, recent papers, current progress and the detailed design of the next experiment. If we arrived as he was concluding a meeting he would introduce each of us to the departing visitor. At the 1<sup>st</sup> Neuroscience Conference in Washington he introduced me to anyone he happened to be speaking to. Wherever possible I have done the same with all my junior colleagues.

In addition to being a solicitous senior colleague he was esteemed as a careful, systematic scientist. Henneman single-handedly shifted the paradigm that motor neurons were recruited at random, or by alternation (‘rotation’) of active motor units. Instead he showed, in a series of experiments notable for their elegance and rigour, that motor units are recruited in an orderly and predictable way.<sup>10-12</sup> This observation fitted perfectly with my own studies, as it suggested that the resultant hierarchy of impulse activity would then be reflected in the properties of the corresponding muscles.

During this time, I redesigned the stimulator, which now fitted into a stainless steel capsule that screwed together, with terminals that allowed the leads to be changed more easily; this device could therefore be reused indefinitely. I also redesigned the electrodes and the surgical technique for implanting them. Pacemaker wire was flexible, but not sufficiently flexible in the space available in a rabbit, so instead I adopted fine, multistrand Cooner wire. I made a simple tool with which to fashion a loop at the end of the lead. The elongated loop was drawn through the muscle tissue underlying the common peroneal nerve inside a hypodermic needle inserted from the opposite side. The other lead was located in the conductive space above the nerve. Each was mounted on a small piece of Dacron™ velour so that it could be secured with a couple of 5/0 sutures. This arrangement ensured that the current field always passed through the common peroneal nerve, and was confined to it. After this we never had any problems with loss of stimulation after closing the skin or during voluntary movements of the hind limb. We could now stimulate reliably for 24 h/day for many months, and a new ‘batteryectomy’ operation allowed us to extend the duration of stimulation still further.

While I was in Boston, I forged a collaboration with Frank Sréter and John Gergely at the Boston Biomedical Research Institute in the city. They brought to bear a range of techniques with which we could study biochemical changes in the stimulated fast muscles. The recent discovery of myosin light chains was of particular interest, as these were distinctly different in fast and slow muscle. They were visualised using SDS gel electrophoresis. We were excited to see that after four weeks of chronic low-frequency stimulation the light chain pattern typical of fast muscles had changed to one that contained two additional bands corresponding to those found in slow muscle. This result was published in Nature.<sup>13</sup> After longer periods of stimulation the fast light chains progressively disappeared, and after ten weeks the stimulated fast muscle exhibited only light chains of the

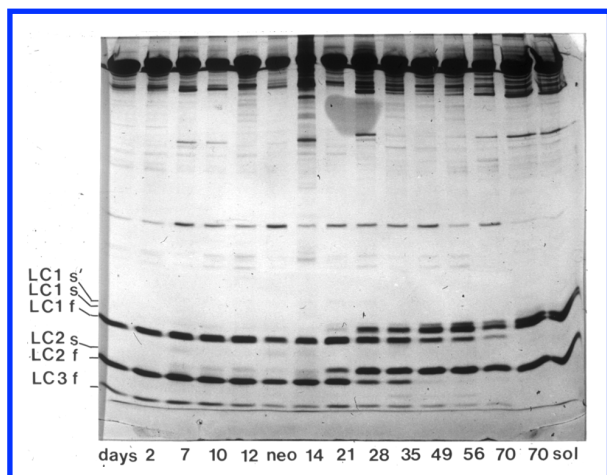
slow muscle type<sup>14,15</sup> (Figure 3). In the soleus muscle of the rabbit a fast component comprises about 4.5% of the light chain fraction, but a soleus muscle stimulated for ten weeks was homogeneously slow<sup>15</sup> (Figure 3).

If stimulation was stopped after six weeks the original fast light chain complement was re-established.<sup>16</sup>

Corresponding changes were seen in the myosin heavy chains.<sup>15</sup> Fast muscles subjected to chronic low-frequency stimulation acquired a light meromyosin paracrystal staining pattern identical to that of slow muscle.<sup>13,17</sup> N<sup>ε</sup>-methyl-histidine, a normal component of the heavy chain of fast, but not slow, muscle, disappeared completely following stimulation. It was restored following cessation of stimulation.<sup>16</sup> Stimulated fast muscle that was stained for myofibrillar adenosine triphosphatase with alkali incubation showed the histochemical appearance of slow muscle.<sup>18</sup>

Although biochemical changes had been observed in enzymes of metabolism in response to endurance exercise<sup>19</sup> and chronic stimulation<sup>20</sup> these were of a quantitative nature. Our demonstration of qualitative changes in myosin light and heavy chains provided the first evidence that a fully differentiated muscle could re-express its genome. We had overturned the first paradigm, namely the notion of ‘terminal differentiation’.

The second paradigm, the ‘chemotrophic theory’, proved to be a more difficult undertaking. The results obtained with the implantable stimulator were themselves too clear-cut to be challenged. But the chemotrophic theory was well established, and the thesis that impulse activity is responsible for the differentiation of fast and slow muscles was not widely accepted. Rather, arguments were advanced to explain the results in terms of chemotrophism. There were, in fact, ten such explanations and I set about disproving all ten. A full account of these experiments may be found elsewhere.<sup>21</sup> Here I will describe how just three of those key objections could be dismissed.



**Figure 3.** SDS-polyacrylamide gel electrophoresis of myosin purified from tibialis anterior muscle after various periods of stimulation. neo: neonatal myosin from 3-day-old rabbit leg muscle. 70sol: myosin prepared from adult soleus muscle after stimulation for 70 days.

## Generalized degeneration and regeneration

Those who raised the first of these objections asserted that chronic low-frequency stimulation was damaging, and fast muscles suffered wholesale degeneration followed by generation of new muscle fibres of the slow type. The following studies addressed this proposition.

In my own laboratory 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, showing no evidence of widespread degeneration. Our electron microscopy studies, described below, would have been impossible had damage featured significantly in the muscles. We also looked at developmental isoforms of myosin. During normal development, muscles change from embryonic to neonatal to fast isoforms, or from embryonic to neonatal to slow isoforms. Regenerating muscle fibres would therefore be expected to exhibit recapitulation of these developmental isoforms. But the transition during chronic stimulation involved neither the neonatal isoforms of myosin light chains nor those of myosin heavy chains<sup>15</sup>, nor did it involve recapitulation of embryonic isoforms.<sup>22</sup> Explanations based on damage or selective proliferation of slow fibres were also incompatible with the observation that normal physiological and biochemical properties were resumed after cessation of stimulation, and the customary mosaic histochemical appearance restored, with no evidence of fibre type grouping.<sup>23</sup>

Yet perhaps the most persuasive evidence that damage was not a prerequisite for type transformation was that it could be seen taking place within existing adult fibres. Partially transformed fibres showed myofibrillar adenosine triphosphatase staining that was intermediate in staining intensity<sup>18</sup>. Gel electrophoresis performed on single fibres taken from transforming muscle revealed the simultaneous presence of myosin light chain isoforms of both the fast and the slow type.<sup>24</sup> Using immunogold electron microscopy we could show that, during transformation, fast and slow MHCs were present at the sarcomere level in the same muscles.<sup>25</sup> The decline of fast and the increase of slow myosin heavy chains (MHCs) could also be visualized in individual fibres by immunogold staining with silver enhancement<sup>26</sup> (Figure 4). The inescapable conclusion is that the transformation of type induced by chronic electrical stimulation is a distinct phenomenon, and any contribution from damage is quite incidental, not obligatory.

## Species-specificity

The original stimulation experiments were conducted on rabbits and initially it was claimed that the phenomenon was unique to that species. To date, however, similar results have been demonstrated in rat, cat, dog, goat, sheep, pig, and man (references at Salmons, 2018).<sup>21</sup> The basic mechanisms of adaptation therefore appear to be the same in all mammalian species. However, larger animals have slower-contracting muscles and they normally activate them at lower frequencies; lower frequencies of stimulation are therefore sufficient to elicit the same extent of adaptive

change. Conversely smaller animals have faster-contracting muscles and full adaptation calls for more demanding regimes of activity.<sup>26,27</sup>

**Cross-reinnervation and stimulation**

By the early 1970s there had been many studies of the effects of cross-reinnervation on fast and slow muscles, and proponents of the chemotropic theory were still inclined to regard the effect of chronic stimulation as a separate phenomenon, one that was quite distinct from that of cross-reinnervation.

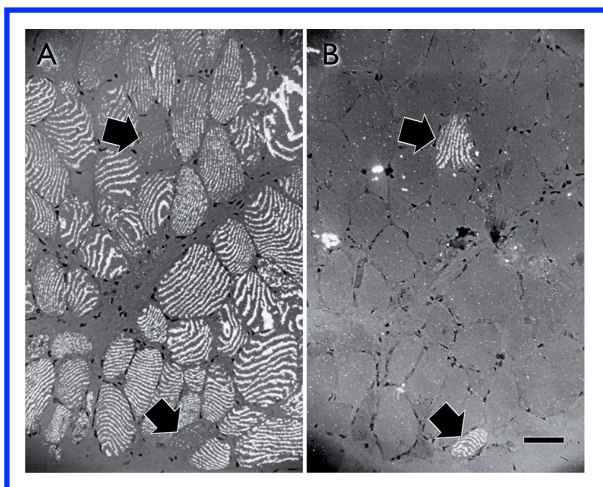
I had conceived of a way of testing this idea some years before, but back then I felt that the stimulation pattern needed to be optimised further before embarking on it. By 1975, however, we had built up a good deal of experience with the stimulation technique and its effects on physiological properties, myosin composition, and histochemical changes in metabolic enzymes. Those effects were so complete I felt the time was right to go ahead.

The experiment consisted of two parts. In the first part, I stimulated the anterior tibial muscles in 2 rabbits continuously for 5 months. 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 changing the innervating nerve produced effects greater than cross-reinnervation in every respect.

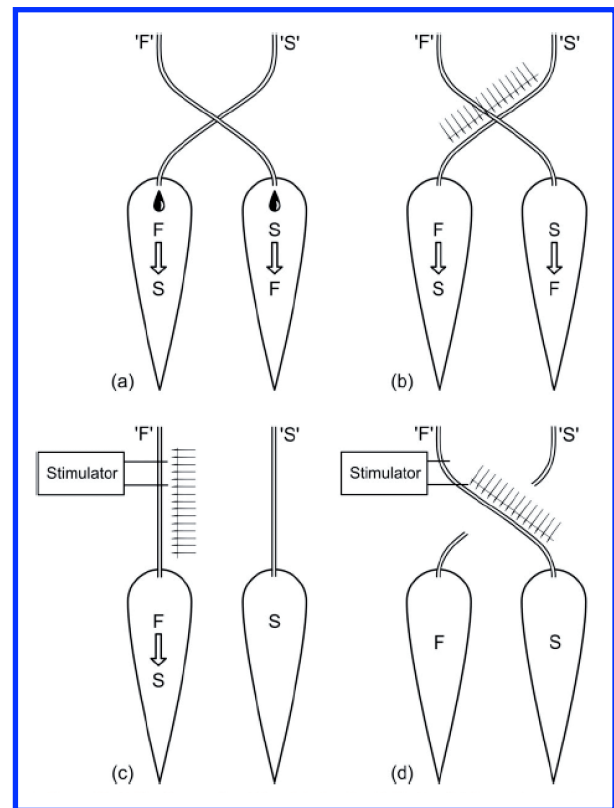
The second part of the experiment placed stimulation in opposition to cross-reinnervation. It involved two groups of rabbits, operated in matched pairs. In both groups, I cross-anastomosed the motor nerve of the fast tibialis an-

terior muscle to the nerve supplying the slow soleus muscle. This was a classical cross-reinnervation procedure although to avoid any pull on the nerves and to eliminate the possibility of reinnervation by the original nerve I performed the cross in one direction only. Electrode leads were implanted in both groups. Eight weeks later a stimulator was connected to the leads in the experimental group, delivering impulses at 10 Hz to the cross-reinnervated soleus muscle in an approximation of its original physiological activity. The leads in the control group were dummies (Figure 5d).

The surgical procedure is worth describing in more detail. The original cross-reinnervation experiments were conducted with muscles in the posterior compartment of the hind limb, muscles such as flexor digitorum longus, whose nerve is not too dissimilar in size to that of the soleus muscle (less than 1 mm in diameter). The stimulation aspect of the proposed experiment, however, meant that I had to use the common peroneal nerve, which is many times larger



**Figure 4.** Thin serial cross-sections of rabbit tibialis anterior muscle after stimulation for 2 weeks, stained by the immunogold technique with silver intensification. (A) Antibody specific for fast myosin (B) Antibody specific for slow myosin. In two fibres (arrows), A-bands that stain lightly for fast myosin also stain heavily for slow myosin, evidence of transformation in progress within intact fibres. Scale bar = 50 µm.



**Figure 5.** Schematic diagram illustrating the experimental models described in the text. (a) Cross-reinnervation interpreted as a chemotropic process. (b) Cross-reinnervation interpreted as an adaptive process. (c) Chronic stimulation. (d) Cross-reinnervation combined with chronic stimulation. F, fast muscle characteristics; 'F', nerve that normally supplies a fast muscle; S, slow muscle characteristics; 'S', nerve that normally supplies a slow muscle.

and carries a sizeable sensory component. This nerve runs under the biceps femoris muscle and dips below a slip of the gastrocnemius muscle. At this point it divides into between 4 and 13 branches and I familiarised myself thoroughly with the destination of all of them. Under a dissecting microscope I could now reliably isolate the motor branch innervating the fast tibialis anterior muscle, using fine watchmaker forceps and handling only the perineural connective tissue between the branches. The nerve to the slow soleus muscle was exposed using a lateral approach, shown to me by A.R. Luff. The two nerves were then cut and anastomosed gently using a single loop of 9/0 or 10/0 nylon. This suture material is used in ophthalmic surgery and is equivalent in diameter to just a few red blood cells, so it would present a minimal barrier to regenerating axons. The material is not easy to handle, however; draughts had to be avoided, and I polished brand new watchmaker forceps with jeweller's rouge to prevent the suture from clinging to their surface.

Again the results were clear cut. Cross-reinnervation alone closely reproduced the results published previously by others. However, 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. Such a detailed complementarity of effect would be very hard to explain other than in terms of an identical underlying mechanism.<sup>14</sup>

I think this paper was the only one I have written for a major publication that was accepted immediately as a full-length *Nature* article, with not even a request to deal with referees' comments. I remember Professor Alan M. Kelly of the University of Pennsylvania saying to me, 'That paper was the nail in the coffin of the chemotrophic theory.'

The second paradigm had indeed been shifted.

### Electron microscopy

Examining the effects of chronic stimulation at the ultrastructural level Dennis Gale and I recorded an increase in the Z-line width, from that typical of fast muscle (39 nm) to that of slow muscle (78 nm).<sup>28</sup> As we later discovered, these measurements correspond closely to the overlap of actin filaments in the mammalian Z-line.<sup>29</sup> We also observed a marked increase in the volume of mitochondria.<sup>28</sup> Using stereological techniques, Brenda Eisenberg and I generated a time course for these changes, together with changes in the T-system, over periods from 6 h to 24 weeks of stimulation.<sup>30</sup> Interestingly, mitochondrial volume increased several-fold over the first 3 weeks of stimulation, then fell rapidly after 7 weeks, although it still remained well above the levels typical of slow muscle. This complete time course agreed with changes in enzymes of oxidative metabolism.<sup>31,32</sup> Together with single fibre studies of metabolites, the changes shed light on adaptations that enabled chronically stimulated muscles to sustain a continuous high level of ATP utilization.<sup>33</sup> This explained the remarkable fatigue-resistance of stimulated muscles, the bioenergetic correlates of which were studied in dogs using *in vivo*<sup>31</sup> P-NMR spectroscopy.<sup>34</sup>

### Reversibility of the effects of stimulation

We had already made some observations of the restoration of fast muscle characteristics following cessation of stimulation, including myosin light chain composition, myosin ATPase, N<sup>ε</sup>-methylhistidine, and Ca<sup>2+</sup>-uptake by sarcoplasmic reticulum.<sup>16</sup> In further experiments we established the time course of recovery, dealing comprehensively with ultrastructural,<sup>35</sup> and physiological, histochemical, and metabolic characteristics.<sup>23,36</sup> These studies established the 'first-in, last out' nature of the response to stimulation, which we'll revisit below.

### Early events – the need for remote switching

We had shown that chronic stimulation of fast muscles resulted in a change in the expression of myosin isoforms.<sup>13-15</sup> We had also demonstrated changes in the ratio, as well as the amount, of phosphorylase kinase isozymes.<sup>37</sup> Others had documented changes in the amount and molecular type of proteins involved in calcium transport and binding.<sup>38,39</sup> The events that gave rise to these changes were, of course, of great interest, and this posed a need to examine changes at the very earliest stages of type transformation. We therefore needed to make sure that the effects of stimulation could be dissociated completely from the consequences of anaesthesia and surgery.

The implantable stimulator worked continuously from the moment of assembly and therefore as soon as it was implanted. Clearly there was a need to switch it on or off remotely after implantation. Initially I tried a miniature capillary switch which, placed subcutaneously, could be operated by a magnet. Later I developed an optical switch that formed part of a redesigned stimulator which could be triggered remotely through the fur, skin, and subcutaneous tissues of the subject by a single discharge from an electronic flash gun (known in North America as a strobe)<sup>40</sup>. This was totally reliable and also more convenient to use. Later still these devices evolved further, making use of integrated circuitry.<sup>41,42</sup> With these modifications we could now allow days or even weeks to elapse for recovery after surgery, and could then activate the implanted device and study the effects of stimulation on its own, which we did for periods ranging from 6 hours to 24 weeks. We will now look at the results.

### Regulatory events

The redifferentiation of skeletal muscle in response to a change in activity provides a valuable experimental model in which to investigate the regulation and coordination of expression of the large number of genes involved.

Our ultrastructural studies had already revealed signs consistent with mobilisation of transcriptional and translational activity.<sup>43</sup> We looked at the basis for these changes, first studying events in the polyamine pathway. The activities of ornithine decarboxylase and S-adenosyl-L-methionine decarboxylase increased markedly between 18 and 48 h of stimulation. These changes in enzyme activities were followed, between 3 and 11 days, by a

sequence of phasic elevations in the concentrations of putrescine, spermidine and spermine.<sup>44</sup> The phosphorylation pattern of cytoplasmic proteins and the activity of cyclic AMP-dependent protein kinase changed significantly by 12 days of stimulation and became indistinguishable from those of slow muscle by 3 to 4 weeks.<sup>44</sup>

To examine early stages in the expression of myosin, Northern blots of mRNA were hybridized with cDNA probes specific to the fast myosin heavy chain gene. These showed greatly reduced hybridization in fast muscles that had been stimulated for as little as 7 days. This indicated that changes in the expression of the corresponding genes were initiated much earlier than might have been supposed.<sup>45</sup>

We used cDNA probes to study the expression of four genes: Carbonic Anhydrase (CAIII), fast Myosin Heavy Chain (MHCf),  $\alpha$ -actin, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH). During 21 days of stimulation the mRNA for CAIII, which is specific for slow muscle fibres, rose significantly, whereas that of MHCf fell markedly. GAPDH mRNA declined steadily to levels typical of slow muscle, which is less dependent on anaerobic glycolysis. There were early changes in actin mRNA, suggestive of coordination with other contractile proteins during the fast-to-slow transition.<sup>46</sup> Focusing more on myosin, we constructed a time course for the reciprocal changes in fast and slow myosin heavy chains during 4 days to 6 weeks of stimulation and 4 to 12 days of recovery after cessation of stimulation.<sup>47</sup> In another study this was extended to myosin light chains.<sup>48</sup> We thus had clear evidence that for several classes of muscle protein, the response to chronic stimulation involves regulatory events at a pre-translational level.

The adaptive response of mitochondrial and cytoplasmic enzymes to chronic stimulation calls for coordinated regulation of genes located within nuclear chromatin and mitochondrial DNA. In two extensive studies with Sandy Williams we demonstrated reciprocal changes in the expression of aldolase and mitochondrial cytochrome b,<sup>49</sup> and changes in nuclear genes that encode mitochondrial proteins.<sup>50</sup>

A definitive pathway for all these phenomena has yet to emerge, although a number of candidate pathways have been described.<sup>51,52</sup> It seems unlikely, however, that adaptive changes are produced by a single pathway. For example, under conditions of chronic stimulation there is a monophasic decline in enzymes of glycolysis,<sup>20,32</sup> but the response of mitochondrial volume and enzymes of oxidative metabolism is biphasic.<sup>30,32</sup> In the latter case, we showed that the secondary, declining phase coincides with the transition from fast to slow MHCs<sup>15</sup> and fails to occur under stimulating conditions that do not produce that transition.<sup>53,54</sup> 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.<sup>50,55,56</sup> Changes in metabolites<sup>33,57</sup> 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 developing field.

### Damage – again

Earlier I provided a number of lines of evidence that enabled us to comprehensively dismiss the notion that the response to chronic stimulation was the result of damage. It therefore came as a surprise when Maier and his colleagues published evidence of damage, amounting to as much as 25% of the muscle, in stimulated muscles. They went on to attribute at least part of the conversion to slow characteristics to a population of satellite cells from which new slow muscle fibers were formed.<sup>58,59</sup>

Why had they observed such serious damage when we had not? In our laboratory the way the electrodes are placed and the use of a constant low voltage confines the stimulating current field to the common peroneal nerve. I understand that the practice in the other group was to increase the stimulating voltage during the initial reduction in palpable contractions. Firstly, this is unnecessary, because the adaptive response takes place independently of force development during this period.<sup>60</sup> Secondly, it is highly undesirable, because of potential spread of the stimulating current to the powerful plantar flexor muscles. Muscles of the anterior compartment are then subjected to simultaneous stretch and stimulation, potentially causing the damage that results from eccentric work.

Although this provided a ready explanation for these authors' findings, it was important to seek a more definitive answer because their reported damage occurred at the very stage when the regulatory events underlying gene re-expression are known to occur. As indicated in the previous section, these phenomena are the subject of considerable research interest. If extensive damage were present, it would alter entirely our perception of observations in this period. It was therefore important to confirm that, in our hands at least, muscle damage was not a factor.

We therefore decided to put the issue on a thoroughly 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 experimental subjects, and we did this for a variety of stimulation patterns.<sup>61-63</sup> We found that the volume percentage of degenerating fibers was 3.4% to 3.8% (mean  $\pm$  SD) for continuous stimulation, and 1.0%  $\pm$  1.0% for intermittent stimulation. Because the tibialis anterior muscle in the rabbit contains no more than 5% of slow (type I) 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 3%–4% of these fibres, so it cannot account for the observed transformation. While we can therefore dismiss the conclusions of Maier et al., their observations should serve as a warning to others of the potential damage that can result from excessive levels of stimulation.

### The threshold hypothesis

Let us return to the original paper of Buller, Eccles, and Eccles.<sup>1</sup> Why, after careful discussion, did they discount the idea that impulse activity was responsible for the differentiation of fast and slow muscle, proposing instead the che-

motrophic theory? Their problem was understandable, for there appeared to be a sharp differentiation of muscle into fast-contracting and slow-contracting types. As we now know, the situation is even more complex: muscle fibres show continuous variation in some parameters (such as enzymes of metabolism) but discontinuous variation in others (such as myosin light and heavy chains). The latter is well illustrated by the mosaic histochemical and immunohistochemical appearance of sections stained to demonstrate myofibrillar ATPase and myosin. How can this be a consequence of motor neurone firing patterns, which are inherently variable?

I presented a new concept to address this problem at a conference on the plasticity of muscle held in Konstanz, 1979. This was published in the book of proceedings.<sup>64</sup> It postulates that some components of muscle (such as enzymes involved in metabolism) respond continuously in a more or less linear way to increased activity. But others, such as the myosin proteins (with their important influence on the speed of contraction) respond along an S-shaped curve. This, as a physicist would immediately perceive, creates a threshold and hysteresis. To elaborate, it explains the stability of the fast and slow fibre types, because for these properties to change, the level of activity must rise above a threshold or fall substantially below it, and intermediate values are not open to them. The explanation is consistent with the orderly sequence of changes brought about both by chronic stimulation and endurance exercise<sup>65</sup> and the 'first-in, last-out' nature of the changes induced by stimulation of a fast muscle and following recovery after cessation of stimulation.<sup>23,35,36</sup> This is illustrated schematically in Figure 6. The horizontal axis denotes the time course of stimulation and recovery and the vertical axis indicates the adaptive response. There is an initial response from more readily inducible phenomena such as the increase in oxidative capacity. This is indicated in the Figure by a notional lower threshold. With a further increase in activity the upper threshold is crossed, initiating transcriptional events associated with the synthesis of myosin isoforms of the slow muscle type. Clearly these two thresholds will be crossed in the reverse order when the level of activity declines.

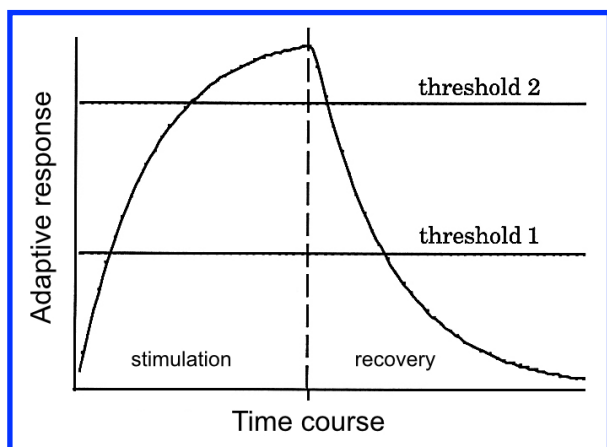


Figure 6. The threshold hypothesis.

After the talk Arthur Buller clapped me on the shoulder and said one word, 'Brilliant!', a significant accolade. Others evidently also embraced the idea because the orderly sequence of changes brought about by chronic stimulation and the threshold notion soon came into general use, together with the notion of adaptation, which I had described in my PhD thesis and early publications twenty years before.

### University of Liverpool

In 1987 I took up the Chair of Medical Cell Biology at the University of Liverpool. Jonathan Jarvis and Caroline Mayne (now Munro) took on the logistical challenges of moving the lab and all its activities to its new home, a task they managed to perfection.

### Clinical applications

At an early stage, physiological observations of the changes induced by chronic stimulation revealed a remarkable increase in the fatigue resistance of the muscle (Figure 7), and we had studied the underlying biology. This knowledge put me in a good position to make a contribution to emerging clinical applications of electrical stimulation.

Initially, I became involved in the society DIENST, and I was invited to become President. I accepted the invitation, provided I could change the name! It became the ISTS (International Society for Therapeutic Stimulation) and attracted members from the USA, France, UK, Germany, Austria, Sweden, Hungary, Switzerland, and Japan, as well as the original strong contingent from Finland. In time the society was supplanted by the very successful IFESS (International Functional Stimulation Society), on which I served as a Board Member.

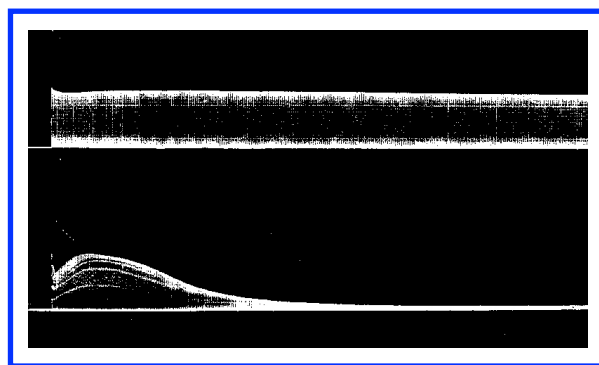


Figure 7. Fatigue resistance of stimulated muscle. The response to a series of tetani, consisting of 500 ms trains of impulses at 25 impulses/s delivered every 1.25 s. The tension developed by the control muscle (lower trace) declines rapidly after an initial phase of post-tetanic potentiation. The stimulated muscle (upper trace), which shows the slight post-tetanic depression characteristic of slow-contracting muscle, can sustain this taxing regime almost indefinitely without decrement.

Every application poses its own functional requirements, and these must be taken into account when designing the corresponding stimulation regime.<sup>66</sup>

### Stimulation of the diaphragm

One focus was on ventilatory insufficiency, such as that caused by injuries to the cervical spine. Studies were already ongoing in which electrical stimulation was used to activate the diaphragm or abdominal muscles to improve tidal volume<sup>67-69</sup>. Such stimulation is unphysiological, resulting inevitably in muscle fatigue. The practice was to avoid this by starting with a prolonged period of intermittent stimulation. At a conference on the subject in Hamburg I proposed an alternative initial regime grounded on my basic studies. In response to these suggestions a period of low-frequency conditioning was tried in 2 patients. Satisfactory resistance to diaphragmatic fatigue was achieved in just 3 weeks instead of 6, and both patients were independent of the mechanical ventilator by 2 months (Baer, personal communication).

### Stimulation of paralysed muscle

Electrical stimulation of muscles in human subjects who had been paralysed by stroke or spinal cord injury was already being employed – commercially in some cases – to restore the forces and coordinated movements needed for posture and movement.<sup>70-72</sup> Just as in the previous application there is a risk that it will result in premature onset of fatigue. Again this can be avoided by preceding the functional stimulation with a less challenging program of stimulation which can be progressively escalated to adapt the muscles, rendering them more fatigue-resistant. In this way periods of grasping and manipulating (upper limb) and standing and walking (lower limb) can be extended safely.<sup>66</sup>

### Artificial sphincters

With suitable prestimulation an artificial sphincter can be created from skeletal muscle to treat patients with fecal incontinence.<sup>73,74</sup> I advised Norman Williams on possible protocols. Pedicled muscle grafts could also be used to provide

better management of voiding in patients fitted with a stoma.<sup>75,76</sup> Following encouraging meetings with Moshe Kon and John Barker we developed a continent stoma in the pig<sup>77,78</sup>, work that demonstrates a strong potential for clinical application.

### Cardiac assistance

A major commitment in my research group was the use of skeletal muscle to augment cardiac muscle impaired by disease. This started with a letter, published in 1975 in response to a paper in which an attempt had been made to use diaphragm muscle for cardiac assist. In the letter I pointed out that the potential for such an application had been underestimated because it took no account of adaptive change.<sup>79</sup> Some six years later the letter was picked up by John Macoviak, a young surgeon working in the laboratory of Dr Larry W. Stephenson, then in Philadelphia. This began a twenty-year collaboration, during which my lab became the British Heart Foundation Skeletal Muscle Assist Research Group (Figure 8).

Normal skeletal muscles cannot sustain cardiac work, a regime that greatly exceeds the functional demands habitually placed on them. But a suitable conditioning regime renders a pedicled graft of skeletal muscle capable of augmenting cardiac function. Our muscle of choice was the latissimus dorsi muscle, which could be mobilised without seriously impairing the patient's mobility and transferred into the thoracic cavity. Our approach was to configure the muscle as an auxiliary ventricle, stimulated to operate in counterpulsation with the heart. The expanding skeletal muscle ventricle would then reduce the load of the patient's weakened heart during cardiac systole, and in cardiac diastole it would contract, assisting the systemic and coronary circulations (Figure 9).<sup>66</sup>



Figure 8. The research group in 1992.

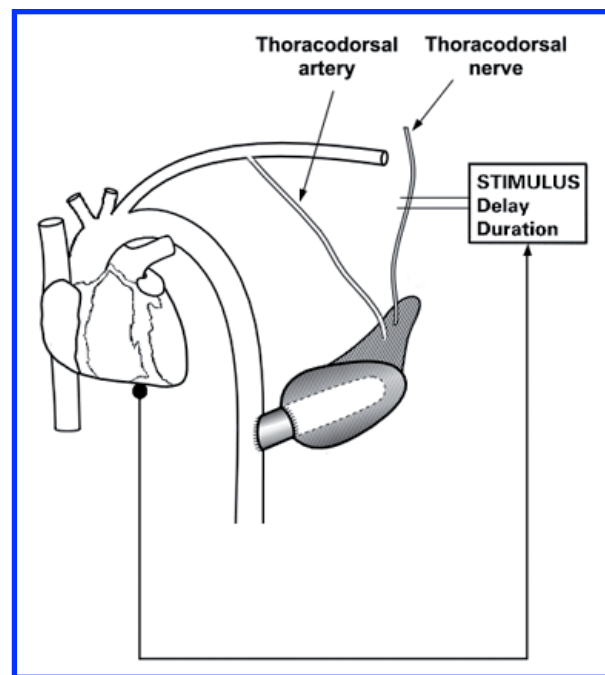


Figure 9. Schematic of a skeletal muscle ventricle.

The accompanying research program included such detailed studies as the effect of varying the frequency and duty cycle of stimulation used for conditioning (reviewed in Salmons 2009),<sup>66</sup> flow patterns that revealed the importance of travelling vortices in the heart and in single-conduit skeletal muscle ventricles<sup>80,81</sup>, and fresh insights into the blood supply of the latissimus dorsi muscle.<sup>82,83</sup> These studies supported steady progress in surgical application, to the point where a skeletal muscle ventricle was still delivering effective cardiac assistance after implantation for four years in a dog<sup>84</sup>. Progress was also being made with a single conduit version of the skeletal muscle ventricle in the pig<sup>81</sup> and we showed that this could provide assistance at least equal to that available from an intra-aortic balloon pump.<sup>85</sup> The idea of using an adaptively conditioned latissimus dorsi muscle was picked up by cardiac surgeons and used in a technique they termed cardiomyoplasty, in which the graft was wrapped around the patient's heart. Some two thousand cases were operated. There were a number of reasons why cardiomyoplasty disappointed initial expectations (for review see Salmons 2008).<sup>86</sup> Unfortunately, this high-profile failure also spelled the end of the skeletal muscle ventricle work, which had, and still has, greater potential.

### *Stimulation of denervated muscle*

As mentioned above, there was already a good deal of research and commercial activity involved in the pursuit of electrical stimulation as a solution to mobility problems caused by stroke or spinal cord injury. However it was Helmut Kern who interested me in a more difficult problem: muscles inactivated by nerve damage, such as that caused by injuries of the brachial plexus or the cauda equina. At first I was skeptical, but I was won over by his preliminary results with an intensive program of stimulation. As a result we embarked on the EU-RISE program, which involved a number of European research groups with the following main clinical objectives: i) Restoration of muscle mass. For cosmetic reasons and to improve skin cushioning; ii) Restoration of muscle force. For standing up and standing of short duration, with associated improvements in local blood flow, skin condition, bone density, and general cardiovascular fitness; iii) Improved fatigue resistance. For standing of longer duration.

The challenges have been discussed elsewhere.<sup>87</sup> Early results were encouraging, affording clear evidence that some patients would benefit in fitness, appearance, and self-esteem.<sup>88</sup> As before my group performed a variety of experiments to provide the necessary knowledge base.<sup>89-92</sup> These long-term experiments were made possible by a remarkable implantable stimulator, developed by the Vienna group, that met the demanding specification for remote operation, power delivery, and operating lifetime yet was small enough to be implanted into a rabbit.<sup>93</sup>

Stimulation of denervated muscles in animals was not new, of course, but to reflect the actual clinical conditions to be encountered our experiments included stimulation of muscles with long-standing denervation<sup>94</sup>. These experiments revealed both the benefits and the limitations of the technique, and stressed the therapeutic advantages of introducing a program of stimulation during the initial,

nondegenerative phase of the muscle response to nerve or root injury.

### **Conclusions**

This journey began with an urge to address, and overturn, two existing paradigms. In the process we established the adaptive capacity of skeletal muscle and developed an understanding of the underlying phenomena. The resultant clinical applications show how this remarkable property can provide a workable basis for developing new therapeutic modalities.

### **List of abbreviations**

EMG, electromyographic signals.  
SDS, sodium dodecyl sulphate.  
CAIII, carbonic anhydrase.  
MHC, myosin heavy chain.  
GAPDH, glyceraldehyde-3-phosphate dehydrogenase.  
IFEES, International Functional Electrical Stimulation Society.

### **Acknowledgements**

I would like to thank my collaborators and those who passed through my research group over the years. Their names are recorded as authors in the relevant references. In particular I must pay tribute to Dr (now Professor) Jonathan C. Jarvis and Dr Hazel Sutherland, who played a crucial role in accomplishing much of the work described here.

### **Conflict of interest**

The author declares no potential conflict of interest, and confirms accuracy.

### **Ethics approval and consent to participate**

Not applicable.

### **Availability of data and materials**

All data generated or analyzed during this study are included in this published article.

### **Corresponding author**

Stanley Salmons, Emeritus Professor, University of Liverpool, Liverpool, United Kingdom.  
ORCID ID: 0000-0002-1392-9869  
E-mail: ssalmons242@gmail.com

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