Environmental Medicine, Vol. 46 No.1, 2, 2002 (page 17 ~ 27) **Original Article**

# "DRY" Immersion Induces Neural and Contractile Adaptations in the Human Triceps Surae Muscle

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**Abstract:** The effects of 7-days of simulated spaceflight, achieved with the technique of "*dry*" water immersion, on human triceps surae muscle function have been investigated in six subjects. After immersion, the maximal voluntary contraction (MVC) was reduced by 19% ($p<0.01$), and the electrically evoked (150 Hz) maximal tetanic contraction ($P_o$) was reduced by 8% ($p>0.05$). The difference between $P_o$ and MVC expressed as a percentage of $P_o$ and referred to as force deficiency has also been calculated. The force deficiency increased by 44% ($p<0.01$) after immersion. The decrease in $P_o$ was associated with increased maximal rates of tension development (7.2%) and of tension relaxation. The twitch time-to-peak was not significantly changed, and half relaxation and total contraction time were decreased by 5% and 3%, respectively, but the twitch tension ($P_t$) was not significantly changed and the $P_t/P_o$ ratio was decreased by 9%. The 60-s intermittent contractions (50 Hz) decreased tetanic force to 57% ($p<0.05$) of initial values, but force reduction was not significantly different in the two fatigue tests: fatigue index was 36.2±5.4% *vs.* 38.6±2.8%, respectively ($p>0.05$). While identical force reduction was present in the two fatigue test, it would appear that concomitant electrical failure was considerably different. Comparison of the electrical and mechanical responses alterations recorded during voluntary contractions, and in contractions evoked by electrical stimulation of the motor nerve, suggests that immersion not only modifies the peripheral processes associated with contraction, but also changes central and/or neural command of the contraction. At peripheral sites, it is proposed that the intracellular processes of contraction play a role in the contractile impairment recorded during immersion.

**Key words:** immersion, muscle, contractile and electrical properties

## Introduction

The physiological and biochemical properties of limb skeletal muscle have been shown to adapt to a variety of experimental conditions.[1-4] Among these has been shown to be the microgravity encountered with spaceflight.[4,5] In these conditions there has been a reduced contraction of the muscles (hypokinesia) as well as a decrease in the force of contraction (hypodynamia). Our knowledge of the effects of disuse on skeletal muscle contractile kinetics appears rather confusing. It has been generally admitted that the twitch time to peak and its time to half-relaxation are shortened in disused slow muscles,[6,7] but there is no consensus with regard to fast muscles. No change of twitch time course has been observed in fast muscles by some authors,[6-8] whereas slowing of the twitch-to-time course has been reported by others.[9,10] Reports of changes in the maximal rates of tension development and of relaxation have also been controversial in respect disused muscle. It have been observed that these parameters increase in slow muscles, whereas they do not change in fast muscles.[10] The interesting finding that the reduction of the mechanical tension has not been proportional to the reduction of muscle mass, fibre diameter, and concentration of contractile proteins,[8] would suggest that changes in electrical activity might have contributed to the reduction of the contraction force in disused muscle.[11]

It is well known that during muscle contraction mechanical force output decreases gradually. This phenomenon, called "*fatigue*", is probably one of the most intriguing observations associated with contractile activity. Human muscle fatigue studies have been performed under a variety of experimental conditions and, in many cases, the concept of fatigue has been applied to assessing deterioration of muscle performance, i.e., "*the failure point*" at which the muscle is no longer able to maintain the required force or work output level[12] or as "*an inability to maintain the required or expected force*".[13] The cause of muscular fatigue remains elusive, despite intensive efforts to settle the problem.[14]

When a muscle is activated by electrical stimulation at a constant frequency, fatigue is defined as a decrease in tension. Few easily measurable, objective, physiological indices of fatigue exist. These maximally stimulated contractions serve as an index of muscle contractility and have been shown to be independent of central drive.[15] Changes in the surface electromyogram (EMG) have been used extensively as an index of fatigue,[16-18] but the relationship between the EMG and fatigue remains unclear and it has been found that changes in

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the EMG often precede muscle fatigue,[17] and interpretations of the relationship between the EMG signal and fatigue are made difficult by the complexity of the EMG signal. Therefore, in spite of extensive use, the exact relationship between the EMG signal and muscle fatigue has not been defined. The EMG recorded from a muscle during volitional activation has been described as the result of a summation and interference of the motor unit action potentials (AP) from all the active motor units[19] and the characteristics of the EMG have been found to be depend on the firing patterns of the motor units as well as on the configuration of the motor units APs.[18]

Up to now, owing to methodological difficulties, the free contractile properties of human skeletal muscles in a true weightlessness environment or during its simulation have been beyond the field of vision of the scientists who in the main have concentrated on examining the mechanical features of the voluntary muscular contractions. This is the first study to make quantitative measurement of the functional properties of neuromuscular system in men exposed to long-term "*dry*" water immersion. The investigation was concerned with the parameters of the mechanical responses of the triceps surae (TS) muscle, which has been shown to be a postural antigravity muscle.[20] Mechanical and electrical parameters were recorded during electrical stimulation of the motor nerve to distinguish peripheral changes from those occurring centrally.

## Methods

### *Subjects*

A total of six healthy male subject-volunteers, were recruited to participate in this present study after explanation of the experimental protocol. Their mean age, body height and mass, were 22.7±3.5 years, 1.76±0.3 m, and 66.4±2.3 kg, respectively. All subjects were habitually active and had a lean body composition. No subjects was taking medication at the time of the study and all were nonsmokers. Each subject served as his own control. All were familiar with the procedures and gave their informed consent. The study was approved by the Human Ethics Committee at the Institute of Biomedical Problems and informed consent was obtained prior to the experiments.

The experimental protocol was approved by the Russian National Committee on Bioethics of the Russian Academy of Sciences and was in compliance with the principles set forth in the Declaration of Helsinki.

### *"Dry" water immersion (DI)*

DI was used to simulate microgravity.[21] Each subject was positioned horizontally in a special bath on fabric film that separated him from the water (Fig. 1). During immersion, the subjects remained in a horizontal position (a angle which make the body and horizontal line, e.g 5° head-up position) continuously for all activities including excretory function and eating. The duration of the DI was 7 days. The water temperature was constant (33.4°C) and maintained automatically at this level throughout the experiment. The subjects were kept under medical observation

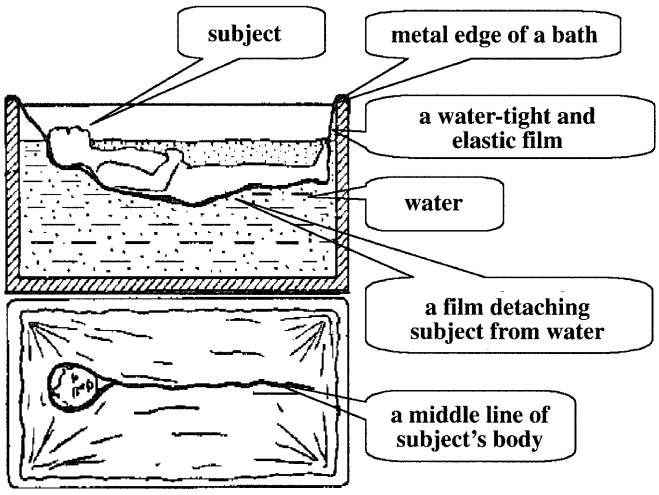


Fig. 1 A scheme of dipping of the man in "*dry*" water immersion environment (on: *Shulzenko & Vil-Villiams*, 1976).

The functional properties of the neuromuscular system were evaluated twice: 8–10 days before the beginning of DI and after it ended. The test protocol was identical for both pre- and post-DI tests.

### *Testing procedure and measurement.*

The mechanical responses of the TS were recorded by tendometry which made it possible to measure the force of a single muscle contraction by the degree of tension change in muscle distal tendon.[22] Measurement of muscle tension using a strain-gauge transducer is based on the physical law of the resolution of forces according to the parallelogram principle (Fig 2, *A*, *insert).* If a strain-gauge transducer is pressed to the tendon, the transducer causes it to bend at an angle. The force ($\mathbf{F}_1$) that is directed along the muscle axis to the proximal point of attaching and originates during the muscle contraction is oppositely directed and equal to the force ($\mathbf{F}_2$) that is directed to the distal point of the tendon attachment. $\mathbf{F}_1$ which is directed across the tendon, operates at the point of the transducer and tendon contraction. If the angle at which the tendon bends is constant, the force ($\mathbf{F}$) recorded by the strain-gauge dynamometer is proportional to $\mathbf{F}_1$ (or $\mathbf{F}_2$). A rigid dynamometer is needed for recording the muscle force using a strain-gauge transducer, because any deformation under tendon pressure will change the transducer position and alter the tendon angle. A steel dynamometer ring was used in our transducer.

The tendometrical sensor was a steel ring of dynamometer with a saddle-shaped special block attached to its surface to support tendon. The degree of pressure between the

tendometrical sensor and the tendon was constant for all the subjects and amounted to 5 kg. To ensure standardization of position and fixation of the limb during assessment, a special set-up was designed as shown in Figure 1, *A*. The apparatus maintained the thigh and lower leg in a standardized position (knee joint angle between tibia and sole of foot at 90 deg). The position of the seat were adjusted to the individual and then firmly secured. A rigid leg fixation ensured isometric conditions for the muscle contraction. The stimulation was performed by supramaximal voltage rectangular wave pulses of 1 ms duration and with the frequency of 150 Hz[22] and 50 Hz.[23]

The isometric twitch and tetanic contractions of the TS muscle were induced by electrical stimulation of the tibial nerve using supramaximal rectangular pulses of 1-ms duration with a frequency of 150 Hz for the tetanic contractions.[22] To stimulate the muscle, the active electrode (cathode, 1 cm in diameter) was located in the popliteal fossa which is the place of the lowest resistance and the anode (a 6 cm × 4 cm in size) was positioned on the lower third of the front of the thigh.

The maximal isometric peak twitch force ($P_t$) was measured from the tendogram of the TS muscle isometric twitch response to a single electrical stimulus applied to the tibial nerve (Fig. 2, *B*). The time from the moment of stimulation to peak twitch (TPT), the time from contraction peak-to-half relaxation time (1/2RT) and total contraction time (TCT) – the time from moment of stimulation to the total muscle relaxation – were calculated from the tendogram of the isometric twitch (Fig. 2, *B*).

On double stimulation when the second impulse was generated at intervals of 3, 4, 5, 10, 20, and 50 ms,[3,22] the maximum amplitude (strength) of the muscle contraction was determined. The maximal strength (amplitude) of the muscle contraction was determined by the maximal amplitude of the second response due to double stimulation where the second impulse was generated at intervals and expressed as a percentage of the twitch contraction.

The maximal voluntary contraction (MVC) was measured from the tendogram of an isometric voluntary contraction performed after the subject had been instructed to contract maximally (Fig. 2, B). The MVC was determined from three contractions of 3–4 s duration separated by 3 min, the largest being considered maximal. During the contractions, the subjects were verbally encouraged and visual feedback was provided. The maximal strength of the contraction ($P_0$) evoked in response to an electrical tetanic stimulation of the tibial nerve innervating the TS muscle at a frequency of 150 Hz was measured from the tendogram as has been described by Koryak.[22,23] The difference between $P_0$ and MVC expressed as the percentage of the $P_0$ and referred to as the force deficiency (Fig. 2, *B*) was also calculated.[22]

The rate of development of increased muscle tension was calculated from the tendogram by the isometric voluntary contraction after the instruction to exert the fastest and greatest tension using a relative scale, i.e. the time of reach 25%, 50%, 75%, 90% of maximal tension.[22,23] Similarly measurements were made for the rate of rise of the evoked contraction, in response to electrical stimulation of the nerve with a frequency of 150 Hz.[22] The maximal rates of voluntary ($dP_{vc}/dt$), and twitch ($dP_t/dt$), and tetanic development ($dP_{ec}/dt$), and tetanic relaxation ($dP_{er}/dt$) were obtained by differentiation of the analogue signal.

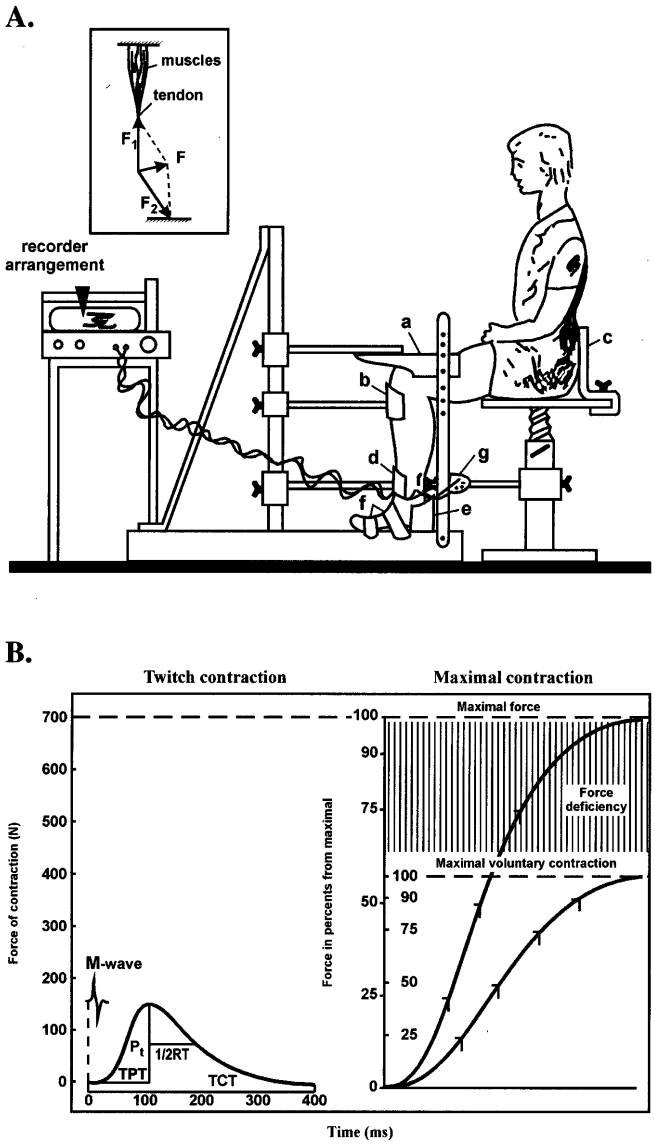


Fig. 2 **A,** A scheme of experimental set-up for measuring isometric voluntary and electrically evoked twitch and tetanic contractions in triceps surae muscle with the principle of tendometry (insert). a, b - supports for knee joint; c – support for hip joint; d – a support for ankle joint; e – a support for heel bone; f – straps for foot; g – a strain-gauge dinamometer. **B,** Measurements of mechanical response parameters in isometric twitch contraction curve (left) and in electrically evoked tetanic tension and voluntary muscle tension development (right). TPT, a time-to-peak; 1/2RT, a half-relaxation time; TCT, a total contraction time; $P_t$, a twitch force.

At the end of the tests, after full rest (4–5 min), the fatiguability of the skeletal muscle was evaluated. The contractile features of the TS muscle were studied during a standard series of 60 1-s electrically evoked (50 Hz) isometric contractions separated by 1-s intervals as has been described.[24] The stimulation frequency was 50 Hz during fatigue tests, because it is within the physiological frequency range of activation of muscle cells during the initial part of strong voluntary contractions,[25] and is the value that gives the maximal isometric tetanic contraction in response to an electrical tetanic stimulation of the nerve innervating the TS muscle.[22]

During the fatigue tests, recording of muscle electrical activity, called electromyography (EMG) or surface action potential (SAP) in the test, was achieved by means of *Ag-AgCl* surface electrodes (8 mm diameter). The two recording electrodes were placed longitudinally over the soleus muscle belly with their centres 25 mm apart. The inter-electrode impedance was less than 5 kΩ. The large grounding electrode (7.5 cm × 6.5 cm) was located in the proximal portion of the leg between the pick-up and stimulating electrodes. Recording of the mechanical (tendometry) and electrical (SAP) responses of the skeletal muscle during intermittent contractions was made during 1-s after the start "of" tetanization of the motor nerve and then for short periods (about 0.2 s) at the end of each subsequent contraction.

The EMG was analyzed from the amplitude of the electrical responses (M-waves, peak-to-peak amplitude of SAP[26,27] as well as the amplitude, duration and area of the first phase of the SAP at the end of 1, 3, 5, 61 and 121 s of rhythmic muscle contraction. To determine the relative extent changes in contractile (C) and electrical *(E)* function the *E:C* ratio was calculated where *E* was the amplitude of the M-wave and C the mechanical response. The *E:C* ratio was calculated at the end of contractions at 1, 3, 5, 61 and 121 s during the electrical fatigue test. The results of the experiment were simultaneously recorded on magnetic tape and the SAP was also recorded on a storage oscilloscope.

The fatiguability of the TS muscle was calculated as the fatigue index, being the mean loss of force of the last five contractions, expressed as a percentage of the mean value of the first five contractions.[24]

### *Data analysis.*

Conventional statistical methods were used for the calculation of means and standard errors (±SE). Differences between baseline (background) values of the subject and those post-exposure were tested for significance by Student's paired *t*-test and Wilcoxon non-paired test. Values are given as mean ±SE in the text and in the figures. Significant differences between means were set at the $p<0.05$ level. The percentage changes for pre- and post-exposure were calculated.

## Results

### *Immersion and strength*

The effects of DI on the MVC is illustrated in Fig. 3, *A*. A decrease in MVC was consistently observed in all subjects by a mean of 18.9% ($p<0.01$). The values of $P_t$ and $P_o$ were not statistically significantly different to the control value [pre 125.6 (±SE 13.7) N compared to post-immersion 139.3 (±SE 18.6) N and 643.5 (±SE 34.3) N compared to post-immersion 590.5 (±SE 56.8) N, respectively]. The force deficiency increased significantly by a mean of 44.1% [pre 25.4 (±SE 3.1) % compared to post-immersion 45.4 (±SE 4.9) %, respectively; $p<0.001$, Fig. 3, *B*].

The mean changes in force of the TS muscle contraction during doublet stimulation in which a second pulse was applied at various intervals, are presented graphically in Fig. 4. The greatest force of contraction under these conditions was an interval of between 4 ms and 10 ms and decreases or

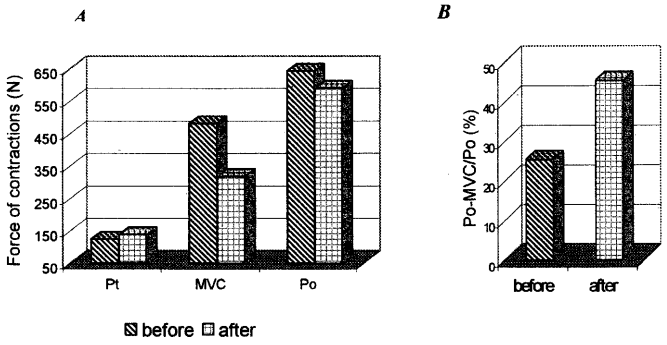


Fig. 3 **A.** The effect of 7-day DI on the maximal twitch response of force ($P_t$), maximal voluntary contraction (MVC) and maximal electrically evoked tetanic contraction ($P_o$, recorded at 150 Hz) in control (before) and disused muscle (after). **B.** MVC force as % $P_o$ after immersion in control and disused muscle.

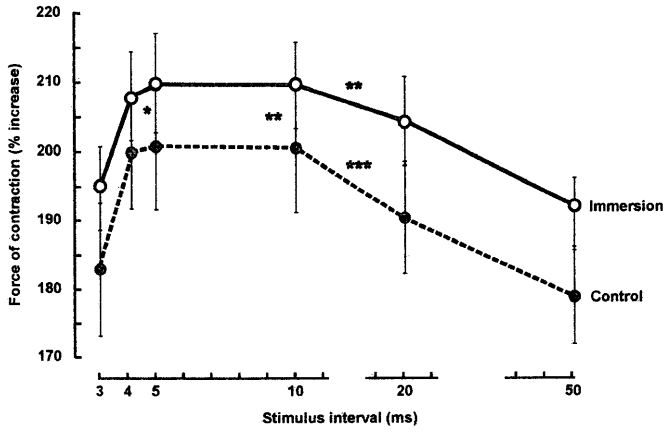


Fig. 4 The maximal contraction (mean ±SEM) of the TS muscle with doublet stimulation at different intervals between impulses, in control and after immersion.
*p<0.05 **p<0.01 ***p<0.001

increases in the interval were accompanied by a considerable decline ($p<0.01$–$0.001$). The relative increase in force as a result of doublet stimulation was significantly greater after long-term of immersion in comparison with the control value ($p<0.05$–$0.01$).

### *Immersion and force-velocity properties*

The decrease in the MVC (19%) was associated with a significant slowing of the rate of tension development during a voluntary isometric contraction (Fig. 5 *top panel*, left) and by a decrease of maximal *dP/dt* [pre 3.29 (±SE 0.39) $N \cdot ms^{-1}$ compared to post-DI 2.25 (±SE 0.39) $N \cdot ms^{-1}$] when measured in absolute terms. The normalized values (% of MVC) of *dP/dt* declined by 9.6% [pre 0.73 (±SE 0.10) $\%P_0/ms$ compared to post-DI 0.66 (±SE 0.10) $\%P_0/ms$] (Fig. 5, *top panel*, right; $p<0.01$).

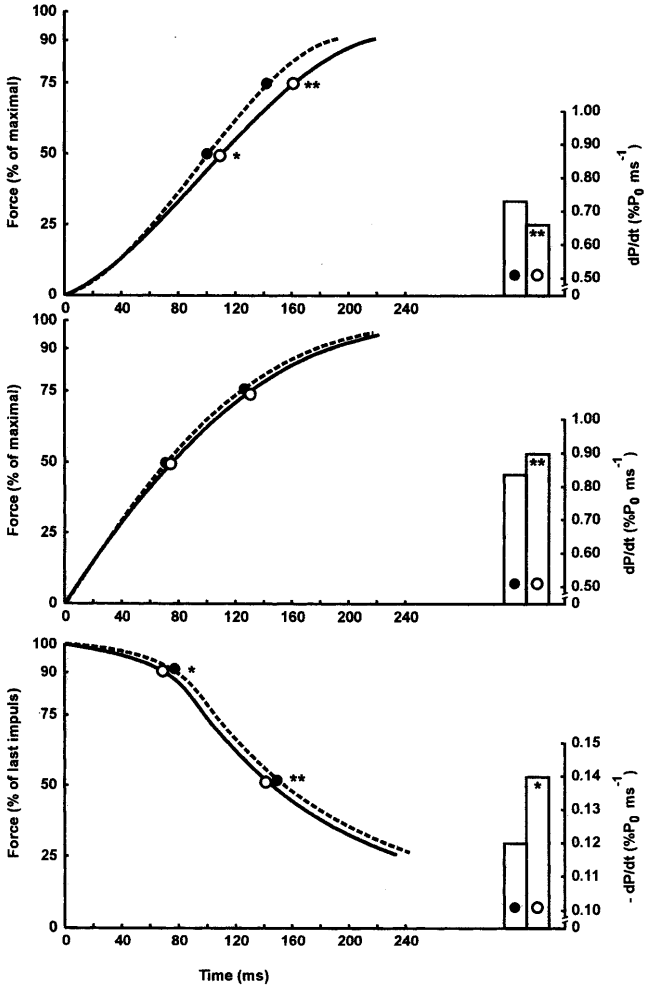


Fig. 5 Average force-time curves for the TS expressed on a relative scale (left) and the maximal rate of rise of tension development (right) while executing explosive voluntary contraction (top panel) and as a result of electrical stimulation at 150 Hz (middle panel), and of the rate of tension relaxation after electrical evoked contraction (bottom panel).
○ - control; ● - after immersion; * $p<0.05$ ** $p<0.01$

Analysis of the *force-time* curve of the electrically evoked contractions did not reveal significant differences (Fig. 5, *bottom panel*, left) while the maximal $dP_{ec}/dt$ was slightly increased by 6.7% [pre 0.83 (±SE 0.06) $\%P_0/ms^{-1}$ compared to post-DI 0.89 (±SE 0.08) $\%P_0/ms$] after immersion (Fig. 5, *bottom panel*, right). The corresponding rate of tension relaxation showed a slight, but significant increase when expressed on a relative scale [pre 0.12 (±SE 0.04) $\%P_0/ms^{-1}$ compared to post-DI 0.14 (±SE 0.05) $\%P_0/ms^{-1}$] (Fig. 5, *bottom* panel, right) and a decrease in time of achievement of maximum value [80 ms *vs.*100 ms].

### *Immersion and fatiguability*

The effects of 7-day DI on the electrically evoked intermittent contractions stimulated at 50 Hz are illustrated in Fig. 6, *A*. In this example, tetanic force decreased gradually to about 57% of its initial value (recorded from the same muscle at 50 Hz). There were no significant differences between the measurements made before and after immersion: the fatigue

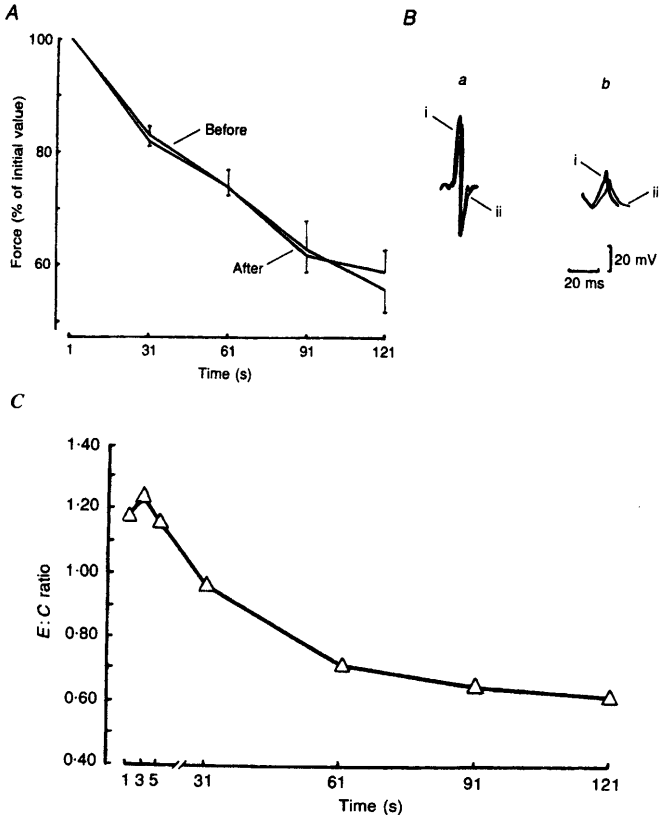


Fig. 6 **A**. Changes in force during 60 intermittent 1 s electrically evoked contractions, separated by 1 s intervals, before and after 7-days immersion. Values expressed as % of initial tetanus force (50 Hz). **B**. Last surface action potential recorded during the 1st (a) and 60th contractions (b) in control conditions (i) and after 7 days of immersion (ii). **C**. Changes in the ratio of electrical responses to muscle contraction force (the E:C ratio) during 60 intermittent 1-sec electrically evoked contractions (50 Hz) after 7 days of immersion.

indices were 36.2±5.4% *vs.* 38.6±2.8%, respectively, (p>0.05). In addition to the loss of force, there were changes in the kinetics of contraction. The time course of the contractile activity of the muscle revealed a number of phases characterized by changes in the speed of contraction. There was an increase in speed compared the initial value at 30–31 s (17%–18%) with a subsequent relative slowing-down at 60–61 s (8%–9%) followed by a slight rise at 90–91 s (11%–12%) with a slowing-down in the last phase (3%–7%). Figure 6, *A* reflects the dynamics of a decrease in force of a muscle. High force isometric contractions interfere with intramuscular blood flow[28] and the different phases of the fatigue curve could be associated with alterations in blood flow as muscle force production changed. It is of particular interest that after disuse, there were no changes in the fatigue curves of the muscle.

***Immersion and electrical processes***

The reduction of muscle force output could be explained by a decrease of electrical activity. Figure 7 shows that in both tests, SAP duration decreased significantly during the first 3 s and the corresponding amplitude and area increased. Thereafter, the duration of the negative SAP phase increased throughout the test, whereas the time courses of SAP amplitude and area were quite different. Their initial increases observed during the first 150 and 250 responses, respectively, were followed not only by a drop to the initial level but by the inversion of the measured parameters as well. At the same time the arrangement of the curves has engaged attention: the increase and/or decrease of the electrical M-waves was greater after DI when compared to the control. Thus, after disuse, with fatiguability both the force of electrically induced contraction and the electrical M-waves (or SAP) were reduced significantly with fatigue. However, after elimination of the gravitational loading, during an intermittent electrically evoked test to induce fatigue there were no differences in the dynamics of the decrease in the TS muscle contraction, but there were significant differences in the dynamics of the changes in electrical responses. The relative extent of the decline in either of these two parameters could be determined from the change in the relationship of the electrical M-wave to the mechanical response of the TS muscle (the *E:C* ratio).

Analysis of the dynamics of the change of the *E:C* ratio revealed a significant difference (Fig. 6, *C*). As shown by this ratio, during muscle work of the same intensity there may have been different changes in the status of the *contractile* and *electrogenic* elements of the peripheral neuromuscular system. The ratio also suggests that, after immersion during the initial 5 s of the test contraction, there was a relatively more marked decrease in the contraction force than of the electrical M-waves. With increasing duration of the work performed there was a relatively more marked decrease in the muscle electrical responses.

***Immersion and excitation-contraction coupling (E-C coupling)***

*E-C coupling* was examined by delivering a single supramaximal stimulus of short duration (1 ms) and studying the resulting response, both of the SAP and the twitch force.

Table 1 and Figure 7, *B* compares *E-C coupling* in extreme situations, before and after immersion. Study of mechanical twitch indicates that with constant $P_t$ and TPT of muscle but maximum $dP_t/dt$ expressed as absolute values decreased significantly. The normalized value (% of $P_t$) $dP_t/dt$ decreased by 5.3% [pre 1.50 (±SE 0.08) $\%P_t/ms^{-1}$ compared to post-spaceflight 1.42 (SEM 0.04) $\%P_t/ms^{-1}$; $p< 0.05$]. The

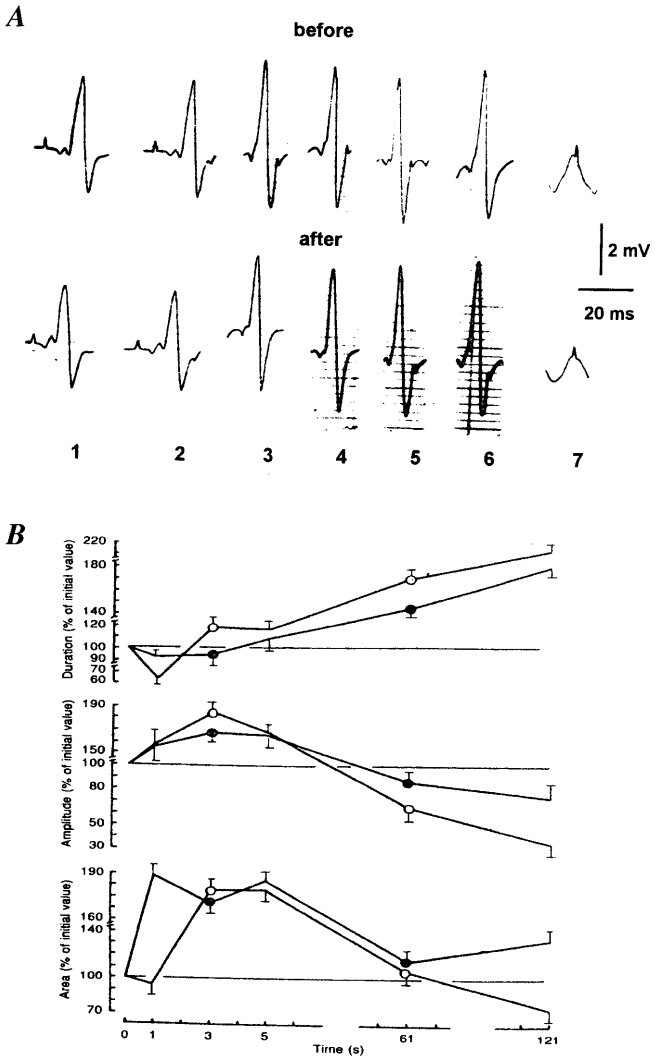


Fig. 7 **A.** Typical record of surface action potentials (SAP) at one subject before and after of immersion.
1 - maximum SAP (M-wave), 2 - first SAP in the rhythmic tetanic (50 Hz) series of contractions; 3–7 - registered at each end of 1, 3, 5, 7, 61 and 121 s before and after of immersion. **B.** Changes of the time course of SAP parameters: duration (top panel), amplitude (middle panel) and area (bottom panel) are expressed as the percentage of their initial value during the fatiguing series of intermittent contractions.
○ - control; ● - after immersion

Table 1 Twitch and SAPs before and after 7-d "*dry*" water immersion

| | Twitch | | | | SAP (*first phase*) | | |
|---|---|---|---|---|---|---|---|
| | TPT (ms) | 1/2RH (ms) | TCT (ms) | $dP_t/dt$ (%$P_t$/ms) | Amplitude (mV) | Duration (ms) | Area (mV·ms) |
| **Control** | 139.3 ±18.6 | 93.7 ±3.3 | 503.7 ±10.1 | 2.06 ±0.29 | 4.6 ±0.6 | 10.4 ±1.0 | 21.6 ±3.6 |
| **Immersion** | 118.8 ±3.4 | 88.7* ±3.8 | 490.3 ±16.8 | 1.77* ±0.19 | 3.5* ±0.5 | 12.8** ±0.7 | 10.2** ±2.0 |
| Δ,% | –14.7 | –5.3 | –2.7 | –14.1 | –23.9 | 23.1 | –52.8 |

*Note:* $\Delta,\% = \frac{control - immersion}{control} \times 100\%$;

$P_t$, Twitch contraction; TPT, Time-to-peak twitch; 1/2RH, Time-to-half relaxation; TCT, Total contraction time; $dP_t/dt$, Maximal rates of twitch development; SAP, Surface action potential;
* and **, $p<0.05$ and $p<0.01$ against control, respectively.

1/2RT, and TCT reduced by 5.3% and 2.8%, respectively. It is interesting that comparison of these mechanical twitch showed no significant difference. The $P_t/P_0$ ratio was reduced by 8.7%. The concomitantly recorded electrical properties indicated that the soleus muscle SAP was significantly changed after immersion (Table 1). The amplitude of SAP was decreased by 14.6% ($p<0.05$) and the duration was prolonged by 18.8% ($P < 0.01$), and the area was found to be decreased by 2.8% after DI.

## Discussion

The effects of reduced motor activity and/or amount of movement on the characteristics of muscular contraction have been studied in relation to morphological, biochemical, and physiological changes.[3,6,7,9,23,29-36] Muscle adaptation to experimental conditions has been thought to result possibly from changes in the motor (neural) command arriving at the muscle,[37,38] and/or peripheral compartments.[34,39] In this context, the question of whether the changes in contractile response of the muscle can be explained only by peripheral processes or whether the neural (motor) command ids also modified during disuse has remained unclear.[34,40,41] At the peripheral sites it is not possible to distinguish between the relative importance of electrical versus mechanical alterations in disused muscles. Another question which remains open is the understanding that although the energetic stores have been found to reduce during immobilization,[7] the kinetics of muscle fatigue have not been found to be proportionally different in disused and control muscles.[8,18]

The most important effect of the experimental conditions in our study was the decreased force of contraction of the extensor muscles of the foot. In this respect the following suggestion can be made. Firstly, the decrease in the force of contraction could have been dictated by a change in the composition of muscle fibres forming the skeletal muscle. It has been found that muscle disuse causes atrophy of muscle fibers[7,42] accompanied by a decline in the synthesis of contractile protein and an increase of its catabolism.[29] However, such a suggestion, is improbable because, as the present results indicate, 7-days of reduced functional requirements did not reveal significant changes in $P_0$ of muscle contraction. Another assumption, which is more probable, lies in the fact that the neural command was changed under the influence of reduced functional requirements. As the findings indicated, the greater decrease of mechanical tension recorded during voluntary contraction, compared with electrically evoked contractions, would support the assumption that the reduction in the strength of contraction during MVC depended to a large extent, if not completely, on changes in the neural command. This point of view is supported by the observation that in response to disuse of the muscle the amplitude was reduced and the interference pattern of the voluntary electromyogram changed.[40,43-45] It has been suggested that a decrease in maximal firing rate could be explained by changes in proprioceptive afferents of motoneurons[46] and/or with slowing of the capacity for activation of the motor units.[34,40] This latter point of view was confirmed by our data indicated that after immersion the value of force deficit has increased almost twofold pointing to a decrease in the neural (motor) command controlling the muscle system. Such observations agree with findings that have been obtained previously.[3,23,36]

Mechanical failure (fatigue) during contraction is probably one of the most intriguing physiological phenomena of muscle capacity. The present work contributes to the discussion of this problem. It examined the specific effects of disuse

on the electrical and mechanical changes in the intact human TS muscle, during intermittent contractions elicited by electrical stimulation of the motor nerve. The possible effects of central (motor) nervous command during these fatigue tests are excluded because of our experimental procedure (cf. METHODS). Our results showed that: (i) the rate of decrease in the force of muscle contraction during rhythmic stimulation to induce fatigue did not differ between control and after disuse, which is in good agreement with previous observations;[8] (ii) stimulation of the SAPs showed a marked decline in amplitude and increase in duration, reflecting changes in the peripheral generation of the action potentials (AP) by the muscular fibres; and (iii) a correlation between the electrical and mechanical responses of the muscle (the *E:C* ratio) indicated that the specific role in fatigue of *electrogenic* and *contractile* elements of the neuromuscular system is changed during the development of peripheral fatigue.

Our findings did not reveal any difference in the reduction of the working capacity after disuse which is in good agreement with previously obtained data[8,41] and confirm Merton's point of view[47] that peripheral mechanisms play an important role in force reduction. Mechanical failure (fatigue) during contraction is probably results from a deterioration in the excitability of the muscle fibres. It has been found that failure of propagation of APs may occur: (i) along the terminal branches of motor nerves,[48] (ii) at the neuromuscular junction,[48] (iii) along the surface of muscle fibres,[15,48,49] and along the T-tubules.[50] The blocking of the fiber APs during stimulation would indicate a failure of excitation, but the location cannot be determined. Blocking of an SAP was invariably preceded by a major change in the amplitude and duration of the fibre AP. An integral index of the state of the *electrogenic* element of the neuromuscular system can be the size (amplitude and area) of the recorded SAP. Comparison of electrical and mechanical failures during intermittent contractions was interesting because this comparison indicated that identical tetanic force reduction after and before disuse is associated with complex SAP change. This observation suggests that muscle intracellular processes must play a major role in the observed contractile failure. Recorded SAP changes during fatigue indicate that different peripheral mechanisms may be involved. The comparison between control and disused muscle indicates that the failure of the electrical processes is different in the two fatigue tests. It appears from Fig. 7 that during first 3 s of contraction, the SAP durations decrease in both tests while the corresponding amplitude and area by the SAP significantly increased. These data suggest that presynaptic and/or end-plate potentials are facilitated, and that the propagation velocity of the AP increases along the muscle membranes[51] and that the dispersion between the fiber APs is reduced.[52,53] The subsequent increase in SAP duration and area, observed without any reduction in SAP amplitude, must be due mainly to the slowing of the conduction velocity along membranes of muscle fibers[15,38] so the SAP broadens in shape.

In the second half of the intermittent contractions, the reduction in SAP amplitude would suggests that presynaptic and/or end-plate failure was then present in intermittent fatigue. This view is consistent with the finding that the SAP area which was previously found to have increased now decreased, although the duration of the SAP keeps increasing throughout the test. It is also consistent with the previous observation that the rise time of the end-plate current has been found to decrease during disuse,[54,55] although the decrease in contraction force was identical in both fatigue intermittent tests. This difference in behavior between nerve and muscle membranes is not suprising, since their architectures are quite different, and recovery of control ionic concentrations must be slower in muscle T (transverse) system compared with nerve membranes. It has been suggested that the presence of a T (transverse) tubular system plays an important role in muscle electrical fatigue.[17,56]

The study of muscle *E-C coupling* is achieved by comparison of twitch SAP and tension development. Our results showed that $dP_t/dt$ was reduced, but $P_t$ was not significantly changed after disuse, whereas changes of corresponding SAP are considerably different. This electromechanical dissociation suggests not only failure of electrical propagation, but that some stage beyond membrane processes changed and played a preponderant role.[47,56] Changes in the configuration of AP have been associated with a depletion in extracellular [$Na^+$], increased [$K^+$], and an increase in [$H^+$] ion of muscle fibres.[15,57] In addition, these suggest that muscle energy metabolism can play an important role in regulating the muscle membrane excitability. An additional factor in enhancing the electrolytes excretion could be that disuse significantly impairs the electrolytic homeostasis as suggested by Noskov et al.[58] and thus affording the reduction of muscle membrane excitability as proposed by Edwards,[13] Bigland-Ritchie et al.,[15] Jones et al.,[57] Lindstrom et al.[59] In the present case, the electrically evoked EMG shown in Fig. 5 strongly supports this hypothesis.

As is evident from our findings, the reduction in the contraction force of the muscle during the intermittent fatigue test was similar in control and after disuse, suggesting, on the one hand, that a developed fatigue cannot be explained by the changes in the contractile apparatus itself as a result of acting factor and, on the other hand, that one of the components (if not the single cause) of developing peripheral fatigue can be the disorders in the *electrogenic* element of the neuromuscular system. The comparison of the changes in mechanical response with the corresponding alteration of electrical response (ratio *E:C)* enables determination of the specific role of the *electrogenic* and *contractile* elements in the development of peripheral fatigue. Analysis of the ratio *E:C* before and after disuse indicated that there occurs the different dy-

namics of changes in the state *of contractile* and *electrical* elements of peripheral neuromuscular system. These results are not contradictory to the above discussions because an interpretation of the *E:C* ratio suggests a linear relation between mechanical responses of muscle, but the relationship between the EMG and fatigue remains unclear[18] and especially when developing fatigue can be impaired. During contraction triggered by electrical stimulation of the motor nerve, the decrease in the contractile force of the muscle cannot be compensated by a modulation of the motor unit firing frequency.[60] The study of electrically evoked fatigue shows that the decrease in force observed during intermittent contractions is not different in control and disused muscles and indicates that fatigue is now not larger after DI. Besides, the normal AP of muscle fiber is several times higher than the threshold value required for an activation of the contractile apparatus,[61] due to this even reduced APs are capable of triggering normal contraction of muscle fibers.

The TPT did not change whereas $dP_t/dt$ was decreased. This dissociation has been observed in many models simulating both intensified use of muscles[62] and disuse created by denervation.[63] The latter evidently may be considered as a nonspecific response of sarcoplasmic reticulum (SR) to functional changes in the muscle. One may usually speculate that TPT and 1/2RT are mainly determined by the changes of SR and capacity of the $Ca^{2+}$ pump[64] whereas *dP/dt* to a greater extent is the function of a myosin-ATPase.[65] Thus, muscle disuse may affect adversely to some extent the cycle of *E-C* coupling possibly due to changes in the sarcolemmal AP, depolarisation of the T-tubule membrane and/or direct effects on the capacity of the SR for $Ca^{2+}$ release and/or re-uptake.

The shape of the *force-time* curve is little affected in this, which is in agreement with the observations[9,35] of relatively constant mechanics of tetanic contraction and the present-day cross-bridge theory of muscle contraction.[66] Reasoning from the statement that the shape of the force-time curve is determined by the net rate of formation and disruption of relation of the cross-bridges[66] which is proportional to the activity of myosin-ATP-ase[67] then it may be assumed that the cycling of cross-bridges and activity of myosin-ATP-ase varies slightly (or not at all) under the effects of immersion. The increase of the normalized rate of tension development ($dP_{ec}/dt$), that we recorded during disuse is in line with the finding that myosin-ATP-ase activity and maximal velocity of shortening are enhanced during immersion.[68]

The cause of the models of weightlessness or spaceflight-induced increase in maximal rate of tension develoment is unknown. Increase in the maximal unloaded shortening velocity might have occurred due to the selective loss of thin filaments.[69] This structural change during spaceflight and models of weightlessness would be expected to increase the distance between the thin and thick filaments, and as a result, the cycling cross-bridges would be expected to detach sooner, which in turn would reduce the internal drag that develops during the final portion of the cross-bridge stroke.[70] Increase in muscle velocity was unable to fully compensate for the reduced force-generating capacity of the muscle.[71]

The increase in the rate of tetanic tension relaxation observed in our experiments allows one to suggest that SR and mitochondria were not less abundant than before immersion and the permeability of the cellular membrane of SR remained unchanged (not increased at least) for ions $Ca^{2+}$ as was noted earlier.[8] Because of this, the assumpion that after muscle disuse there is a decrease in the myosin-ATP-ase activity of SR[31] and the number of $Ca^{2+}$ pumps controlling the rate of tension relaxation[72] can be denied.

It was concluded that the alterations in muscle contraction observed during disuse resulted from changes in neural command and in the peripheral mechanisms associated with the contraction. At the peripheral sites, changes in muscle intracellular processes would appear to play a dominant role in the control of mechanical impairment recorded in disused muscles. Mechanical failure during intermittent muscle contraction involves peripheral sites and mechanisms associated with membrane and intracellular processes. The comparison of peripheral electrical and mechanical changes during intermittent electrically triggered contractions in both contractions indicates that the slowing of AP conduction along nerve and muscle membranes, do not explain the observed mechanical failure.

The comparison between the electrical and mechanical responses, recorded in control and disused muscle, supports the proposition .that electrical changes do not closely control the mechanical failure. Thus among the peripheral neuromuscular changes observed during short duration exercises, muscle intracellular processes must play the dominant role in relation with the observed force decrease during fatigue after disuse.

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