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STUDY ON CONTRACTION AND RELAXATION OF EXPERIMENTALLY  
DENERVATED AND IMMOBILIZED MUSCLES: COMPARISON WITH  
DYSTROPHIC MUSCLES

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16. Abstract  This study surveyed the contraction-relaxation mechanism of experimentally denervated and immobilized muscles of the rabbit. Results are compared with those of human dystrophic muscles, in order to elucidate the role and extent of the neurotrophic factor, and the role played by the intrinsic activity of muscle in connection with pathogenesis and pathophysiology of this disease.			
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Original article

Study on contraction and relaxation of experimentally denervated and immobilized muscles: Comparison with dystrophic muscles.

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Summary: It has been well known that various characteristics of muscle cells are under neural control. On the other hand, the muscle itself possesses some control mechanism. Under the concept of active state, this study surveyed the contraction-relaxation mechanism of experimentally denervated and immobilized muscles of the rabbit, and the results are compared with those of human dystrophic muscles, in order to elucidate the role and extent of the neurotrophic factor, and the role played by the intrinsic activity of muscle in connection with pathogenesis and pathophysiology of this disease. The findings include that: (1) A decrease in acceleration of generation of simple contracting force that reflects the state of  $Ca^{++}$  of muscle vesicles, or abnormal

reactions to epinephrine through B-receptors, are noted only in the denervated muscle. (2) Not only changes in state of  $Ca^{++}$  of muscle vesicles, abnormalities in various parameters such as the process subsequent to  $Ca^{++}$ -binding by troponin, the prolongation of relaxation time of simple contraction force induced by forced contraction loading, of simple contracting tension relaxation time that reflects the state of contracting protein, staircase phenomenon, and forced contracting tension and its speed of generation, are noted both in denervated and immobilized muscles, but are more prominent in the latter. (3) Denervated muscles exhibited subnormal reaction, similar to that seen in human dystrophic muscles, to caffeine that acts on the dissociation-combination process of  $Ca^{++}$  in muscle vesicles. (4) Normal reactions are shown by dystrophic, denervated, and immobilized muscles to dantrolene that relaxes muscles through steps partially different from those with caffeine. From the foregoing observations, it is suggested that pathogenesis of this disease is complex with the combination of nervous and muscular abnormalities, rather than single factors at work.

#### Introduction.

The role of neurotrophic factors in controlling electrical, chemical, and mechanical characteristics of the muscle has been studied through cross dominance experiment of nerves, as well as causing changes in neuromuscular synapse, axon flow, and muscle activities. This concept of neurotrophic factor has given some support for the neural origin theory for pathogenesis of progressive muscular dystrophy, but has not given definitive conclusions. Since muscle contraction could be changed and controlled by various

treatments such as disuse, and overuse while maintaining the normal neuromuscular relationship, some role, at least partially, played by muscle activity itself, has been considered. Based on experiments that show controlling of muscle contraction is possible by the application of electric currents of different frequencies artificially, some researchers state that the results of neural crossing dominance experiments are not due to the presence of chemotropic factors specific to the motor neurons, but due to variations in the nerve impulse patterns (phasic, tonic). However, experiments on muscle activities cannot change fast and slow muscles completely. Previously we carried out, in human dystrophic muscles, especially in peripheral muscles, in the early phase of the illness, prior to the appearance of physiologically detectable abnormalities in items expressed by electric parameters such as neuromuscular transmission and muscle surface membrane properties, analyses of muscle tension curves. The results revealed the presence of abnormalities in muscular intracellular structures and in the catecholamine reactivity mediated by their membrane receptors (1).

As the pathological process progresses, electrical parameters such as action potential show some changes. Analyses of muscle tension at such a stage would not render true results, as the expression of cell structures would be modified by electrical changes on the surface membrane. When the pathological process is far advanced, nonspecific changes will become more prevalent, thus possibly masking specific changes of dystrophic muscles.

Thus in this study, comparisons are made between various changes of experimental rabbit muscles observed at different periods of time, and those of dystrophic muscles, so as to elucidate which

are reflecting neural factors and which are reflecting intrinsic muscle factors among various abnormalities of contraction and relaxation of dystrophic muscles. For study of neural factors, muscles for which dominant nerves have been severed for a given period of time are used. For study of intrinsic muscle factors, muscles immobilized by joint fixation (hereinafter referred to as immobilized muscles) are used. In the latter, the peripheral nerve and the neuromuscular synapse are intact, and the cholinergic synapse and other factors that might have some effects on muscles are maintained normally while muscles are immobilized. Under these circumstances, if changes are noted on cell membrane of muscles or various intracellular functions, they could be attributed to the effects of intrinsic muscle activities.

#### Method.

The tibialis anterior muscle of rabbits (body weight 2-2.5kg) anesthetized with pentobarbitone sodium (20mg/kg) is used. For denervation experiment, the right sciatic nerve in 10 rabbits is severed at about 8cm proximal to the tibialis anterior muscle, and the skin of the incised area is sutured aseptically. Analyses are made 20-24 hours later, when amplitude of muscle action potential induced by neural stimulation is lowered but that induced by direct muscle stimulation is unchanged (that is when abnormalities in neuromuscular transmission level start, preceding the muscle surface membrane level). Using needle electrode electromyography, the absence of fibrillation and increase in insertion action potential is confirmed.

For immobilization experiment, the right knee and foot joints in 10 rabbits are fixed (aseptically) at 90° with pieces of Kirchner

steel wire, and observations are made in 4 weeks.

In both of these experiments, the left tibialis anterior muscle is used for control. After physiological experiments, the experimental muscles, along with the control muscles from the contrasting side, are used for weight determinations and histopathological examination (hematoxylin-eosin and masson-trichrome stains and myofibrillar ATPase activities).

Electrophysiological experiments are carried out by placing the rabbit in the supine position on a table, fixing the knee and foot joints, anesthetized with pentobarbitone (20mg/kg), with muscular temperature held constantly at 33°C. For indirect stimulation of the tibialis anterior muscle, action potential is induced by placing platinum wire electrode on the fibular nerve exposed at the popliteal fossa. For direct stimulation, bipolar needle electrode is inserted directly into the muscle to cause electric stimulation by rectangular waves. For recording, silver plate surface electrodes fixed at the mid-belly and at the tendon (diameter 1.5mm) are used. With direct stimulation, 0.03% d-tubocurarine chloride is injected into the ear lobe vein with electric powered micordrip during experiment in order to avoid stimulation of intramuscular nerves.

The tibialis anterior muscle tension is determined by an isometric strain gauge myograph, to which an end of tendon and a portion of the muscle removed from the surrounding structures, are connected with a piece of stainless steel wire in the horizontal direction. The methods of stimulation and recording are as noted above. The stationary tension is fixed with the tension such that the maximum single contractile force could be obtained. From the single contractile tension curve, we obtain the maximum single contractile force (Pt),



time  $T_{\frac{1}{2}R}$  from the generation of tension through  $P_t$  to the relaxation of  $\frac{1}{2}$  of the maximum force, time  $T_d P_t/dt$  from the generation of action potential to the first power differential peak value of the tension (maximum speed of tension generation), and the second power differential peak value of the tension (maximum acceleration in tension generation)  $d^2 P_t/dt^2$ . Also, the forced contractile tension curve is constructed by repeated stimulation at 100Hz, and its maximum value  $P_o$  and the first power differential peak value  $dP_o/dt$  are recorded. The significance of these biological parameters on muscular contraction and relaxation, based on the concept of active state, are shown in table 1 (1-3).

Table 1.

Abbreviations	Measurements	Active state properties
$P_t$	Maximum single contraction force	Force generated by contractile component plus series elastic component
$T_{\frac{1}{2}R}$	Time from generation of tension, through the peak single contractile force, to half that amount of tension	Decay of active state
$T_d P_t/dt$	Time from generation of action potential to the peak value of the first power differential of the single contractile tension curve	Duration of active state
$d^2 P_t/dt^2$	The peak value of second power differential of single contractile tension curve (maximum rate of acceleration in generation of single contractile force)	Active state intensity of shortening
$P_o$	The maximum strength of forceful contractile tension generated by repeated stimulation with 250/100Hz	Active state intensity of load-bearing
$dP_o/dt$	The peak value of first power differential of the forceful contractile tension curve (maximum speed in generation of forceful contractile tension)	Force-velocity relation; rate of formation of cross-bridges; series-elastic component

The staircase phenomenon in single contractile force is computed by comparing the first and 120th contractions with repeated 1Hz stimuli for 2 minutes. The phenomenon of increase in single contractile force induced after forceful contractile loading (post tetanic potentiation) is computed by comparing the single contraction induced a minute after 10 seconds of repeating 50Hz stimuli for 10 minutes with that induced prior to the loading. In each case, the conditions for simultaneously recorded muscle action potentials are set so that they would not change before and after stimulation loading, and induced single contractile tension would return to the preloading levels within 10 minutes in the former, and 15 minutes in the latter.

For pharmacological testing, isoproterenol (5mg/kg), caffeine (50mg/kg), and dantrolene (4mg/kg) are injected through the ear lobe vein, various parameters of muscle tension curves are measured before and after the administration of medication, and results are compared. The results of tension measurements of reaction of human dystrophic muscles to epinephrine have been reported elsewhere (1). For the responses to dantrolene which are presented in detail in this report, 5 cases of dystrophic long muscles of limb without evident abnormalities in the electric parameters in the peripheral muscles, and 5 cases of normal control (age-matched) are used. Three hours after oral administration of 100mg of this medication, the isometric single contractile force and forceful contractile tension (repetitious stimuli at 250Hz) of the adductor pollicis muscle induced by stimulating the ulnar nerve are measured,

differentially analyzed, and pre-medication and post-medication results are compared, along with simultaneously induced muscle action potentials.

Results.

### I. Forces of single contraction and forceful contraction

The measurements obtained from the normally innervated tibialis anterior muscle of rabbits (20 cases) included the maximum single contractile force (Pt) 104-144g, half relaxation time ( $T_{1/2}R$ ) 37-48msec, and maximum force of forceful contraction (Po) 690-1000g. Along with the results of differential analyses of each, these are shown in figure 1 (shaded portion).

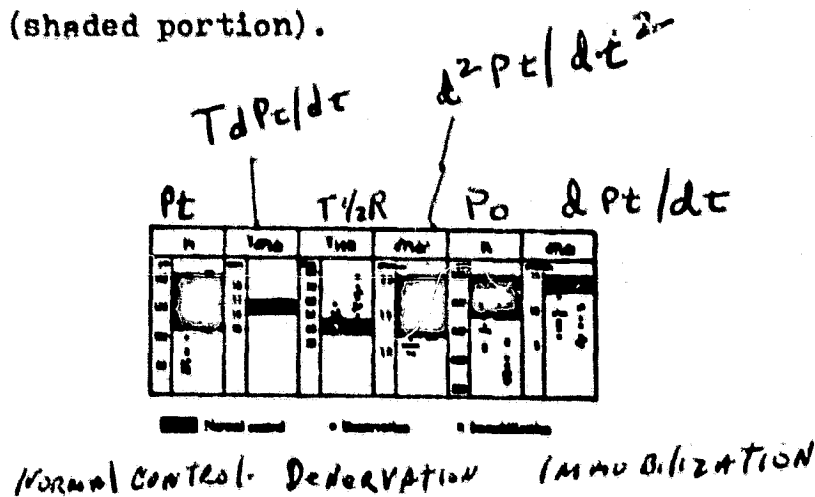


Fig. 1.

The values of muscle tension analyses for the muscles denervated by resection of the sciatic nerve (10 cases, marked  $\bullet$  in figure 1) are chosen for the time during which the muscles had amplitudes of muscle action potential induced by direct muscle stimulation to be within normal range (22-26mV), but those induced by neural stimulation are lowered (8.8-19mV). With the immobilized muscles which are fixed for 4 weeks (10 cases, marked X in figure 1), the amplitudes of induced muscle action potentials are equal and within

normal range for both neural and direct muscle stimulation. The most remarkably abnormal parameter in the denervated muscle is the decrease of single contractile force (Pt) due to the decrease in the rate of acceleration of tension generation ( $d^2Pt/dt^2$ ). Noted in the immobilized muscle are the prolongation of relaxation time in the single contractile tension curve ( $T_{1/2}R$ ), and the decrease of forceful contractile force (Po) and rate of speed of its generation ( $dPo/dt$ ). Examples of muscle contractile tension curves for the normal, denervated, and immobilized muscles and their analyses are shown in figure 2.

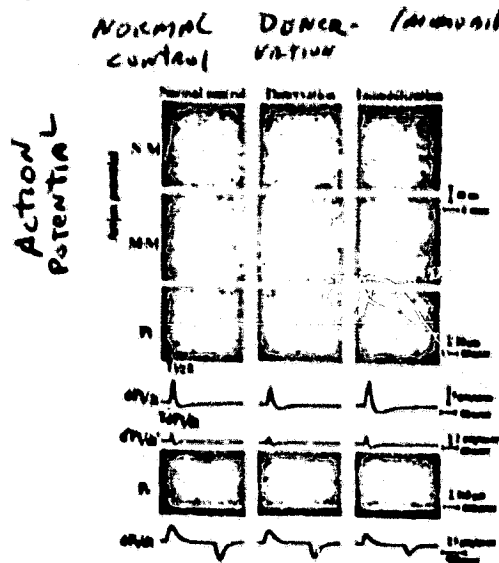


Fig. 2.

Histochemical examination revealed type II fiber dominant in the normal muscle, while fiber type atrophy and -11 ~ -21% weight loss, without increase in connective tissue, in the immobilized muscle.

## II. Staircase phenomenon and posttetanic potentiation.

The normally innervated rabbit tibialis anterior muscles (16 cases) are repeatedly and directly stimulated with 1Hz for 2 minutes. This resulted in the increase of single contractile force (Pt) (115-139%), accompanied by the increase in the rate of acceleration of the generation of single contractile force ( $d^2Pt/dt^2$ ) (125-160%)

and the shortening of the relaxation time ( $T_{\frac{1}{2}R}$ ) (87-94%) (Figure 3 SC; figure 4 left, shaded portion).

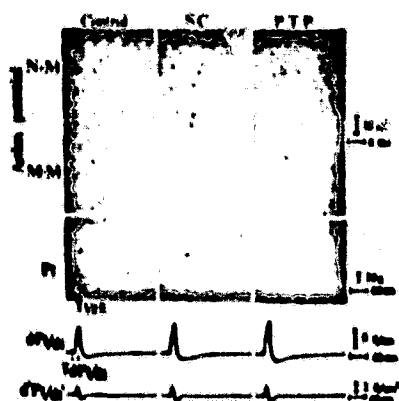


Fig. 3

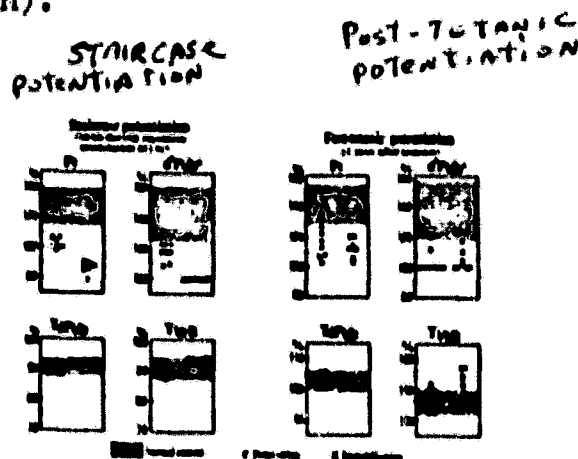


Fig. 4.

Also, the single contractile force ( $P_t$ ) induced 1 minute after forced contractile stimulus loading with 50Hz for 10 seconds, revealed the increase (128-154%) accompanied by the increase in the rate of acceleration of the generation of tension ( $d^2P_t/dt^2$ ) (120-157%) and the prolongation of the relaxation time ( $T_{\frac{1}{2}R}$ ) (102-110%) (posttetanic potentiation) (Figure 3 PTP; Figure 4 right, shaded portion). These phenomena are observed with the denervated muscle (8 cases, marked ●) and with the immobilized muscle (8 cases, marked X). Figure 4 summarizes these results, compared with the normal controls (shaded portion). The staircase phenomenon showed less than normal degree of increase or even a decrease to below the pre-stimulation loading level (negative staircase) in both denervated and immobilized muscles, but these findings are especially marked quantitatively in the latter. In both denervated and immobilized muscles, posttetanic potentiation are less than normal range, but abnormal prolongation of single contraction relaxation time ( $T_{\frac{1}{2}R}$ ) is prominent in the immobilized muscle.

III. Reactions of muscle tension curves to isoproterenol, caffeine and dantrolene.

1) Reactions to isoproterenol.

The single contractile force (Pt) of the normally innervated rabbit tibialis anterior muscle (10 cases) is increased (+5~+23%), accompanied by the prolongation of the relaxation time ( $T_{1/2}R$ ) (+10~+27%) by intravenous injection of isoproterenol (5ug/kg, via ear lobe vein) (Figure 5 left, figure 6 left, shaded area).

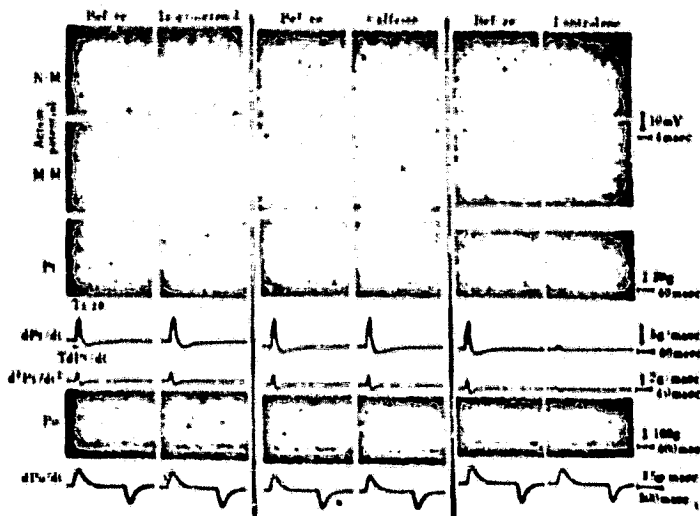


Fig. 5.

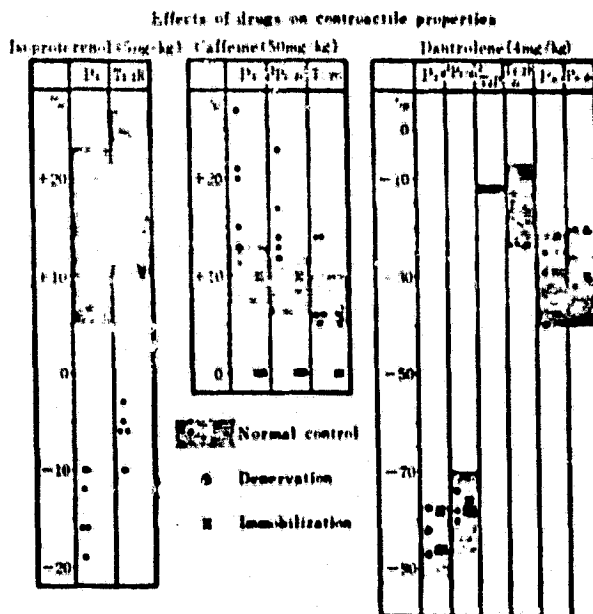


Fig. 6.

No changes are noted before and after the administration of isoproterenol in the forceful contractile force ( $P_o$ ), its rate of generation ( $dP_o/dt$ ), and action potentials induced by neural and direct muscle stimulation.

The immobilized muscle (5 cases) (Figure 6 left, marked X) showed normal responses, but the denervated muscle (5 cases) (figure 6, left, marked  $\odot$ ) showed decreasing reaction of single contractile force ( $P_t$ ), accompanied by the shortening of relaxation time ( $T_{\frac{1}{2}R}$ ). For each muscle group, action potentials and forceful contractile forces are not different from those in the normal control.

### 2) Reactions to caffeine.

The single contractile force ( $P_t$ ) of the normally innervated rabbit tibialis anterior muscle (10 cases) is increased (+7~+13%), accompanied by the increase in the rate of acceleration of the generation of tension ( $d^2P_t/dt^2$ ) (+6~+12%), and the prolongation of relaxation time ( $T_{\frac{1}{2}R}$ ) (+5~+10%) by intravenous injection of caffeine (50mg/kg) (figure 5 center, figure 6 center, shaded portion). In 4 out of 5 denervated muscles (figure 6 center, marked  $\odot$ ) showed hypersensitive reaction exceeding the normal range, while 3 out of 4 immobilized muscles (figure 6 center, marked X) showed no change, and the remaining 1 showed reaction within normal range. For each muscle group, no changes are noted before and after caffeine administration in induced muscle action potentials, forceful contractile forces and their velocity curves.

### 3) Reactions to dantrolene.

The single contractile force ( $P_t$ ) of the normally innervated rabbit tibialis anterior muscle (7 cases) is markedly lowered by intravenous injection of dantrolene (4mg/kg) (-77~-92%) (figure 5

right, figure 6 right, shaded portion). The major change is the decrease in the rate of acceleration of the generation of tension ( $d^2Pt/dt^2$ ) (-70~-87%). Changes in time factors such as contraction time ( $TdPt/dt$ , -11~-13%) and relaxation time ( $T\frac{1}{2}R$ , -7~-24%) are only slight. Also, changes in forceful contractile force ( $Po$ , -21~-40%) and its rate of generation ( $dPo/dt$ , -20~-40%) are less compared to those in single contractile force. Changes caused by this agent in various factors related to single contractile and forceful contractile forces seen in the denervated muscle (3 cases) (figure 6 right, marked  $\odot$ ) and in the immobilized muscle (3 cases) (figure 6 right, marked X) are within the ranges seen in the normally innervated muscle noted above. In each group, dantrolene administration caused no changes in the simultaneously induced muscle action potentials.

Figure 7 shows effects of dantrolene on the human adductor pollicis muscle of 5 patients with muscular dystrophy of the extremities (whose induced and needle electrode electromyography are normal), in terms of single contraction, forced contractile force, and their analytical values, compared with 5 cases of normal control (100mg by mouth, determination made 3 hours later). The human dystrophic muscles showed responses within normal range. In all cases, no differences are noted before and after the administration of medication in simultaneously induced muscular action potential.



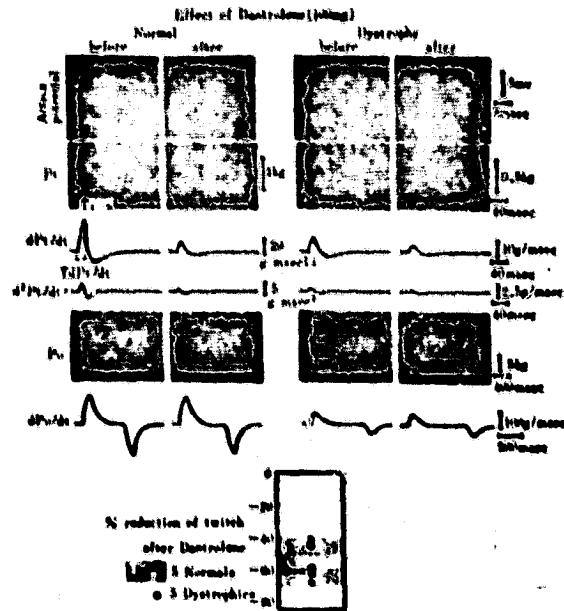


Fig. 7.

### Discussion.

Over the several MSECs between the generation of action potential on the surface membrane of a muscle and its mechanical reactions (tension) appears, there is a series of events taking place, including the depolarization of T-tubules,  $Ca^{++}$  dissociation and combination in the muscle vesicles, troponin-tropomyosin complex, and actin-myosin crossbridges. In order to study these intracellular factors in the muscle physiologically, we have defined various physiological parameters utilizing differential analyses of isometric single contraction and forceful contractile force curves recorded by a strain gauge (action potentials are also recorded simultaneously), in light of the concept of active state (table 1), (1-3). In peripheral muscles of patients with early phase of muscular dystrophy in which nerve-muscle transmission and electrophysiological parameters of muscle surface membranes are still normal (not mentioning biochemical changes of the membrane, or

changes in the internal membrane system and muscle vesicular membranes), the presence of abnormalities in muscle contraction-relaxation has been demonstrated (1), and the findings are summarized in figure 8.

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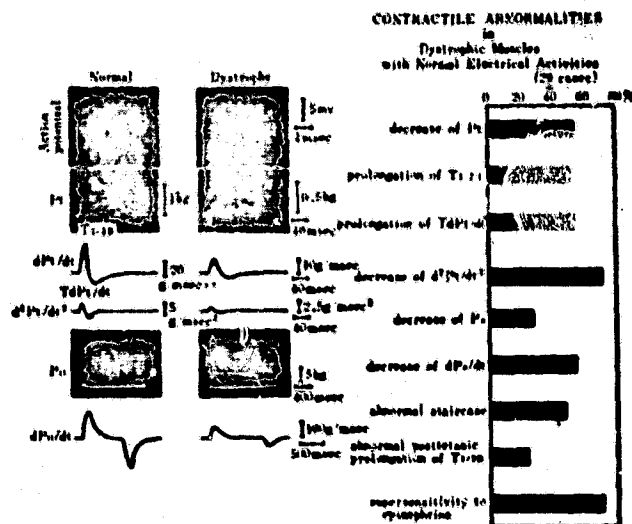


Fig. 8.

Using the rabbit tibialis anterior muscle, we studied how far the recently proposed concept of neurotrophic factor could be applied to these findings. The study utilized the denervated muscle, as well as the measurements of contractility of the immobilized muscle, rendered immobile by joint fixation with intact nerve-muscle synapse. The findings are compared with those of the human dystrophic muscle, and study is made also on the involvement of abnormalities of the intrinsic muscle factors.

Figures 1-2 show effects of denervation (marked ●) and immobilization (marked X) on various factors of muscle contraction and relaxation. In the denervated muscle, marked lowering of single contractile force (Pt) along with a decrease in the rate of acceleration of the generation of single contractile force ( $d^2Pt/dt^2$ ) is noted.

These are normal in the immobilized muscle. The decrease in  $d^2Pt/dt^2$  is an abnormal finding seen in 72% of human dystrophic muscles (figure 8). This parameter expresses the "strength element" (intensity of shortening) of the active state, and thought to reflect the amount of  $Ca^{++}$  dissociated from the muscle vesicles by stimulation (4). Our observation of abnormality in this parameter in the denervated muscle coincides with the report that pointed out abnormalities in  $Ca^{++}$  dynamics from the biochemical standpoint. From these findings, it could be inferred that the pathological process in the subcellular  $Ca^{++}$  transport system, suggested by abnormalities in this parameter, noted frequently in the human dystrophic muscle, reflect the abnormalities in the nerves (neurotrophic factor).

The abnormal prolongation of the relaxation time of single contractile force ( $T_{1/2}R$ ) is more prominent in the immobilized muscle than in the denervated muscle (figures 1, 2). It was noted in 55% of human dystrophic muscles (figure 8). Based on the concept of active state, this parameter expresses the active state decay, reflecting the relationship between the recombination of  $Ca^{++}$  removal from troponin and myovesicular  $Ca^{++}$ , and  $Ca^{++}$  binding by troponin and ATP binding by myosin (6). The prolongation of the relaxation time of single contractile force has been reported with denervated muscles (7), and since it occurs with cholinergic synapse blocking by botulinum toxin, it has been inferred that it suggests the abnormalities in neurotrophic influence which utilizes acetylcholine as the transmitter of information (8). However, the results of this study confirmed the prolongation in the denervated muscle (at the stage in which neuromuscular transmission is abnormal while

muscular surface membrane is normal, as seen through the electrophysiological parameters), While only slight changes are noted in the immobilized muscle. (with normal nerve-muscle synapse). Denervation experiments thus far mostly made observations 2-3 weeks or later, after nerve resection or pharmacological blocking, so that the possibilities of changes in electric characteristics of membrane surfaces or effects of chronic disuse on muscle tension curves, could not be ruled out. Histochemical examination revealed that in the normal rabbit tibialis anterior muscle findings are type II fiber dominant, and in the immobilized muscle, sporadic atrophy of muscle fibers is noted, but specific fiber type atrophy could not be determined. Accordingly, the prolongation of relaxation time seen with immobilization is a reflexion of uniform intracellular changes within muscle cells regardless of fiber types, rather than the uncovering of the characteristics of slow muscle fibers that function normally, in face of the profound atrophy of fast muscle fibers.

The reduction of forceful contractile force ( $P_0$ ) (6) which reflects the "strength element" (intensity of load bearing) of active state, and quantity and strength of the actin-myosin cross bridge formation, is seen in both denervated and immobilized muscles, but more prominently in the latter (figures 1,2). This parameter is seen in 28% of human dystrophic muscles (figure 8). The results of this study suggest that intrinsic abnormalities of muscles are more strongly expressed. The decrease in the rate of the generation of forceful contractile force ( $dP_0/dt$ ) is noted in 55% of human dystrophic muscles (figure 8). This is considered to be a parameter that expresses the force-speed relationship of muscular contraction,

actin-myosin crossbridge formation rate, and series elasticity element (9). In this study, as in  $P_o$ , this parameter is lowered in both denervated and immobilized muscles, but more prominently in the latter, suggesting strong reflexion of abnormalities in intrinsic muscular factors. Also, the weight loss in the immobilized muscle in this study is -11 -22% in comparison to the control group, which is not parallel to the prominent decrease in  $P_o$  (figure 1). Accordingly, the main courses of decrease in  $P_o$  are likely to be due to the activation process in individual muscle fibers, or physiological and biochemical changes in contractile proteins rather than the disappearance of functioning muscle fibers. Also, histopathological examination failed to show an increase in connective tissue (series elasticity element). The decrease in  $dP_o/dt$  also could be construed to reflect changes in contractile elements themselves.

During low frequency, repetitious stimulation or after high frequency stimulation (forced contraction) loading of fast twitching muscles, the isometric single contractile force increases. These phenomena are called staircase phenomenon and posttetanic potentiation respectively, and are not accompanied by changes in action potential. As such, these are useful in studying intracellular mechanisms. The staircase phenomenon is considered to accompany the increase of "element of strength" (intensity of shortening, related to sarcoplasmic  $Ca^{++}$  dissociation) of the active state (10). In this study with the normal rabbit tibialis anterior muscle, this phenomenon is observed with an increase in the rate of acceleration of the tension generation ( $d^2P_t/dt^2 = \text{active state intensity of shortening}$ ), and it is accompanied by the decrease in contraction and relaxation

time ( $TdPt/dt$ ) ( $T\frac{1}{2}R$ ) (figure 3 SC; figure 4 left, shaded portion).

The posttetanic potentiation is thought to be caused by the  $Ca^{++}$  dissociation process of muscle vesicles (11), or by the steps following  $Ca^{++}$  binding by troponin (12). In our study with the normal rabbit tibialis anterior muscle, we found an increase in single contractile force without change in the action potential after forceful contractile loading. Analyses of this observation reveal the accompanying increase in the rate of acceleration of tension generation ( $d^2Pt/dt^2$ =active state intensity of shortening) and prolongation of the relaxation time ( $T\frac{1}{2}R$ =active state decay), which are not in disagreement with the concepts mentioned above (figure 3 PTP; figure 4 right, shaded portion).

In analyses of human dystrophic muscles, abnormalities in staircase phenomenon are noted in 48% of cases, and abnormal prolongation in relaxation time accompanying posttetanic potentiation in 24% of cases (figure 8). High rates of abnormalities in these phenomena are noted both in experimentally denervated and immobilized muscles, and quantitatively these are more prominent in the latter (figure 4). These phenomena are seen usually in the normal fast muscle. In the normal slow muscle, the negative staircase phenomenon with lower level than the pre-conditional loading single contractile force, and posttetanic depression are noted. Specifically, it has been reported that posttetanic changes could be controlled for increase or decrease by the crossing of neural dominance (13). Accordingly, these are thought to be phenomena that express intracellular muscular mechanism under control of the neurotrophic factor. The responses of denervated muscles in our study appear to be in agreement with this concept, but the

results of analyses of study of immobilized muscles suggest that intrinsic muscle factors are also reflected strongly. The abnormal appearance of these phenomena noted in human dystrophic muscles should be considered in light of these points.

Study of effects of epinephrine on various factors of the active state and of reactions on muscle tension curves are useful means in obtaining information on membrane receptors and intracellular muscular mechanisms. The actions of epinephrine in these areas include the B-receptor mediated effects on contraction-relaxation cycle through  $Ca^{++}$  dissociation and combination in muscle vesicles (at least partially dependent on cyclic AMP), and activation of phosphorylase promoting glycogenolysis (at least partially cyclic AMP dependent) (1). In general, the single contractile force of fast muscles increases with the prolongation of contraction-relaxation time (especially the latter), while that of slow muscles decreases with the shortening of relaxation time, and maximum forceful contraction forces are not changed, by epinephrine administration (14). Our study on the human dystrophic adductor pollicis muscle revealed abnormal lowering reaction with epinephrine, exceeding the normal range, in 71% of cases (figure 8) (1). In this study, attempts are made to clarify whether this is a reflexion of neural factors or that of intrinsic muscle factors, using isoproterenol (B-stimulant). With the normally innervated rabbit tibialis anterior muscle, the single contractile force ( $P_t$ ), as has been reported previously, increased with the prolongation of its relaxation time ( $T_{\frac{1}{2}R}$ ), and no changes in forceful contractile force ( $P_o$ ) and action potentials are noted (figure 5 left, figure 6 left, shaded portion). However, the single contractile force ( $P_t$ ) of the denervated muscle

is decreased as in a normally innervated slow muscle, accompanied by the shortening of relaxation time ( $T_{\frac{1}{2}R}$ ) with administration of isoproterenol (figure 6 left, marked  $\odot$ ). On the other hand, the immobilized muscle showed increasing reaction, as in normal muscles (figure 6 left, marked X). These observations suggest that the abnormal B-receptor mediated reactions to epinephrine by intracellular mechanisms of the dystrophic muscle noted above are the results of abnormalities in the trophic influences of the motor neuron on the muscle.

Caffeine is known to enhance  $Ca^{++}$  dissociation from muscle vesicles and inhibit  $Ca^{++}$  uptake by muscle vesicles, thus affecting muscle tension (contracture, twitch potentiation) without affecting electric reactions of skeletal muscles (15). The normally innervated rabbit tibialis anterior muscle in our study exhibited the increase in the rate of acceleration of tension generation ( $d^2Pt/dt^2$ ) and the prolongation of relaxation time ( $T_{\frac{1}{2}R}$ ), along with the increase in single contractile force (Pt) (no changes in forceful contractile force), without changes in neurally or directly induced action potentials (figure 5 center; figure 6 center, shaded portion). The findings are not in disagreement with the mechanism of action of this agent as noted above. In diseased muscles, muscular tension responds to this agent abnormally. It has been reported that human dystrophic muscles exhibit subnormal reaction (16) while muscles of patients with motor neuron diseases (16) and experimentally denervated muscle (17) show greater than normal reaction. With blocking of neural axon flow by colchicine, abnormal responses to caffeine with these muscles are not seen, and such responses are said to be seen only when acetylcholine dissociation from nerve terminals and neural



conductivity are blocked (18). In our study of the experimentally denervated muscle, reactions to caffeine when blocking of neuromuscular transmission occurred, included the increase of single contractile force (Pt) beyond normal range, accompanied by abnormal increases in the rate of acceleration of tension generation ( $d^2Pt/dt^2$ ) and relaxation time ( $T_{\frac{1}{2}R}$ ) (figure 6 center, marked  $\odot$ ). On the other hand, the immobilized muscle showed normal or subnormal responses in these items (figure 6 center, marked X), coinciding with the findings reported in an in vitro study of human dystrophic muscles (16), suggesting that these observations reflect abnormalities in intrinsic muscle factors.

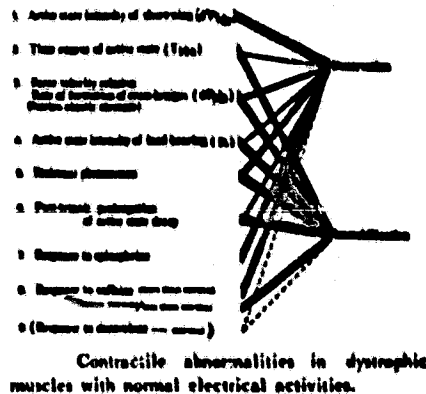
Dantrolene is a muscle relaxant widely used clinically because of its absence of central nervous system effects. It is known to cause lowering of single contractile force by inhibiting  $Ca^{++}$  dissociation in muscle vesicles without affecting neuromuscular transmission or electric properties of muscle cell membranes (it exerts little effects on forceful contractile force or muscle contracture due to high  $K^+$  concentrations), suggesting the mechanism that inhibits the process of trigger  $Ca^{++}$  influx  $\longrightarrow$   $Ca^{++}$ -induced  $Ca^{++}$  release from sarcoplasmic reticulum (19). Our findings on effects of dantrolene on the tension reactions of the normal rabbit tibialis anterior muscle mainly is the decrease of single contractile force (Pt) accompanied by changes in the rate of acceleration of tension generation ( $d^2Pt/dt^2$ =active state intensity of shortening, related to  $Ca^{++}$  dissociation in muscle vesicles). Effects on contraction-relaxation time, forceful contractile force and rate of its generation ( $TdPt/dt$ ,  $T_{\frac{1}{2}R}$ ,  $Po$ ,  $dPo/dt$ ) are only slight (figure 5 right, figure 6 right, shaded portion). Accordingly, effects of dantrolene are

partially similar to those of caffeine, but some antagonism is also suggested. However, effects on  $\text{Ca}^{++}$  uptake by muscle vesicles thought to be present with caffeine are not found in dantrolene. The caffeine-contraction which is held to be due to the enhancement of  $\text{Ca}^{++}$  dissociation in muscle vesicles caused by high concentrations of caffeine is profoundly affected by dantrolene. The antagonism of dantrolene against the increase in single contractile force due to the inhibition of  $\text{Ca}^{++}$  uptake by muscle vesicles caused by low concentrations of caffeine, is weak. Also, it is said that single contractile force enhanced by  $\text{NO}_3^-$  is affected more strongly by that enhanced by caffeine (20). In light of antagonism of dantrolene against  $\text{NO}_3^-$ , it is possible that dantrolene acts in connection with T system. Dantrolene does not necessarily act on the same location as caffeine, thus responses of diseased muscles to this agent are studied. The results showed that human dystrophic muscles reacted within the normal range for the normal human adductor pollicis muscle (figure 7). The denervated and immobilized rabbit muscles showed responses which are within normal range seen in the control group (figure 6 right, marked ● and X). It is suggested that a certain process in E-C coupling, which is the target of pharmacological effects of dantrolene is not functionally compromised in pathological processes including dystrophy, denervation, or decreased muscle activities.

Figure 9 summarizes the relationship between various abnormalities of intracellular mechanisms seen in muscle cells of human dystrophic muscles and the experimental results obtained from denervation and immobilization. It suggests that with partial exceptions, pathogenesis of muscle dystrophy is combination of neural and intrinsic

muscle factors, and not necessarily simple and unitarian. From results of the present study above, when neural factors are in question, it could not be concluded whether the trophic influence to that muscle could be due to acetylcholine itself or due to trophic substance (s) other than acetylcholine. Also, when activity of muscle itself is in question, it must be determined whether the intracellular abnormalities seen in the immobilized muscle is due to trophic effect caused by changes in nerve activity pattern (changes in phasic, tonic neural electric discharge patterns) or due to changes in mechanical stress to which the muscle is subjected.

Fig. 9



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