Simultaneous Recording of Electromyogram, Impedance and Zeta potential Change in Muscle

LIAO Ten Jen*, SUZUKI Hitoshi***, NISHIKAWA Hiroyasu**, TASAKI Kyoji*** and YUKIMACHI Toshinori*

* *Department of Acupuncture and Moxibustion, Meiji College of Oriental Medicine*
** *Department of Physiology, Meiji College of Oriental Medicine*
*** *Department of Physiology, School of Medicine, Tohoku University*

Summary: A method to record the EMG, impedance change and zeta potential simultaneously was described in this study. These three measurements were carried out using a 20-kHz alternating current on the surface of muscle tissue during stimulation and an insulated acupuncture needle as a probe. Each was separted out with high-pass, band-pass and low-pass filters. Impedance changes were detected in muscle tissue of frogs and humans with and without excitation. Impedance change may be due to the change the extracelluar space in muscle, because the EMG is detected early than impedance change.

Key Words: Acupuncture Needle, Impedance, Electromyogram, Isometric Contraction, Isotonic Contraction, Muscle Tissue, Zeta Potential.

1. Introduction

Electromyograms (EMG) are widely used to diagnose nerve and muscle diseases in humans. Piper[1] used surface electrodes to record EMG firstly, then Adian and Bronk[2] developed the needle electrode. Vallbo[3] succeeded in recording the action potential of peripheral nerves using a fine insulated tungsten electrode without a tip. Recently, many kinds of electrodes have been used in clinical examinations to avoid causing pain while taking measurements. Although EMG recording provides accurate information on the neural activity of muscle, other information such as the change in tonus and shape of muscle without excitation can not be obtained. An electrical impedance change in muscle tissue during muscle contraction was reported by Bozler[4] and Dubuisson[5], and this impedance change may effect the intensity of neural activity and tension of muscle tissue. If many signals could be recorded individually with a fine electrode without involving much pain, it might be valuable for the research and clinical examination of muscle tissue.

This study reports a new method of simultaneously measuring impedance, Zeta potential[6], and EMG changes in muscle tissue with an insulated acupuncture needle. A series of basic experiments were performed to investigate the extent to which these three measurements can be used to indicate the physicochemical changes presumed to be taking place in the tissue during stimulation.

2. Materials and Methods

Two sets of experiments were performed. The main experiments involved ten bullfrogs (Rana Catesbeiena), and a preliminary set of experiments was performed on seven healthy human adults to fully investigate the physicochemical and impedance changes that occur in muscle under different conditions.

Bullfrogs (weighing 250 g) were used in the experiment. Frog Ringer's solution containing NaCl, 6.43 g, KCl, 0.14 g, $NaHCO_3$, 0.21 g, $CaCl_2 . 2H_2O$, 0.176 g, $NaH_2PO_4 . 2H_2O$, 0.013 g per liter of distilled water was used. Gastrocnemius nerve-muscle of bullfrogs were used. After pithing, the gastrocnemius nerve-muscle was dissected and placed in a chamber filled with liquid paraffin.

Volunteers (seven healthy adults of both sexes) were tested lying in a semireclined position in an armchair in a quiet room. A detailed explanation of the procedures was given and the subjects made themselves as comfortable and relaxed as possible.

The leading electrode (both for humans and frogs) was a sterilized acupuncture needle (200um), insulated with formvar except for 2 mm of the tip. It was inserted into the muscular tissue, and picked up signals of neural activity, alternating the current change and zeta potential.

The surface electrode (for humans) was an EEG dish electrode (NE-103, Nihon Koden, Japan). Besides, a needle electrode (for frogs) as reference was placed on the epidermis 2 cm from the leading electrode, and connected to an oscillator supplying a 20-kHz signal at 100 nA. The amplitude of this signal at the leading electrode changed according to the impedance of the intervening tissue.

In order to decrease the external noise of this method, a low input impedance amplifier was used in this study. Fig.1 shows the power spectra of these three signals, namely the impedance, EMG and zeta potential recorded simultaneously. The power spectrum range of the EMG was approximately between several Hz and 3kHz, with the most desirable frequency range for recording being 20 to 200 Hz[7]. Therefore a low-pass filter, a band-pass filter and a high-pass filter[8] were used to separate it from the 20-kHz carrier and zeta potential. The procedure described in Fig. 2 concerns a 20-kHz signal (INTERSAL 8038 oscillator, Japan) applied simultaneously to the surface electrode and full-wave rectifier C. The signals (EMG, 20-kHz and movement of the needle) from the leading electrode were passed through a current limiter, the high-pass filter and fullwave rectifier B to differential amplifier 3, to show contrast with the reference oscillation from rectifier C. The output from the differential amplifier gave a continuous measurement of the muscle impedance. Signals for the EMG and zeta potential were also fed through the current limiter and high-pass filter. The EMG signals were passed through full-wave rectifier A, which integrated, and through amplifier 2. The zeta potential of the electrode was passed through the low-pass filter to remove frequencies above 5 Hz, and then through amplifier 1.

(a) Impedance change versus isotonic contraction

The gastrocnemius muscle of a bullfrog was anchored in gum with its nerve at one end and at the other end attached to a weight by

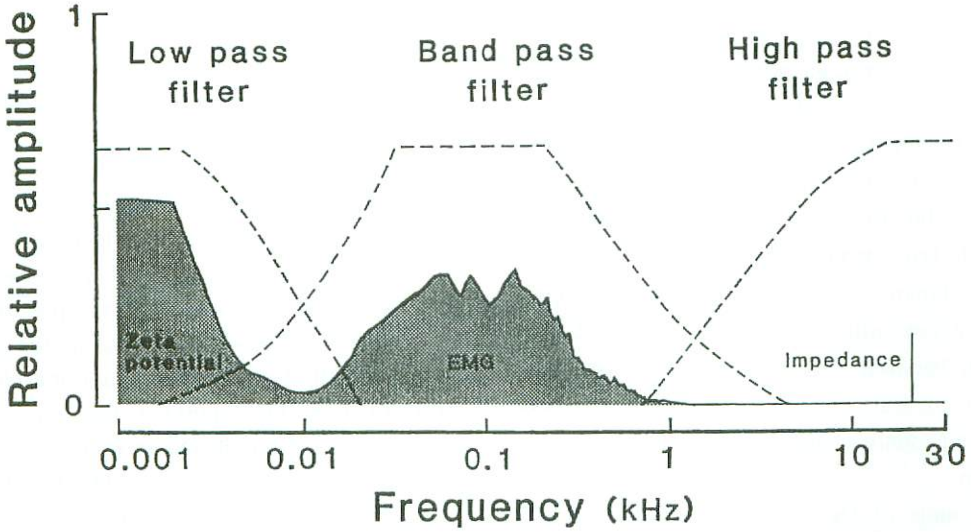

Fig. 1 This diagram shows the principle of the recording method actually applied to the gastrocnemius muscle of humans during voluntary contraction with an alternating current of 20-kHz. The frequency ranges of the EMG, zeta potential of the needle and 20-kHz carrier were separated using a low-pass filter, band-pass filter and highpass filter.

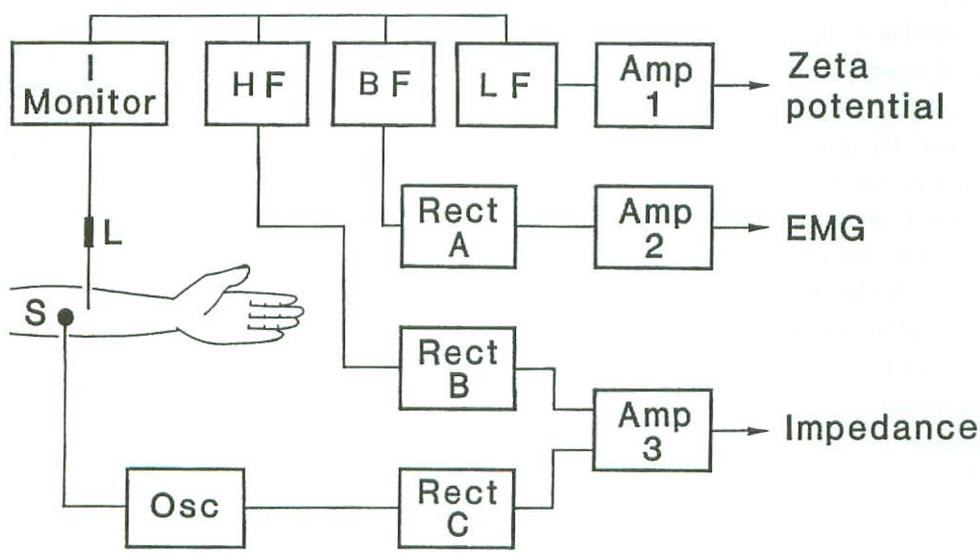

Fig. 2 Block diagram of the three measurements. HF : high-pass filter, BF : band-pass filter, LF : low-pass filter, Rect : rectifier, Amp : amplifier, L : lead-ing electrode, S : surface electrode.

a nylon thread, via a pulley. The leading electrode was inserted into the belly of the muscle and connected to the current limiter. The other needle electrode was placed in the muscle tendon to transmit the 20-kHz reference oscillation. Single square-wave pulses of 1 ms duration were applied to the nerve by a electric stimulator (SEM-3201, Nihon Koden, Japan). Alteration of the voltages to the nerves allowed a measurement of the relation between the impedance changes and isotonic contractions.

(b) Impedance change versus isometric contraction

Both ends of the gastrocnemius muscle of a bullfrog were fixed in gum with its nerve hanging on two small silver hooks, while the other experimental conditions were the same as in (a). The stimulating conditions were divided into two groups, twitch and tetanus (30 Hz), to find the impedance of isometric contractions.

(c) Impedance change versus morphological change of muscle

Both ends of the gastrocnemius muscle were fixed, and the electrodes were located and connected in the same way as in experiment (a). A force, which was perpendicular to the axis of contraction, was applied to the muscle around the leading electrode by means of a plastic plate attached to weights via a wire and pulley. Alternation of the weights allowed measurement of the impedance change surrounding the needle electrode.

(d) Impedance change versus change in muscle tension

The experimental conditions in this step were almost the same as those in (a), i. e. one end was fixed, but the other end was pulled by various forces instead of electrically stimulating the nerve.

Two kinds of muscles (tibialis anterior and flexor carpi radialis muscle) of human volunteers were investigated in this study. It was impossible to set the same conditions for isotonic contractions in the human muscles as in the frog cases. However, the location of the reference and leading electrode were the same as in the frog experiment. After preparation and fixation of these electrodes, in the volunteer cases, (A) the tibialis anterior muscle was used to detect three signal changes during voluntary contraction. (B) The forearm was placed on a plate, taking into account that the elbow served as an axis at which to bend (or extent) the palm gradually in order to measure the impedance change of the flexor carpi radialis muscle with isotonic contraction. (C) To stretch the palm, a plate which was connected with a tender-meter (Kinoshitas, Tokyo Suzuki, Japan) was used to increase the force gradually to give a measurement of the impedance change of the ulnar flexor carpi radialis muscle. (D) The forearm was placed horizontally on a plate and a force was applied to the area surrounding the needle, which was inserted through a cup with a hole into the flexor carpi radialis muscle on the forearm to register the impedance during muscle shape changes.

3. Results

Isometric and isotonic contractions were performed in the gastrocnemius muscle of frogs to observe the impedance, EMG, and zeta potential change. In Fig. 3, stimulations of (A) isometric and (B) isotonic contractions were divided into several steps and executed in the bullfrog gastrocnemius muscle. It was possible for the amplitudes of these three responses to obtained according to the

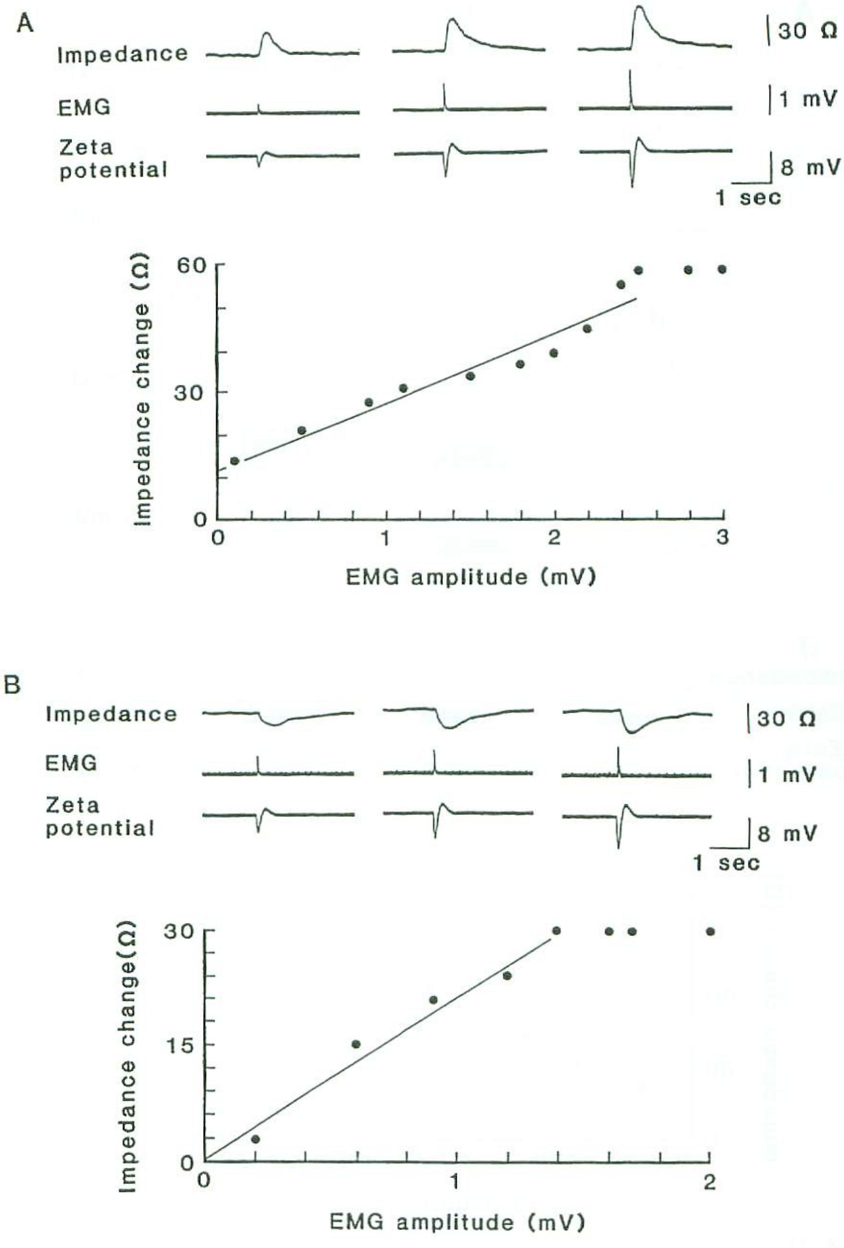

Fig. 3 Upper three traces of (A) are the impedance, EMG and zeta potential which were recorded at a gastrocnemius nerve-muscle of a frog under isometric contraction. These were stimulated with different voltages of 20, 30, and 40mV. The average relationship of the impedance and EMG change were plotted under these traces. The same conditions as (A) were performed on muscle of other frogs (n=6) under isotonic contraction, and the relation between the impedance change and EMG amplitude is also described in (B) with the direction of impedance change reverses to the results of (A).

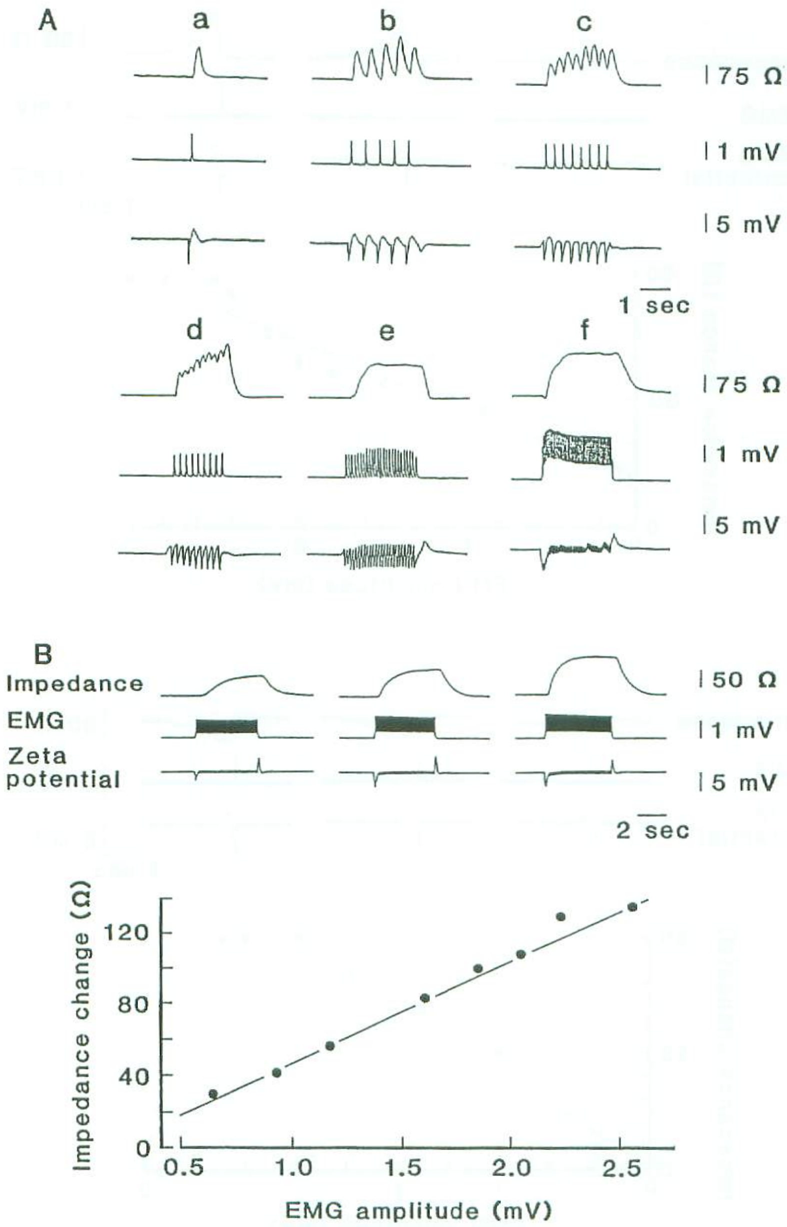

Fig. 4 (A) The upper trace is the impedance, the middle trace is the EMG and the bottom trace is the zeta potential of the needle in isometric contraction by stimulation of a gastrocnemius nerve-muscle of frog. The increasing of the frequency intensity of the stimulation from a to f (1, 5, 8, 9, 25 and 30 Hz), the impedance, EMG and moving of the needle had a significant correspondence to one another. (B) The upper traces were parts of the results of tetanic stimulation (at 30 Hz, n=6, and with a different intensity of 20, 30 and 40 mV), and the amplitude relation of impedance and EMG was plotted under these traces.

degree of stimulation. Amplitude saturation occurred when the stimulation reached a certain level. Before saturation, the amplitudes of the EMG and impedance show good linearity.

In the case of tetanic stimulation, in Fig. 4(A), the experimental conditions were the same as for Fig. 3(A), and the process was divided into the stimulation of six frequencies (a to f) of 1, 5, 8, 9, 25 and 30 Hz. Notwithstanding the variety in the frequency of stimulation, the amplitudes of the impedance, EMG and zeta potential changes are in proportion to the intensity of the stimulation. In Fig. 4(B), starting under 30 Hz and increasing the tetanic stimulation and voltage gradually, the impedance kept good linearity with the EMG change. In other words, the facts of the impedance change and EMG of this method are also applicable to the experiment of tetanic stimulation.

The frog gastrocnemius was extended and pushed, and the results are shown in Figs. 5 (A) and 5(B). The impedance change kept pace with the length and force, even in the period of electrical silence. Both results reveal that the impedance change can offer a good recording of any condition of muscle contraction, except that of EMG. The linearity and direction of changes in the above mentioned model experiments became clearly understood, and these facts which are applicable to humans are described in Fig. 6. In this figure, (A) tibialis anterior muscles of the volunteers were used to a great extent such that an acupuncture needle was inserted and a gradual voluntary contraction of the muscle was performed. In addition, the amplitude of the EMG, impedance and zeta potential were parallel to the strength of this contraction. There is also good linearity between the impedance changes and the forces around the needle electrode in 6(B), extending the palm actively as an isotonic contraction in 6(C) and stretching the palm in 6(D). In other words, the same results were obtained with the recording of these phenom ena both in the bullfrog muscles and humans.

4. Discussion

Electromyogram is well-known as the electrical activity of muscle, when it is excited by external environmental stimulation. It is possible that the shape, tonus and intercellular space of the muscle might change simultaneously, and these differences would cause the impedance to increase or decrease. Any change in these phenomena does not prove it causes a firing of the action potential, it might mean that the stimulation can not reach the firing threshold. Most likely, other factors such as the physicochemical reaction of the extracellular fluid may cause these morphological changes. According to the above mentioned results, the impedance can always reflect these morphological changes closely in contradistinction to EMG. Dubuisson reported that any change in the shape of muscle has a great influence on the resistance[5]. Bozler also reported that the impedance change increases or decreases during isometric contraction or isotonic contraction[4]. Our results correspond with theirs, and impedance change is detected later than EMG which indicates that the direction of these impedance changes depends upon the conditions of stimulation and the position of the needle tip in the extracellular space, resulting in a change in the current density. According the results, the zeta potential can

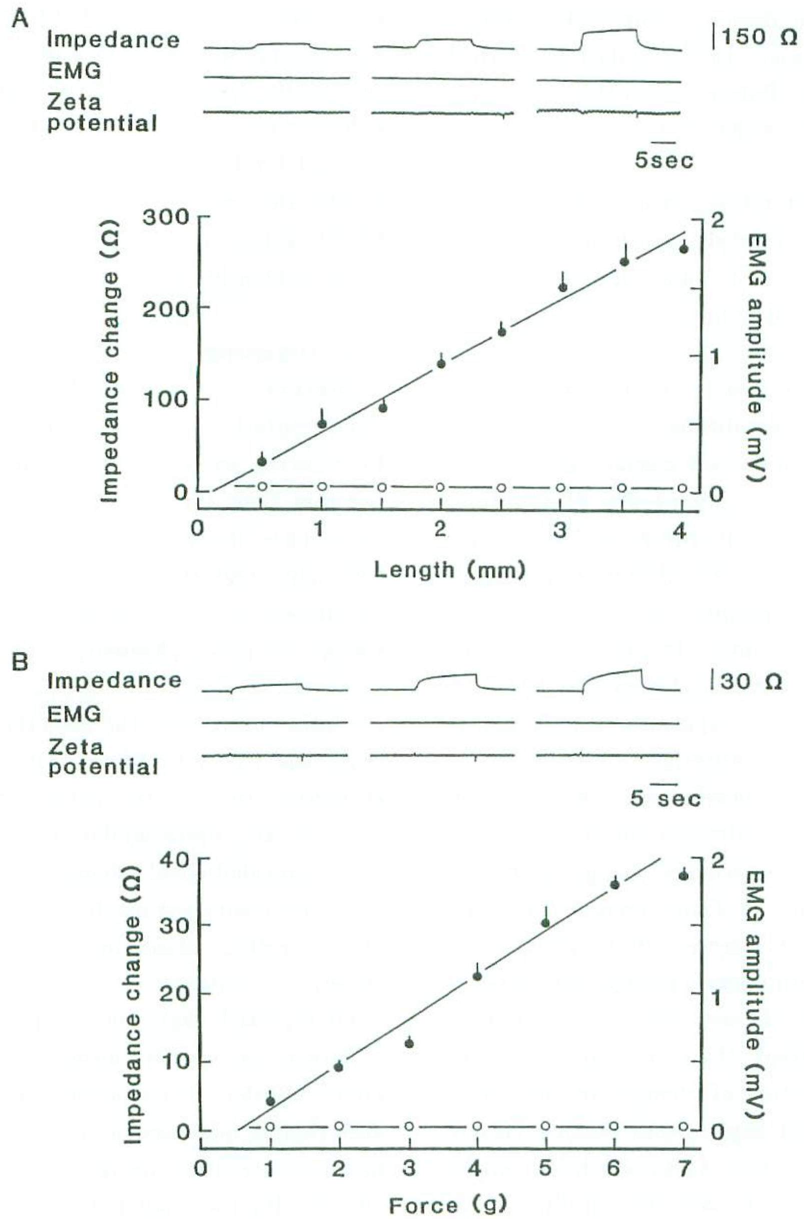

Fig. 5 (A) Alternating the length of the frog muscle (n = 6) under isotonic conditions within 1, 2, and 3 mm of the muscle extensions, recording the impedance, EMG and zeta potential on the upper traces. The relation of the impedance (solid circle, mean+SD) and EMG (open circle) respones measured by changing of length with 8 steps is described under the upper traces. (B) Pressing with forces of 2, 3 and 5 g to the surrounding area of the frog muscle (n=6) in sometric condition and recording the responses on the upper traces and the relationship between impedance and EMG under these traces as (A). In (A) and (B), the EGM were silence.

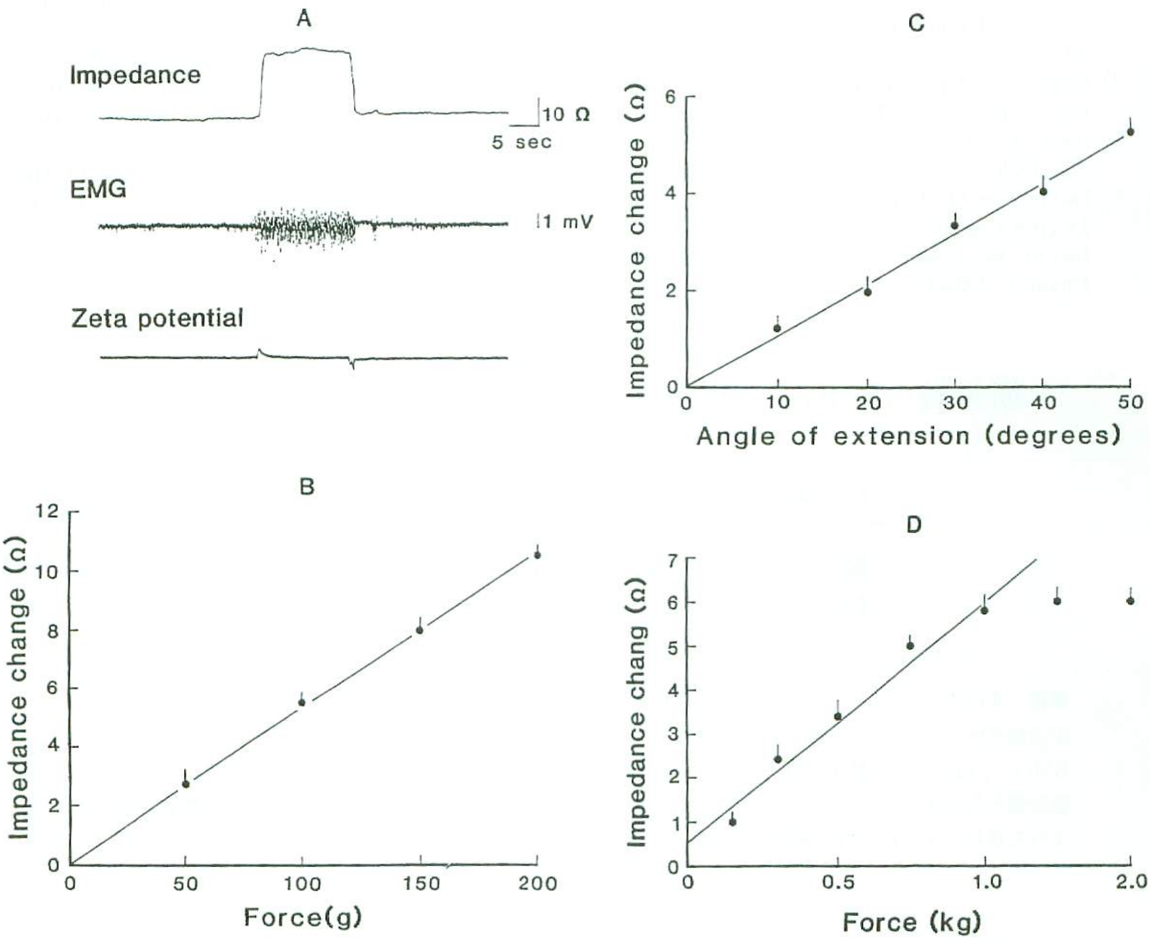

Fig. 6 (A) The impedance, EMG and zeta potential were recorded from the tibialis anterior muscle of humans while performing voluntary contraction. These three respones corresponded to one another. (B) The force for flexor carpi radialis muscle stimulation on the area surrounding the needle, in humans (n=7), and the relationship of applying force and impedance (mean+SD) were plotted. (C) Humans (n=7) extending the wrist actively to different degrees as an isotonic condition to measure the relationship of extension degree and impedance (mean+SD) in the flexor carpi radialis muscle. (D) To stretch the palms of humans (n=7) passively using a plate with different force, and the relationship of the stretch force and impedance (mean+SD) in the flexor carpi radialis muscle were also plotted.

also be used as an indicator to detect the movement of the needle inserted into muscle tissue. This was elicited by the movement of the needle and tissue fluid.

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筋組織のEMG、ζ電位とインピーダンスの同時検出法

*明治鍼灸大学　東洋医学教室　**明治鍼灸大学　生理学教室
***東北大学　医学部・生理学第二教室
廖　登稔*　行待　寿紀*　西川　恭弘**
鈴木　均***　田崎　京二***

要旨：本研究では20KHz の交流を生体に加えながら筋組織を刺激し，同時に信号検出プローブとして絶縁鍼電極を用いて筋組織から得られた信号を高域，バンド及び低域フィルタ（筋インピーダンス，EMG，ζ電位）で分離する方法を確立した．筋の等尺性収縮や等等張性収縮，または興奮を伴わない筋の変形など様々な条件下でインピーダンスの変化が得られた．インピーダンスの変化は筋電図の変化よりも遅れて見られるので前者は膜興奮に伴うのではなく，細胞外間隙の変化によると言える．