

³¹P-MRS Study of Change in Intracellular pH during Sustained Static Contractions in Human

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³¹P-MRS spectra were obtained from human first dorsal interosseous muscle during and after the voluntary static abduction of the index finger. Endurance tasks were performed at randomly assigned contraction levels of 15, 20, 30 and 40% of maximal voluntary contraction (MVC). Muscle pH was calculated according to Taylor et al. (1983) using chemical shift between inorganic phosphate (Pi) and phosphocreatine (PCr) on the ³¹P-MRS spectra. Mean values of endurance times of static contractions were 7.25, 5.33 and 3.08 minutes for 20, 30 and 40%MVC, respectively. At 15%MVC, all of the four subjects maintained contraction for 30 minutes, and the contractions were terminated at 30 minutes. Muscle pH at the onset of contractions were 7.12, 6.98, 7.01 and 7.08 for 15, 20, 30 and 40%MVC, respectively. At the end of contractions when the subject could not maintain the force level, muscle pH were 6.07, 5.97 and 5.94 for 20, 30 and 40%MVC, respectively. There was no significant difference in muscle pH at the end of contractions between three conditions by one-way ANOVA. In conclusion, there was a critical muscle pH of about 6.0 where static contractions could not be maintained.

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The definition of muscle fatigue is difficulty to maintain the muscle force or power output (Porter and Whelan, 1981). In dynamic muscular contraction, blood flow to working muscle is kept well by muscle pump and activation of cardio-vascular system. Endurance of dynamic contraction depends on mainly muscle glycogen content as energy source (Bergstrom et al., 1967). On the other hand, static contraction causes an increase in blood pressure and total peripheral resistance. Muscle lactate production associated with glycolysis is thought to be one of the factors causing muscle fatigue. Donaldson and Hermansen (1978) reported that metabolic acidosis in muscle tissue following lactate accumulation inhibits activities of glycolytic enzyme and the release of calcium from sarcoplasmic reticulum.

However, Sahlin (1986) suggested that inhibited resynthesis of ATP leading a muscle fatigue is due to a decrease in phosphocreatine and a decreased enzymatic activity should be recovered by an increase in ADP or AMP.

Many studies concerning static muscular work have been done using electro-myography and measuring of force output and work endurance time. Since Hermansen and Osnes (1972) established the application of muscle biopsy technique into the study of muscle physiology, muscle fatigue has been studying at cellular level by measuring biochemical changes in muscle.

³¹P-MRS (magnetic resonance spectroscopy) is very useful to study the energy metabolism in human muscle because this technique is non-

invasive and able to collect data continuously during an experiment. We can obtain the intracellular pH of a muscle from chemical shift between phosphocreatine (PCr) and inorganic phosphate (Pi) on the spectra. As described above, muscle pH related with muscle fatigue, and be able to become an important parameter to study the muscle fatigue.

The purpose of this study is to evaluate the mechanism of muscle fatigue during sustained voluntary contractions in human with special reference to the change in muscle pH.

METHODS

Subjects :

Four healthy males volunteered as subjects. The first dorsal interosseous muscle of right hand was tested in each subject. This muscle is the only one which produces abduction of the index finger (Ranatunga et al., 1987).

Measurement of ^{31}P -MRS during the endurance task :

Maximal voluntary contraction force (MVC) was measured in abduction of the index finger isometrically by a straining device connected to proximal interphalanx joint of the index finger (see Figure 1).

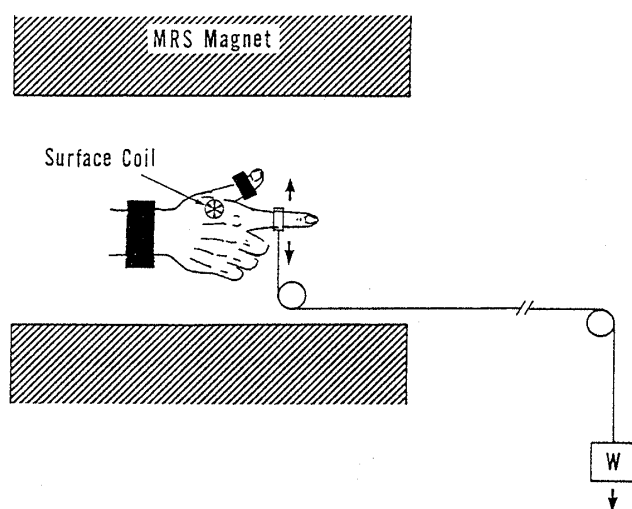


Fig. 1 A diagrammatic representation of the ^{31}P -MRS measurement. W shows the weight corresponding to 15, 20, 30 and 40% for MVC of each subject.

The MVC were determined in triplicate and highest value of them was taken the MVC, to which the load used in the endurance task was determined.

Right hand was placed in the bore of MR spectrometer and stabilized on the wooden plate horizontally as shown in Figure 1. Endurance tasks were performed at randomly assigned contraction levels of 15, 20, 30 and 40% of MVC. ^{31}P -MRS spectra were obtained before (for 2 minutes), during and after (for 5 minutes) the endurance task. During the endurance tasks, the isometric contraction was sustained until the subject could not maintain the force level. The duration of the sustained contraction was called the endurance time. If the endurance time exceeded 30 minutes, the contraction was terminated at 30 minutes.

Each subject performed one endurance task in a day. In each experiment, the MVC was first measured, and contraction level in the endurance tasks was recalculated to this value.

The MR spectrometer used in this study has a 1.9 T, 27 cm bore superconducting magnet, operating at resonance frequencies of 34.05 and 84.13 MHz for ^{31}P and ^1H , respectively. Radiofrequency pulses were applied at 2 seconds intervals and each spectrum represents the accumulation from 60 pulses. Muscle pH was calculated from the chemical shift between PCr and Pi according to Taylor et al. (1983). Pi-to-PCr ratio (Pi/PCr) was calculated from the peak area measured by a planimeter.

Measurement of blood lactate during the endurance task :

Blood lactate concentrations in one of the four subjects were measured during endurance tasks in the same manner of ^{31}P -MRS study. Venous blood was sampled from median cephalic vein at the midpoint of forearm every 2 minutes during contraction and 2nd, 4th and 5th minutes of recovery. Under the condition of 15% MVC, venous blood was taken every 4 minutes during contraction because all of the four subjects performed the contraction for 30 minutes. Blood lactate was obtained by an enzymatic

analysis of the blood.

RESULTS

Table 1 shows the endurance time of each subject at four contraction levels. As described above, endurance times were 30 minutes in all the four subjects at the level of 15% MVC.

^{31}P -MRS spectra of the first dorsal interosseous muscle at rest and at the end of contractions of 20, 30 and 40% MVC are shown in Figure 2. At the end of contractions, PCr almost disappeared and Pi increased obviously.

Figure 3 shows the change in muscle pH during endurance tasks and recovery periods. Though all of the 4 subjects could keep the force level for 30

Table 1 Maximal voluntary contraction force (MVC) and endurance time (ET) in each subject.

Subj	Age (yrs)	MVC (N)	ET (min)			
			15%MVC	20%MVC	30%MVC	40%MVC
A	31	46	>30	5.67	3.67	2.33
B	29	40	>30	9.00	5.00	4.33
C	28	37	>30	10.33	9.00	3.33
D	25	42	>30	4.00	3.67	2.33
Mean	28	41		7.25	5.33	3.08
SE	2	2		1.46	1.26	0.48

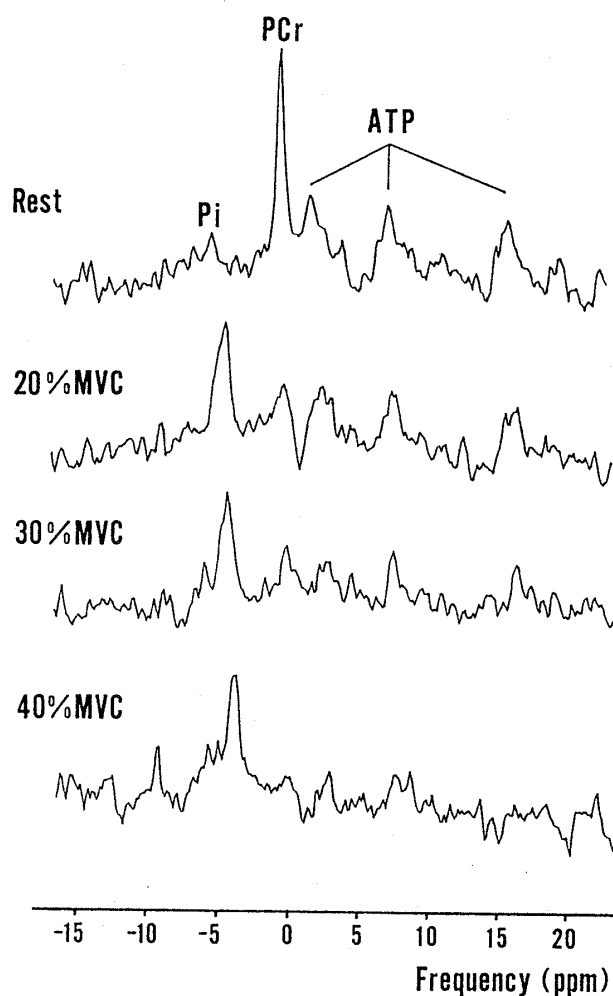


Fig. 2 ^{31}P -MRS spectra of one of the four subjects taken at rest and the end of contractions at 20, 30 and 40% for MVC.

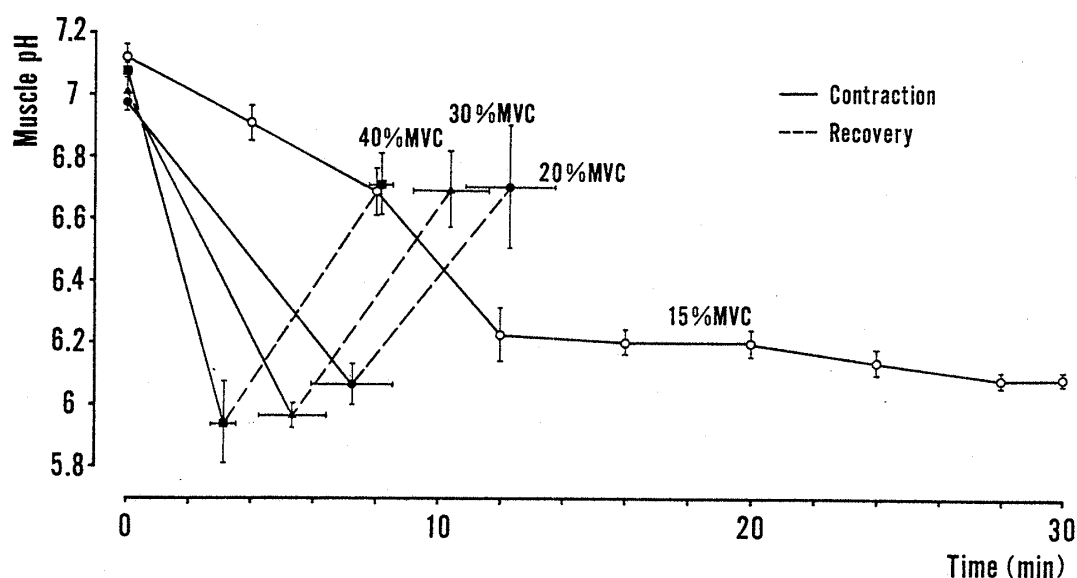


Fig. 3 Change in muscle pH during contraction and after the contractions of 15, 20, 30 and 40% for MVC. Values are means $\pm$ SE.

minutes at 15% MVC, muscle pH decreased to 6.41 at the 10th minute and to 6.10 at 30th minute. At the end of contractions, muscle pH reached to 6.07, 5.97 and 5.94 for 20, 30 and 40% MVC respectively. And in these three contraction levels, it was recovered to 6.7 at 5th minute of recovery. There were no significant differences in muscle pH between them by one-way ANOVA ($F=0.419$ at the end of contraction and 0.005 at 5th minute of recovery, $d.f.=2, 9$). These results describe that at the end of contractions when the muscle force could not be maintained, muscle pH showed about 6.0 in spite of the contraction level, and that the decrease in muscle pH due to contraction recovered quickly and showed almost the same recovering velocity between the three conditions.

At the end of contractions of 20, 30 and 40% MVC, mean values of Pi/PCr were 2.13, 3.44 and 4.07, respectively. These values also did not show significant difference by one-way ANOVA ($F=0.480$, $d.f.=2, 9$).

In the Figure 4, muscle pH and Pi/PCr at the end of contractions of 20, 30 and 40% MVC were plotted against the recalculated %MVC in each subject.

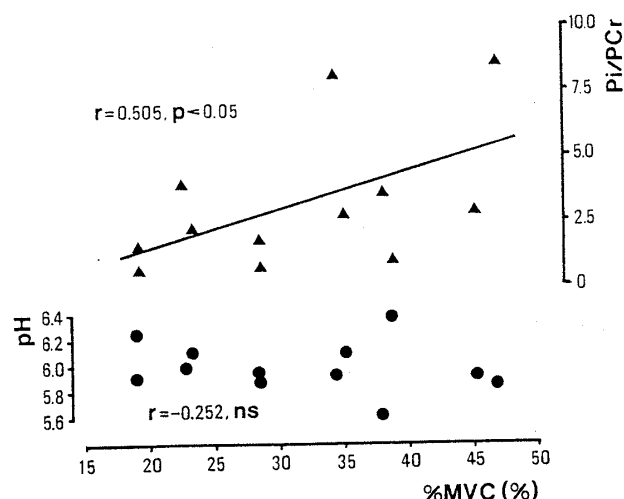


Fig. 4 Pi-to-PCr ratio (Pi/PCr, top) and muscle pH (pH, bottom) at exhaustion in relation to relative force (%MVC).

There was a significant relationship between Pi/PCr and %MVC. This result describes that energy metabolic state shown by an increase in Pi and a decrease in PCr did not show the critical level though the contraction could not be maintained.

Figure 5 shows the change in blood lactate during endurance tasks and recovery period. At 15% MVC, blood lactate maintained the resting level during

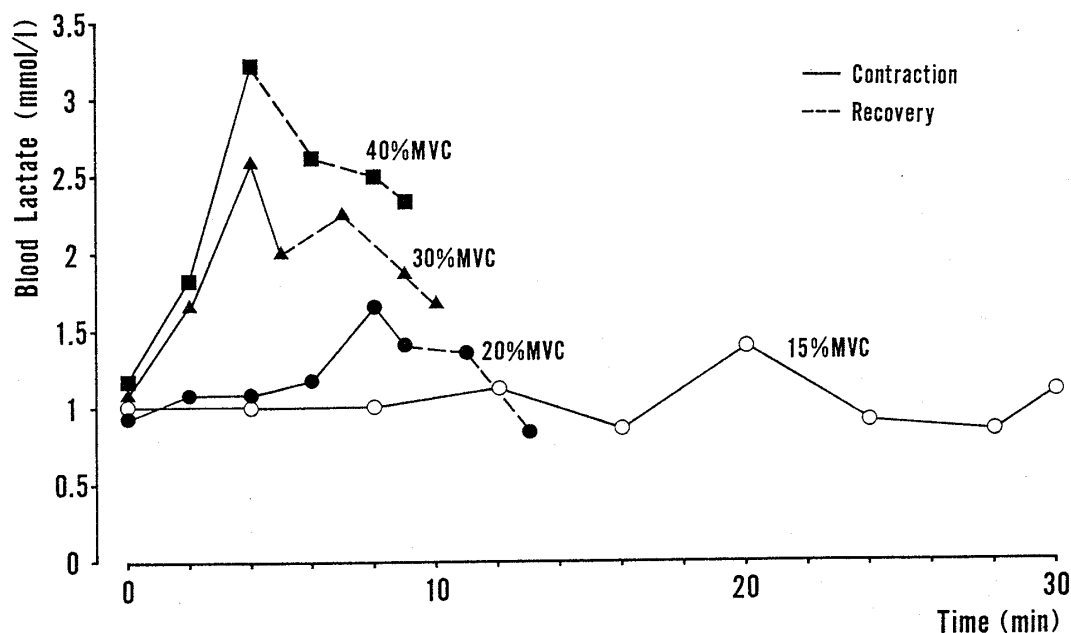


Fig. 5 Change in blood lactate concentration during and after the contractions of 15, 20, 30 and 40% for MVC in one subject.

contraction. At 20, 30 and 40% MVC, blood lactate increased during contraction and depended on the contraction levels at the end of contractions. These results show that blood lactate concentration did not reflect the muscle tissue acidosis taking the change in muscle pH into consideration.

The relationship between contraction level (% MVC) and endurance time for the four subjects is presented in Figure 6. The regression of the endurance time (Y) on %MVC (X) was obtained by the following equation: $\log Y = a + b \log X$. In Figure 7, the endurance time is plotted against the decrease rate of muscle pH (dpH/dt , X) for the four subjects in the same manner as Figure 6. In these regression analysis, endurance time at 15% MVC was regarded as 30 minutes as a matter of convenience. Significant correlation coefficients were obtained in both regressions.

DISCUSSION

Sahlin (1986) described that a decrease in muscle tissue pH was dependent on lactate production due to glycolysis, and that 85% of released hydrogen (H^+) was explained by lactate production. Karlsson (1971) showed about 16 mmol/kg wet muscle of lactate at the exhaustion when the endurance time was within 7 minutes. In the present study, the muscle tissue pH at the end of contraction was about 6.0 in all contraction levels except for 15% MVC. So, in these three contraction levels, almost the same degree of lactate accumulation in the tissue was supposed to occur. However, Karlsson and Ollander (1972) demonstrated that during isometric contraction of quadriceps lactate accumulation in the muscle was increased in relation with contraction level within 50% MVC. In the report,

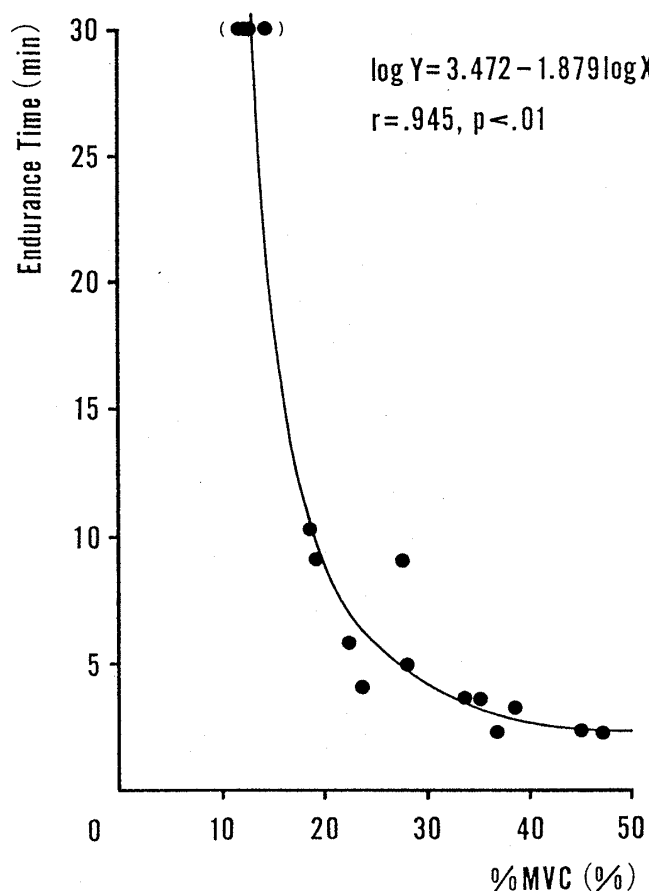


Fig. 6 Relationship between endurance time for sustained contraction and relative force (%MVC).

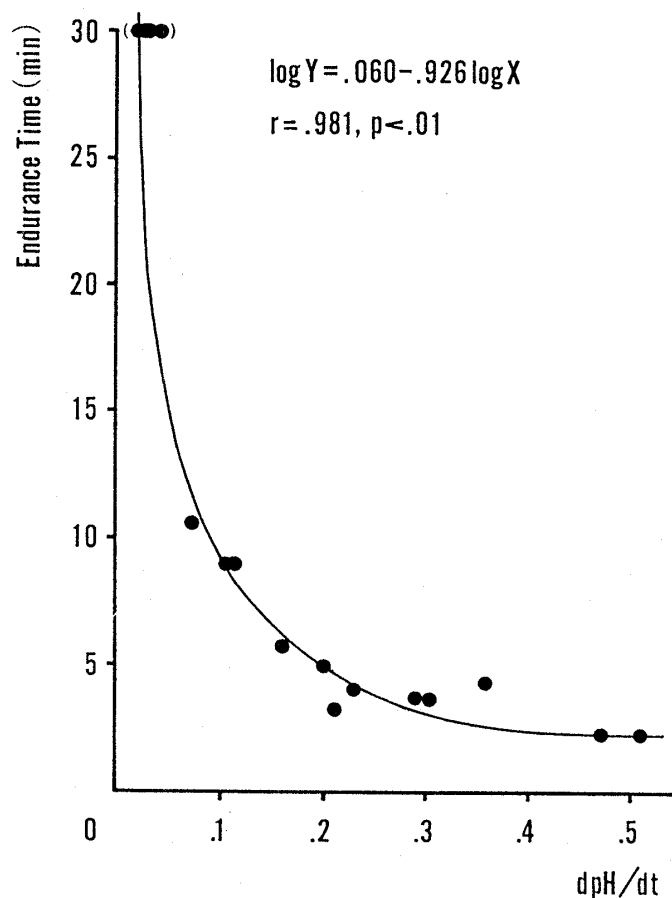


Fig. 7 Relationship between endurance time for sustained contraction and changing rate of muscle pH (dpH/dt).

muscle tissue pH was not measured, and the quadriceps which they evaluated was a large muscle group in which recruitment and activation of muscle fiber could be changed with a progressive muscle fatigue. Thus, the vastus lateralis from which the muscle tissue was sampled did not always show the state of the whole working muscle.

A part of lactate produced by glycolysis is converted to pyruvic acid in the muscle tissue and utilized in aerobic metabolic pathway (Karlsson and Jacobs, 1982). Jorfeldt et al. (1978) studied the lactate released from muscle tissue into blood in relation of muscle lactate production at submaximal exercise using bicycle ergometer. In the report, though lactate released into blood in proportion to muscle lactate within 4 or 5 mmol/kg wet muscle, the muscle lactate was released at constant rate and accumulated in the tissue above more than that level of muscle lactate. Ahlborg et al. (1972) showed the linear relation between lactate production and contraction level during the isometric contractions in human skeletal muscle. In the present study, though muscle tissue pH was almost the same at the end of contraction in the three contraction levels, blood lactate was increased in proportion to contraction level. This results shows the difference in lactate production velocity between contraction levels. At 15% MVC, though muscle tissue pH was decreased, blood lactate did not show the change during contraction. So, at this level of isometric contractions, lactate produced in muscle tissue was assumed not to release into extracellular fluid and decreased muscle tissue pH. But, we did not know the lactate concentration in lymphatic flow which might be another way of lactate efflux from the muscle (Karlsson and Jacobs, 1982).

Hermansen and Osnes (1972) reported the decrease in muscle tissue pH to 6.4 by maximal bicycle exercise. And, another report concerning muscle fatigue by dynamic exercise showed muscle tissue pH decreased to about 6.6 (Jones et al., 1977; Sutton et al., 1981; Costill et al., 1984). Sahlin et al.

(1975) studied the isometric contractions of human skeletal muscle showed 6.56 of tissue pH at muscular fatigue. In these studies, a chemical analysis was used to evaluate the tissue pH. On the other hand, Wilkie et al. (1984) showed 6.1 of tissue pH at muscular fatigue evaluated using ^{31}P -MRS. This result is well consistent with that of the present study. Though there were variations in muscle measured and condition of muscle contraction in the studies described above, tissue pH at muscle fatigue tend to be higher by chemical analysis than by ^{31}P -MRS. It is suggested that there may be systematic error in analytical procedure between the two methods, and that there is a critical muscle pH of about 6.0 where the voluntary muscle contractions could not be maintained.

The relations between muscle fatigue and decrease in tissue pH have been discussed with reference to the following: inhibition of phosphorylase activity by increase in H^+ (Ahlborg et al., 1972; Donaldson and Hermansen, 1978), decrease in PCr and increase in ADP (Sahlin, 1986), and decrease in blood flow according to increase in peripheral resistance of vessels (Sinoway et al., 1989). Sahlin (1986) proposed the following hypothesis: PCr is essential to phosphorylation of ADP and increased ADP according to decrease in PCr inhibits the Na-K-ATPase activity followed by increase in extracellular K^+ . Certainly, when the electrical stimuli on efferent nerve makes muscle contraction in vitro, muscle tension decreases gradually in relation with the change in PCr. In the present study, however, muscle tension was kept constant until exhaustion although PCr decreased during contraction, and Pi/PCr tended to increase in relation with contraction level at exhaustion. Thus, the change in PCr and Pi should play an important role in muscle fatigue, but it is difficult to conclude that the state of energy metabolism is the intrinsic limiting factor of continuance of muscle contraction.

The endurance time of voluntary isometric contractions has a logarithmic relation with contrac-

tion level expressed in % MVC (Sato et al., 1984). In the present study the endurance time showed the same relation with decreasing rate in muscle tissue pH in addition to % MVC (Figure 6 and 7). This relationship also shows the effect of a decrease in muscle pH on maintenance of muscle force. One of the mechanisms of this effect is assumed to inhibit phosphorylase activity by an increase in H^+ . However, the muscle tension is maintained by an increase in efferent nerve activity, so it is difficult to explain muscle fatigue in human voluntary contraction only from the view point of change in energy metabolism.

It may be assumed that at the muscle fatigue when it was impossible to keep the tension constant, there were some changes in excitation-contraction coupling in neuromuscular unit in addition to some chemical changes in muscular cell per se. Nakamura and Schwartz (1972) reported that an increase in Ca^{++} binding protein produced by an increase in H^+ inhibits the ATPase activity. In addition, an increase in extracellular K^+ induced by lactate efflux from the muscle inhibits the depolarization on muscle membrane and chemical transmission at endplate.

In conclusion, 1) a decrease in muscle tissue pH can be found by lactate production even at low level of static muscular contraction, and 2) endurance of voluntary contractions is mainly affected by a decrease in muscle tissue pH. And, there is a critical muscle pH of about 6.0 where static contractions could not be maintained.

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