

Gerta Vrbová, a guide and a friend for a generation of neuro-myologists – Her scientific legacies and relations with colleagues

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Abstract

Gerta Sidonová - Vrbová, (Trnava, Slovakia, November 28, 1926 - London, UK, October 2, 2020) has been a key neuroscientist, who for almost half a century has contributed important findings and hypotheses on the relationships between motoneurons and skeletal muscle fibers, in particular on the differentiation and extent of plasticity of the peculiar characteristics of the different types of fibers present in mammalian muscles. This issue, *Ejtm* 31 (1), 2021, opens with the personal obituary authored by Dirk Pette, who remember his lifelong collaboration with Gerta, describing the many molecular and metabolic events that occur by changing the pattern of activation of adult muscle fibers through neuromuscular low frequency electrical stimulation. To honor the many scientific legacies of Gerta Vrbová and her impact on a generation of researchers studying myology and managements of neuromuscular disorders I add here additional examples of Gerta's scientific heritage and of her relations with colleagues.

Key Words: Gerta Vrbová; muscle development differentiation and plasticity; discharge patterns of motoneurons; electrical stimulation of skeletal muscles.

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Gerta Sidonová -Vrbová, Trnava (Slovakia) November 28, 1926 – London (UK) October 02, 2020.

Gerta Vrbová was a key neuroscientists who for more than half-century contributed results and hypotheses on the mutual relations between motoneurons and skeletal muscle fibers, i.e., about differentiation and maintenance of the characteristics of the motoneurons and of the muscle fiber types of mammalian muscles. Implication and transfer of her personal conclusions to managements of neuromuscular disorders were her second main interest. Gerta Vrbová made a career out of studying nerves, though her own were made of steel. Twice she escaped brutal regimes: once by jumping from a window to flee the Nazis, and later by crossing from Czechoslovakia to Poland on foot with two young children in tow to escape the communists. Her troubles began in her home town of Trnava, in western Slovakia, in 1939. Jewish people faced discrimination and the 12-year-old was excluded from school. Rudi Vrba, an old school friend who was two years her senior, helped with her studies. She recalled a bicycle trip one summer day in 1939 with another friend, Marushka, who announced that they could no longer meet because Gerta was Jewish. We leave to others to stress the courage and determination of Gerta to achieve scientific results and to

overcome tremendous personal obstacle along her long life.^{1,2} This issue, *Ejtm* 31 (1), 2021, opens with the personal obituary authored by Dirk Pette who remember his lifelong collaboration with Gerta, describing the many molecular and metabolic events that occur by changing the pattern of activation of adult muscle fibers through neuromuscular low frequency electrical stimulation.³

To honor Gerta Vrbová and her scientific legacy, I add below my own memories. I meet Gerta for the first time in 1980, when she was visiting Prof. Massimiliano Aloisi at the Institute of General Pathology of the University of Padova, Italy. As the last young fellow of Prof. Aloisi, I was invited to present her my first-name paper on long term denervated rat hem-diaphragm.⁴ She was very pleased to hear that in the six-month denervated hemidiaphragm (a very mixed muscle) almost only fast-type Myosin Light Chains were present, a molecular result fully in agreement with the Gerta's seminal observations that denervation, depriving the slow-type muscle fibers of the continuous stimulation of the slow-type motoneurons, shortened the contraction time of the slow-type muscles.⁵⁻⁷ Her warm attention was the main support to my commitment to continue those studies during the following decades, independently from other



Fig 1. Gerta Vrbová at the 2017 Padua Muscle Days: Translational Myology for Impaired Mobility – *Thermae of Euganean Hills, Padua, Italy, March 23-25, 2017. From left to right: Ugo Carraro, Fanny Pette, Gerta Vrbová, and Dirk Pette.*

international researchers, but heartened from many concordant results of prestigious groups, including, beside Gerta and Dirk Pette,⁸⁻¹⁰ Stanley Salmons,¹¹⁻¹³ and Terje Lomo.¹⁴⁻²⁰ Specifically, Terje had pioneered, often in collaboration with Stefano Schiaffino,²¹ the experimental model of low-frequency full-day electrical stimulation of denervated muscle in the rat model, to avoid the criticism that, stimulating the nerve, an antidromic adaptation of the motoneurons may occur before transformation of the innervated muscle fibers of the motor units. Further strong evidence negating that option were convincingly discussed by Terje Lømo during a Lecture at the 2014 Padua Muscle Days.²²

The criticisms of course remained that our observations were restricted to the denervated hemi-diaphragm, a peculiar experimental model in which the denervated muscle fibers continued to be passively stretched by the innervated contralateral hemi-diaphragm. Encouraged by the suggestion of Gerta that that was further evidence that the denervated muscle fibers were able to respond to induced passive-stretch in absence of direct contact with the motoneuron terminals and by the accumulating evidence that the muscle fibers may develop and partly differentiate *in vitro* in the absence of neural contacts [see for a recent short account of the history of this topic]^{23,24} soon after the visit of Gerta to Padova, we firstly extended our observations to long term denervated and

reinnervated leg muscles,²⁵ then in a conclusive paper in the *Journal of Cell Biology* (1985),²⁶ we demonstrated a substantial slow to fast transformation of the denervated rat hemi-diaphragm by electron microscopy analyses (evidence of severely decreased mitochondria, pathological features of membranes of sarcoplasmic reticulum (SR) and regeneration of muscle fibers), by single fibers analyses of myosin heavy chains (MHC) (evidence that MHC of fast type accumulate at the expense of the slow type) and by 2D SDS Gel electrophoresis of myosin light chains (MLC) and parvalbumin (again, clear prevalence of the fast type characteristics). With our surprise, we were able to analyze a large numbers of muscle fibers up to 16 months after phrenectomy.²⁶ All those results indicated that, after reaching a severe atrophic status 3 months after denervation, all types of denervated muscle fibers: i) maintain a residual 10% mass; ii) resting fast fibers continue to show their features, while iii) previously slow fibers acquire partially or almost completely fast type molecular characteristics.

In 1986, under the influence of results of Terje Lømo, we developed an independent experimental model of electrical stimulation of denervated rat leg muscle, showing that the fast muscle fibers of the fast-type extensor digitorum longus (EDL) rat muscle, submitted to continuous slow-like electrostimulation, switch-on the

genes of slow myosin in denervated fast-type muscle fibers.²⁷ Thus, adult fast and slow skeletal muscles are composed of a large number of fibers with different physiological and biochemical properties that under neuronal control can respond in a plastic manner to a variety of stimuli. Although muscle cells synthesize muscle-specific contractile proteins in the absence of motoneurons, after innervation the type of motoneuron controls the particular set of isoforms subsequently synthesized. However, agreement had not been reached on the mechanism, either chemotrophic or impulse-mediated, by which the nerve influences gene expression of the skeletal muscle fibers. In that study,²⁷ we reported the effect on isomyosins of continuous, low-frequency (a protocol mimicking the discharge pattern of the slow motoneuron) direct electrical stimulation of a permanently denervated fast muscle, the extensor digitorum longus of adult rat. After several weeks, unlike sham-stimulated muscle, the stimulated muscle showed a dramatic increase of the slow myosin light and heavy chains. Myosin light chains were identified by two-dimensional gel electrophoresis. The slow myosin heavy chains were clearly distinguished from fast and embryonic types by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis and orthogonal peptide mapping. The myosin changes could be restricted to a portion of the muscle by the position of the stimulating electrodes. Taking into account the morphologic appearance of the electrostimulated muscle and the large body of evidence demonstrating the absolute dependence of slow myosin on specific innervation, our observations indicated that at least the slow motoneuron influences the isomyosin genes' expression by the kind of activity it imposes on developing muscle fibers. I am still wondering if the protocol we used in Padova, that was able to induce in the denervated fast-type EDL of rats high levels of expression of slow-type light and heavy chains found their rational in the fact that we increased the current duration of each impulse from 0.4 to 4.0 milliseconds.²⁷ Meantime Gerta Vrbová and Stanley Salmons proposed the use of electrical stimulation to increase resistance to fatigue of skeletal muscles for different managements of neuromuscular disorders, among others Duchenne Muscular Dystrophy,^{28,29} and for the support of insufficient circulation by Cardiomyoplasty,³⁰ or of sphincter muscles.³¹ Meanwhile, electrical stimulation of the diaphragm has become an accepted clinical approach for patients with respiratory paralysis and intact phrenic nerves. Indeed, continuous simultaneous pacing of both hemi diaphragms with low-frequency stimulation and a slow respiratory rate is a satisfactory method of providing full-time ventilation support.³²⁻³⁶ In collaboration with the University of Bologna (Italy), we have been involved in a ten-year research project on cardiomyoplasty, testing the concept of dynamic cardiomyoplasty on demand. We were inspired by the differential effects on the contractile properties, population of fibers, myosin light chains and

enzymes of energy metabolism of different periods of intermittent or continuous electrical stimulation of fast-twitch muscles (Gerta Vrbová and Dirk Pette).³⁷ Both in normal sheep,³⁸ and in patients suffering with chronic heart failure, the latissimus dorsi responded to daily intermittent electrical stimulation reaching intermediate contractile characteristics during its fast to slow transition induced by the on Demand (intermittent) stimulation protocol.³⁹ The partially maintained fast type contractions of the patient muscle wrapped around the falling heart allowed to synchronize the pace-maker-induced tetanic contractions with the heart systole, avoiding interference with the diastolic function. Specifically, during assisted systolic contractions cardiac ejection fractions of heart failure patients were increased.⁴⁰⁻⁴²

Always supported by interest and suggestions of Gerta, from 2000, in collaboration with Prof. Helmut Kern of Vienna (Austria) we were able to show in a study supported by the EU Commission [(RISE - Use of electrical stimulation to restore standing in paraplegics with long-term denervated degenerated muscles (QLG5-CT-2001-02191)] that a home-base protocol using long currents (up to 150 milliseconds) is able to reverse severe atrophy of permanent denervated human muscles, up to a level to allow stimulation-induced standing and pacing-in-place exercise.⁴³⁻⁴⁹ For an example see the supplementary material in Kern H, Carraro U (2020)⁵⁰: Home-based Functional Electrical Stimulation (hbFES) assisted stand-up exercise.⁵¹

One of the effects of spinal cord injury (SCI) is rapid loss of contractile force and muscle mass, but atrophy of leg muscles is particularly severe when the injury destroys the soma of the lower motoneurons and, hence, the contacts between skeletal muscle fibers and motoneurons are permanently lost. Within weeks after SCI, muscles become unable to sustain tension during tetanic contractions induced by electrical stimulation.⁵²⁻⁵⁴ Within months after a complete injury of the *conus medullaris* and *cauda equina*, the muscles are no longer excitable by commercially available electrical stimulators.⁵⁵ This is because they have undergone severe disorganization of contractile elements (i.e., of the myofibrils) and of the excitation-contraction coupling (ECC) apparatuses. Finally, after several years of LMN denervation, human muscle fibers are almost completely replaced by adipose and fibrous tissues.⁴⁴⁻⁴⁶ This severe degeneration of muscle tissue does not occur in patients with upper motoneuron lesions even 20 years after thoracic-level SCI.⁵⁶ To substantiate the functional and molecular mechanisms that allow muscle fibers to survive long-term denervation, we meantime performed experiments in a rat model of long-term denervation by analyses not possible in humans for obvious ethical concerns. The results are summarized in the abstract of a paper published by Squecco R, et al. (2009).⁵⁷

To define the time course and potential effects of electrical stimulation on permanently denervated

muscles, we evaluated ECC of rat leg muscles during progression to long-term denervation by ultrastructural analysis, specific binding to dihydropyridine receptors, ryanodine receptor 1 (RYR-1), Ca²⁺ channels and extrusion Ca²⁺ pumps, gene transcription and translation of Ca²⁺-handling proteins, *in vitro* mechanical properties and electrophysiological analyses of sarcolemmal passive properties and L-type Ca²⁺ current (ICa) parameters. We found that in response to long-term denervation: i) isolated muscle that is unable to twitch *in vitro* by electrical stimulation has very small myofibers but may show a slow caffeine contracture; ii) only roughly half of the muscle fibers with voltage-dependent Ca²⁺ channel activity are able to contract; iii) the ECC mechanisms are still present and, in part, functional; iv) ECC-related gene expression is upregulated; and v) at any time point, there are muscle fibers that are more resistant than others to denervation atrophy and disorganization of the ECC apparatus. These results support the hypothesis that prolonged “resting [Ca²⁺]” may drive progression of muscle atrophy to degeneration and that electrical stimulation-induced [Ca²⁺] modulation may mimic the lost nerve influence, playing a key role in modifying the gene expression of denervated muscle. Hence, these data provide a molecular explanation for the muscle recovery that occurs in RISE SCI patients in response to the rehabilitation strategies developed on the grounds of empirical clinical observations.⁴⁴⁻⁵¹

Gerta maintained her interest for the myology activities of the Interdepartmental Research Center of Myology of the University of Padova (CIR-Myo), even after she was more than 85 years old. Our relations were strengthened, indeed, after we discovered (getting out from a night bus after a dinner in Central London) that she was living in Muswell Hill just 10 walking-minutes from the house of my son's family.

She joined several times the PaduaMuscleDays, a meeting mainly devoted to translational research for skeletal muscle biology, management and rehabilitation. Last time it was in 2017, when she went together with Dirk Pette. During that meeting she accepted also to be interviewed on the importance for old people to stay physically and mentally active. She was, indeed, a witness (and herself a strong evidence, being in her ninety years) of the value of a very active life for the oldest olds. Readers may follow her advices at the YOUTUBE link:⁵⁸ <https://www.youtube.com/watch?v=NJ9BPLquPWw>

During her career Gerta Vrbová published more than 270 scientific papers, but her main role was to inspire a generation of successful researchers. Some were her postdoctoral fellows Maggie Lowrie,⁵⁹ Angela Connold,⁶⁰ Linda Greensmith,⁶¹ Roberto Naverrete,⁶² Antal Nógrádi,^{63,70} and Katarzyna Sieradzan⁶⁴, but independent scientists, among which I mention researchers studying nerve regeneration after partial or complete nerve injuries (Tessa Gordon, Canada)⁶⁵ electrical stimulation and muscle plasticity (Dirk Pette,

Germany),^{3,8,9,66} electrical stimulation of denervated muscle in animal models, e.g., Terje Lomo, Norway,⁶⁷ and myself in Italy, working in both animal models and humans,⁶⁸ and finally aging human muscles (Helmut Kern, Austria).⁶⁸ Many of her pupils were brilliant enough to continue independent scientific careers and make major contributions to the fields of neuron diseases and injury, including amyotrophic lateral sclerosis and spinal muscular atrophy (Linda Greensmith in London),⁶¹ Parkinson's, Huntington's and Pompe diseases and epilepsy (Katarzyna Sieradzan in Bristol),⁶⁴ ventral root avulsion (Antal N Nógrádi in Szeged, Hungary)^{63,70} and in the field of locomotion (Urszula Slawinska in Warsaw, Poland).⁷¹ Her medical background explains her interest in translating experimental results into possible treatments for childhood genetic diseases of muscular dystrophy and spinal muscular atrophy, in collaboration with neurologists, Victor Dubowitz in London, UK,⁷² Milan Dimitrijevic in Houston, USA,⁷³ and Irena Hausmanova-Petrusewicz in Warsaw, Poland.⁷⁴ Most of the latest opportunities found grounds on just two of her publications that inspired her a working hypothesis that changed the perspectives of interactions between skeletal muscle fibers and motoneurons, starting 50 years of studies still in need of further investigations.

As is often the case in science and even more in translational research, there are now more open questions and hypotheses than before. Firm conclusions for some of the above discussed topics remain open to further researches, worth of significant founding by international sponsors. What is certain is that Gerta's legacy remains among the key preliminary results for supporting those grant applications.

I will never forget Gerta's friendship and support.

List of acronyms

CIR-Myo - Interdepartmental Research Center of Myology of the University of Padova
 ECC - excitation-contraction coupling
 EDL - extensor digitorum longus
 EU - European Union
 hbFES - Home-based Functional Electrical Stimulation (hbFES)
 MHC - myosin heavy chains
 MLC - myosin light chains
 RISE - Use of electrical stimulation to restore standing in paraplegics with long-term denervated degenerated muscles
 RYR-1 - ryanodine receptor 1
 SCI - spinal cord injury
 SR - sarcoplasmic reticulum

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Conflict of Interest

The author declares no competing interests.

Ethical Publication Statement

I confirms that I have read the Journal's position on issues involved in ethical publication and affirms that this report is consistent with those guidelines.

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