Calcium and actin activated myosin ATPase after compensatory hypertrophy in the plantaris muscle of the rat.

Graeme John. Lang
University of Windsor

Follow this and additional works at: https://scholar.uwindsor.ca/etd

Recommended Citation
https://scholar.uwindsor.ca/etd/1343
NOTICE

The quality of this microfiche is heavily dependent upon the quality of the original thesis submitted for microfilming. Every effort has been made to ensure the highest quality of reproduction possible.

If pages are missing, contact the university which granted the degree.

Some pages may have indistinct print especially if the original pages were typed with a poor typewriter ribbon or if the university sent us a poor photocopy.

Previously copyrighted materials (journal articles, published tests, etc.) are not filmed.

Reproduction in full or in part of this film is governed by the Canadian Copyright Act, R.S.C. 1970, c. C-30. Please read the authorization forms which accompany this thesis.

THIS DISSERTATION HAS BEEN MICROFILMED EXACTLY AS RECEIVED
CALCIUM AND ACTIN ACTIVATED
MYOSIN ATPase
AFTER COMPENSATORY HYPERTROPHY
IN THE PLANTARIS MUSCLE
OF THE RAT

By
GRAEME JOHN LANG

A Thesis
submitted to the Faculty of Graduate Studies
through the Faculty of
Human Kinetics in Partial Fulfillment
of the requirements for the Degree
of Master of Human Kinetics at
The University of Windsor

Windsor, Ontario, Canada
1983
To Mum, Dad, and Jan ...
ABSTRACT

CALCIUM AND ACTIN ACTIVATED MYOSIN ATPase
AFTER COMPENSATORY HYPERTROPHY IN THE PLANTARIS MUSCLE OF THE RAT

by
GRAEME JOHN LANG

Sixteen male Wistar rats (200g), underwent surgery to induce compensatory hypertrophy of the plantaris muscle. A unilateral procedure was employed, with the contralateral limb serving as a control. At 30 days the animals were killed and the control plantaris (CP) and hypertrophied plantaris (HP) were excised and weighed. HP was 83% heavier than CP (P<.0005). Myosin was extracted from each muscle and Ca and actin activated ATPase assays were conducted. Ca\textsuperscript{++} ATPase (37\textdegree C) of HP was reduced 7% when compared to CP at both pH 7.4 (P<.005) and pH 9.8 (P<.025). Actin activated ATPase assays were conducted at 5-8 different actin concentrations (0.3-10.8\textmu M) and at two ionic strengths (I=0.075 and I=0.095). Lineweaver-Burk plots showed linear dependence (mean r=0.97, n=32). Regression analysis
of the Eadie-Scatchard plots were used to determine $V_{\text{max}}$ and $K_m$ for each myosin sample. $V_{\text{max}}$ for HP myosin was decreased 36% at $I = 0.075$ ($P < 0.005$) and 31% at $I = 0.095$ ($P < 0.005$) when compared to CP. $K_m$ for HP myosin was reduced 32% at $I = 0.075$ ($P < 0.005$) and 29% at $I = 0.095$ ($P < 0.05$) when compared to CP. The results suggest that the altered functional demand on the muscle has induced a change in the myosin molecule whereby its affinity for actin is increased. The functional significance of this can be viewed as a longer cross-bridge engagement time between actin and myosin resulting in an increase in efficiency during isometric contractions.
ACKNOWLEDGEMENTS

I would like to Acknowledge Dr. Earl G. Noble for his Guidance, Direction and Friendship during the course of my Masters Degree and my stay in Canada.
TABLE OF CONTENTS

DEDICATION ................................................. iii
ABSTRACT ...................................................... iv
ACKNOWLEDGEMENTS .......................................... vi
LIST OF TABLES ............................................... viii
LIST OF FIGURES ............................................. ix
LIST OF APPENDICES ........................................ vii

INTRODUCTION ............................................... 1

- The Morphology of the Myosin Molecule ........... 1
- The Plasticity of Mammalian Skeletal Muscle ....... 4
- Myosin ATPase and Speed of Shortening .......... 10
- The Biological Significance of the Myosin Light Chains ... 12
- Statement of the Problem ......................... 14

METHODS AND PROCEDURES ................................. 15

- Surgical Techniques .................................. 15
- Biochemical Techniques ............................. 17
- Analytical and Statistical Procedures .......... 24

RESULTS ......................................................... 26

DISCUSSION OF RESULTS .................................. 33

SUMMARY AND CONCLUSIONS ............................... 41

APPENDICES ..................................................... 43

- A. Raw Data .......................................... 43
- B. Calculation of the Expected Reduction in ATPase .... 46
- C. Calculation of Ionic Strength .................. 47
- D. Determination of the % Decrease in V .......... 48

REFERENCES .................................................... 49
LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td></td>
</tr>
</tbody>
</table>

| I | Muscle Wet Weights and Myosin Yields | 28 |
| II | Calcium Activated Myosin ATPase     | 28 |
| III| Actin Activated ATPase              | 28 |
LIST OF FIGURES

Figure                                                                 Page
1a. Reaction Velocity versus [A]. I= .075 ........ 29
1b. Reaction Velocity versus [A]. I=.095 ........ 29
2a. Lineweaver-Burk Plot. I=.075 .................. 30
2b. Lineweaver-Burk Plot. I=.095 .................. 30
3a. Eadie-Scatchard Plot. I=.075 .................. 31
3b. Eadie-Scatchard Plot. I=.095 .................. 31
4. % Decrease in Reaction Velocity at any [A] .......... 32
INTRODUCTION

THE MORPHOLOGY OF THE MYOSIN MOLECULE

Myosin is a mult-subunit protein composed of two heavy chains of molecular weight 200,000 daltons (1), and two pairs of light chains (2). One pair of these light chains is common to all three types of myosin (fast, slow and cardiac), and is in the range of 18,000-19,000 daltons (3). Removal of these light chains with 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), does not significantly alter the ATPase activity of the myosin, they are hence known as inessential, or in view of their dissociation characteristics, DTNB light chains. These light chains, which, when phosphorylated may play some regulatory role in the actin-myosin interaction (4), will not be discussed further, except to say that there is one on each globular "head" of myosin (5).

The other pair of light chains vary according to the type of myosin being investigated. Fast myosin contains two chemically distinct light chains which can be dissociated under alkaline conditions, hence their name alkali light chains (6). These are usually designated Al (25,000 daltons) and A2 (16,000 daltons) (7). These two light chains may exist together on the same myosin, giving the Al.A2 heterodimer, or they may exist in either of the two homodimeric forms Al.Al or A2.A2 (8).
In slow muscle myosin, the analogue of the A1 and A2 light chains are light chain la (LC1a) and light chain lb (LC1b) of molecular weight 27,500 and 26,500 daltons respectively (9). These two light chains travel as a doublet during gel electrophoresis (10). Because of the chemical differences observed between these light chains and the alkalai light chains, they are by definition products of different genes (10), but are closely related.

Because of the size of the heavy chains, knowledge of their peptide sequences is at present limited to specific regions (11). It has been shown however, that the 3-methylhistidine content of slow, fast and cardiac myosin is different (12), thus giving conclusive evidence for the heterogeneity of the heavy chains in different fiber types.

The myosin heavy chain can be subjected to proteolytic digestion by papain and trypsin to yield various subfragments (13). Products of digestion include the tail region, known as light meromyosin (LMM), the head region, heavy meromyosin (HMM), and subfragment 1 (S1). Two S1 subfragments, each of which has been shown to contain an actin binding site and a separate site for ATP hydrolysis (14), are obtained from each myosin molecule. The S1 preparations, unlike myosin itself, are soluble at physiological strength, and have therefore been used extensively in kinetic experiments involving actin binding and ATP hydrolysis (15,16,17,18). HMM is insoluble at low ionic strengths.
Myosin is thought to be the most important contractile protein in the myofibrillar structure, because it hydrolyses ATP to yield the energy for muscular contraction, and the movement of its globular head in relation to its filamentous tail is thought to be the cause of filament sliding. Although the major regulatory mechanism proposed for skeletal muscle is the steric blocking model (19), much of the regulation is known to be myosin based (20,21,22). Consequently, when physiological adaptations take place in response to a given stimulus, alterations in the myosin molecule are often involved.
THE PLASTICITY OF MAMMALIAN SKELETAL MUSCLE

The relative plasticity of mammalian skeletal muscle has been demonstrated in response to a variety of stimuli, which include cross-reinnervation, electrical stimulation, hypergravity stress, exercise, thyrotoxicosis and compensatory hypertrophy. Cross-reinnervation and electrical stimulation are the most effective means of inducing a complete transformation in fiber type, whereas the effects of the other stimuli are more circumscribed. A brief outline of these models is given as an introduction in an attempt to gain some insight as to the mechanism(s) responsible for the observed adaptations.

A. CROSS-REINNERVATION;

The cross-reinnervation experiments of Buller et al. (23), and Close (24,25), helped establish that neural influences are largely responsible for the differentiation of mammalian skeletal muscle into fast and slow types. Cross-union of the motor nerves between fast and slow muscle brings about reciprocal changes in the intrinsic speed of shortening and the force-velocity properties (26,27), and also the biochemical properties (28) of muscle. These studies could not, however, differentiate between the influence of a trophic substance released from the nerve ending, or the stimulation pattern, having an effect on the expression of different fiber types within the muscle.
B. ELECTRICAL STIMULATION;

Since the existence of a chemotrophic substance could not be discounted in cross-reinnervation experiments, a technique whereby a muscle was chronically stimulated with an impulse pattern resembling that of a fast or slow muscle was developed. Salmons and Vrbova (29), and others (30,31), demonstrated that over a period of months, chronic low-frequency electrical stimulation of a fast-twitch muscle resulted in a response whereby the muscle became indistinguishable from slow twitch muscle by a large number of physiological, histochemical, and biochemical criteria. Since then, much work has been directed toward revealing the effects of various impulse patterns on the structure and function of muscle.

Lomo et al. (32) found intermittent 100 Hz (high frequency) stimulation makes a normally slow muscle fast, while continuous 10Hz (low frequency) stimulation makes it slower than normal. A high frequency stimulus train induced fast contractile properties whether it was delivered often or very seldom. Fatigue resistance however, required frequent stimulation and both 10Hz and 100Hz were effective.

These observations suggest that the pattern of muscle activity per se, and not neural influences, play an important role in determining the contractile properties of a skeletal muscle (32).
C. HYPERGRAVITY STRESS;

Changes qualitively similar to those brought about by chronic stimulation may be observed in the muscles of rats kept for six months under hypergravity conditions (33). This finding would tend to reinforce the hypothesis that it is the overload on the muscle, expressed through the pattern of muscle activity which determines its contractile properties. It is interesting that the range of activity elicited by the nervous system is sufficient to induce the same changes as occur under the more artificial conditions of motor nerve stimulation.

D. THYROTOXICOSIS;

Muscle has also been shown to respond to a hormonal stimulus, as shown by the thyrotoxicotic, models of Pitts (34) and Ianuzzo (35). A shift in fiber population from 15% to 60% fast was demonstrated in the rat soleus following thyrotoxicosis. This change however was not accompanied by a concomitant alteration in ATPase activity or maximal shortening velocity, the reasons being unclear. It was hypothesized that an incomplete transformation of the myosin phenotype could account for the differences.

E. EXERCISE;

Exercise represents a less continuous stimulus to the muscle than does chronic electrical stimulation,
cross-innervation or hypergravity stress and its effects are diminutive in comparison. Therefore, while the response of skeletal muscle to endurance exercise is to elicit adaptations measurable in terms of enzyme activities of the various metabolic processes, it has not been demonstrated to elicit significant changes in muscle fiber type (36,37). Recent investigations by Saltin et al. (38) has revealed changes within the broader classes of fast and slow fibers may be induced by exercise, with little conversion being displayed between these types of fibers.

It seems that only when the departure from the normal biological functioning of the muscle is both radical and sustained is the full adaptive potential of the muscle revealed.

F. COMPENSATORY HYPERTROPHY;

Without resorting to electrical stimulation or direct intervention with the motor nerve, ablation of synergistic muscles has been employed to overload the remaining muscles. Compensatory hypertrophy, which can be induced by total ablation (39,40), partial ablation (41), tenotomy (42,43), and denervation of synergistic muscles (44,45), induces hypertrophy of the intact synergist, the greatest size increase being elicited in the total ablation technique. As with the artificial stimulation and cross-reinnervation procedures,
compensatory hypertrophy of the fast-twitch plantaris muscle, results in the muscle exhibiting a decreased calcium activated ATPase activity, and an increase in the number of histochemically determined alkaline labile fibers, as well as an altered light chain pattern (46,47,48). Although these adaptations are prominent, they are not as complete as those observed with cross-reinnervation and electrical stimulation.

Twitch contraction times and the force velocity properties of the compensatory hypertrophied muscle have been reported as unaltered (49). Although the maximal speed of shortening was shown to decrease by these investigators it was assumed to be a result of increased fiber angle in the pennate plantaris muscle following hypertrophy. More recent investigation employing ablation of both the soleus and the gastrocnemius muscle have revealed that virtually all the contractile properties are altered to resemble more closely those of slow muscle. These include a longer time to peak tetanic tension, longer half relaxation time, and a lower mean maximal shortening velocity (40). The question arises as to the advantage of synthesizing slow muscle myosin in a fast muscle. Goldspink et al. (50) have suggested that fast muscles have evolved for efficiency in performing external work, whereas slow muscles have evolved for efficiency in maintaining isometric tension. The plantaris muscle, during normal use, is involved specifically in locomotion. Following the ablation of the
synergystic gastrocnemius however, the plantaris may be more active and assume some role in postural maintenance. Hence the muscle may be called upon to maintain isometric tension and the increase in efficiency due to the synthesis of slow myosin would be an obvious advantage. It must be noted however, that although the percentage of alkaline labile fibers has been observed to increase from 8% to 24% in the experimental plantaris (47), only a minor reduction in the Ca++ activated ATPase has been reported by some investigators (47,51). These observations parallel those with the thyrotoxic model, and similarly it may be hypothesized that an incomplete transformation in the myosin phenotype has occurred.

In line with Lomos' conjecture (32) that the pattern of muscle activity that determines its biochemical and physiological properties, it is thought that the changes observed in this model are a result of the functional adaptation of the muscle to chronic overload and there is therefore no reason to suspect that the response is different to that of hypergravity stress, as neither model interferes directly with the neural input of the muscle.
MYOSIN ATPase AND SPEED OF SHORTENING

Bárány et al. (52) were the first to demonstrate a high positive correlation between the contractile and biochemical properties of skeletal muscle. They found that the ATPase of myosin correlated well with the intrinsic speed of shortening of the muscles, especially when actin was used to activate the myosin. However, a much lower correlation was found when calcium was used as an activator especially at high ionic strength. Employing cross-reinnervation experiments (38), it was concluded that neural influences, which determine the dynamic properties of the contractile apparatus, probably do so by affecting the ATPase site of myosin. As the energy delivery from ATP hydrolysis may be rate limiting to muscle contraction, any change in the contractile characteristics may be reflected by alterations in the ATPase of myosin.

Myosin, in the absence of an activator, is a very weak ATPase. A variety of cationic conditions stimulate the ATPase activity of myosin 200 to 300 times its basal rate, thus bringing it to the order of magnitude it would be operating at in vivo (14). Magnesium is the important cation for the physiological activity of actin activated ATPase (53), yet it inhibits the ATPase activity of pure myosin and so cannot be studied in the absence of actin. The three most commonly used activators of myosin are Ca$$^{++}$$, K$$^{+}$$-EDTA, and Mg$$^{++}$$-actin, of these only Mg$$^{++}$$-actin can be deemed physiological in nature.
Distinct differences may be observed depending on the type of ATPase activity measured. For instance, Malhorta (54) observed that the removal of light chain two (LC2) from cardiac myosin resulted in no change in the Ca$^{++}$ activated ATPase, a fall in the K$^+$-EDTA ATPase and an increase in the Mg$^{++}$-actin ATPase. As will be discussed in more detail later, if the influence of the light chains is to be reflected in the resultant ATPase measurements then Ca$^{++}$ is an unsatisfactory activator.
THE BIOLOGICAL SIGNIFICANCE OF THE MYOSIN LIGHT CHAINS

As previously stated, removal of LC2 from cardiac myosin results in an increase in Mg\(^{++}\)-actin activated ATPase, with no apparent change in the Ca\(^{++}\) activated value (32). This suggests the light chains are involved in the actin-myosin interaction in a regulatory capacity, and hence any assay performed in the absence of actin will not allow the light chains to confer their regulation on the system. Much work in this regard has been conducted by Wagner and Weeds (55), who employed a hybridization technique to manufacture S1 subfragments of myosin containing only A1 or A2 light chains. Using these hybrids at low ionic strength Ca\(^{++}\) ATPase was almost identical in both cases whereas the actin activated ATPase value of S1(A2) was twice that of S1(A1). Wagner and Weeds then went on to explore the role of these light chains by recombining them with either fast, slow or cardiac heavy chains. S1 containing a fast myosin heavy chain and either cardiac or slow twitch muscle light chain had ATPase activities measured in the absence of actin which was almost identical to that in a control fast twitch muscle S1. However in the presence of actin, the ATPase activity reflected not only the source of the heavy chain but also that of the light chain. Similar observations were found in the hybrid containing cardiac heavy chains and A2 light chains.
Stracher (56) found that after dissociating the light chains from fast myosin, there was a resultant loss of ATPase activity and actin combining properties of the molecule. Upon recombination, the ability of the myosin to combine with F-actin was restored to the same extent as was the ATPase activity, suggesting both biological functions of myosin are mediated through an interaction of the light and heavy chains.

Pope et al. (57) have conducted experiments on whole myosin with similar results. The lack of differentiation in the ATPase activity of the A1.A1 homodimer of myosin and that of the A2.A2 homodimer at physiological ionic strength led these authors to conclude that there is at present no obvious reason for the existence of polymorphic forms of myosin light chains in either fast or slow twitch muscle, but as Weeds (58) points out, all studies to date have neglected the highly organised structure of the intact myofibrillar system.
STATEMENT OF THE PROBLEM

In view of the fact that the percentage of histochemically determined alkaline labile fibers increases from 8% to 24%, and the calcium activated ATPase decreases by only 7% following compensatory hypertrophy (47), investigation of the ATPase of myosin using the physiological activator—actin, is warranted.

In studying the actin activated ATPase activity of control and hypertrophied muscle myosin, it is anticipated that an alteration in this measurement will be observed in the hypertrophied muscle. It is further postulated that this measure will provide a more appropriate indication of in vivo adaptation than the Ca++ activated indice.

The second and most important aspect of this study is the kinetic analysis of the actin activated ATPase data. It is anticipated that this will aid in understanding how the actin-myosin interaction is affected by hypertrophy.

It is anticipated that the results obtained may be interpreted with regard to the altered efficiency characteristics of the hypertrophied muscle.
METHODS AND PROCEDURES

SURGICAL TECHNIQUES;

Sixteen male Wistar rats (approx. 200g), aged 30-40 days, underwent surgery to induce compensatory hypertrophy of the plantaris muscle. A unilateral procedure was employed with the contralateral limb serving as a control. Animals were anaesthetized with sodium pentobarbital (50 mg/kg ip), and the surgical region washed with 70% isopropyl alcohol. Surgical myectomy was accomplished by the method of Ianuzzo et al. (39), and is briefly outlined here.

A midline incision through the skin from the popliteal fossa to the achilles tendon was made with a scalpel. A second midline incision was made through the muscle sheath which overlays the posterior aspect of the gastrocnemius muscle. The medial and lateral heads of the gastrocnemius muscle were then separated with a blunt probe. The tendon attachment for the lateral head was transected first, since the plantaris muscle is embedded mainly in the medial head. The lateral head was then folded back and totally removed by transecting it as close as possible to its origin without unduly traumatizing other tissue in that region. The insertion of the medial head was then transected and folded back, making sure the plantaris remained intact. This also was sectioned as close to the origin as possible. The skin incision was then sutured, and the surgical region disinfected.
The sham operation consisted of the midline incisions, followed by manipulation of the gastrocnemius with a blunt probe, followed by suturing.

The animals were returned to their cages, fed and watered ad libitum for thirty days, after which time they were killed by cardiac incision whilst under sodium pentobarbital anaesthesia.

The control and hypertrophied muscles were removed, trimmed of excess connective tissue, blotted and weighed. The muscles were stored in 70% glycerol at -30 °C until their time of use.
BIOCHEMICAL TECHNIQUES

A. MYOSIN ISOLATION;

Myosin isolation was accomplished by the method of Ianuzzo et al. (59), with some modification in the centrifugation procedure.

The muscles were removed from the 70% glycerol solution, placed in a small beaker with 30ml of muscle rinsing solution (40mM KCl, 50mM Tris, pH 7.0), and stirred vigorously with a glass rod. This and all subsequent operations were carried out at 4°C or less unless otherwise stated. The muscles were removed from the rinsing solution, minced coarsely with scissors, and homogenized in 15ml of homogenizing solution (10mM KCl, 50mM Tris, pH 6.6) using a Tekmar Tissuemizer. Two 15 second bursts at a rheostat setting of 65, separated by 30 seconds was standard procedure. The homogenizing tube was immersed in an acetone bath at -20°C during this step. Fifteen ml of myosin extractor solution (590mM KCl, 2mM EDTA, 2mM DTT, 20mM MgATP, 300mM Phosphate buffer, pH 6.6) was then added. The tubes were kept in an ice bath and vortexed frequently during the next 10 minutes. After this time they were centrifuged at 30,000 x g for 3 hours. The recommended centrifugation protocol is 1 hour at 150,000 x g, but 90,000 x g for 3 hours, and more recently, 30,000 x g for 3 hours has been employed often, with no differences being apparent.
in either Ca++ or actin activated ATPase values from myosin obtained using the three procedures.

After centrifugation the fluid was decanted into a small (80ml) beaker and 22.2ml of ammonium sulphate solution (41.12% (NH₄)₂SO₄, 50mM Tris, pH 6.8) was added at a rate of two drops per second from a burette. The solution was then centrifuged at 20,000 x g for 10 minutes to precipitate the 34-45% fraction. The supernatant was decanted into an 80ml beaker and another 16.6ml of ammonium sulphate solution was added from the burette with vigorous stirring. This precipitate had the 45% fraction. After stirring for 10 minutes, the solution was again centrifuged at 20,000 x g for 10 minutes, the pellet being retained and suspended in 10ml of myosin dissolving solution (600mM KCl, 50mM Tris, pH 7.4). This solution was then placed in a dialysis bag which had been previously decontaminated by boiling in 25mM EDTA for 10 minutes and washed thoroughly in single and double distilled water. The solution was dialysed overnight against 2 litres of dialysis buffer (1mM EDTA, 10mM KCl, 1mM Tris, 0.05mM DTT, in single distilled water, pH 7.2). The myosin precipitate was collected by centrifugation at 20,000 x g for 10 minutes and the pellet suspended in 500mM KCl, 50mM Tris, pH 7.4 to a concentration of approximately 2mg/ml. The myosin was usually used within a week of preparation during which time it has been found to be relatively stable (60).
B. ACTIN ISOLATION;

An acetone dried powder was prepared by the method of Feuer et al. (61), and the actin extracted from it by the method of Spudich and Watt (62).

One large rat (400g) was killed as described previously and the hindsection rapidly skinned. The muscle from the hindlimb and back was collected and extraneous fat removed before mincing the muscle through a meat grinder. The muscle (60g) was immediately suspended in 200ml of ice cold Guba and Straubs solution (300mM KCl, 150mM KH₂PO₄, pH 6.5) and stirred for 10 minutes at 0-5°C. After this time 800ml of double distilled water was added and the mixture immediately pressed through a thin cloth. The remaining muscle tissue was suspended in 300ml of a solution containing 0.4% NaHCO₃ and 0.1mM CaCl₂ at 22-25°C and kept at this temperature with constant stirring for 30 minutes. This fluid was again removed through a thin cloth. The residue was then suspended in 100ml of a cold solution (10mM NaHCO₃, 10mM Na₂CO₃, and 0.1mM CaCl₂) and was thoroughly stirred for ten minutes during which time the temperature never exceeded 10°C. After this time 1000ml of double distilled water at 22-25°C was added and the fluid removed through a thin cloth as fast as possible. The residue was weighed and for every 10g of it, 30ml of acetone (22-25°C) was added. After stirring for 10 minutes, the acetone was gently pressed out through a thin cloth and 1/3 the original volume of acetone was added and the
mixture again stirred for 10 minutes, pressed down and the residue dried in air at room temperature.

Feuer et al. (61) state the powder is stable for at least a week. It was found here that good yields of actin are only obtained when extraction takes place within 12-18 hours of powder preparation.

Five grams of the powder (total yield) was suspended in 100ml of buffer A (2mM Tris-HCl, 0.2mM ATP, 0.5mM 2-mercaptoethanol, 0.2mM CaCl$_2$, pH 8.0 at 25°C) at 0-2°C (63) and the solution stirred for 30 minutes. The mixture was then passed through a thin cloth and the fluid retained. The muscle residue was washed with 50ml buffer A and passed through a thin cloth once more. The fluids were combined and cleared by centrifugation at 10,000 x g for 1 hour. The white layer of phospholipid was removed carefully from the surface of the solution using a pasteur pipette and discarded. The solution (100ml) was decanted into a conical flask and KCl and MgCl$_2$ were added to a final concentration of 50mM and 2mM respectively. After initial stirring to dissolve the KCl, the actin was allowed to polymerize without stirring for 2 hours, after which time KCl was added to a concentration of 600mM and the solution degassed. The solution was stirred gently for the next 1.5 hours. It has been shown that few, if any proteins associate with actin at ionic strengths this high. The solution was then centrifuged at 90,000 x g for 3 hours and the pellet suspended in 6ml of buffer A using a glass
homogenizer. This solution was then dialysed against 500ml of buffer A for the next three days, changing to fresh buffer A every 24 hours. The solution thus obtained was then centrifuged at 80,000 x g to remove impurities and then repolymerized by the addition of KCl to 50mM. The yield was usually 10 mg of actin per g of acetone powder.

C. DETERMINATION OF TOTAL PROTEIN;

Protein was determined by the method of Lowry et al. (64). Fifty µl of myosin solution, or 20µl of actin solution was brought to 500µl with double distilled water. Five ml of alkaline copper reagent (0.04% CuSO₄, 2H₂O, 0.08% Na-Tartrate, in 3% Na₂CO₃) was added, and after vortexing, the solution was allowed to stand for 10 minutes. One-half ml of diluted Folin-Ciocalteau reagent (2:1) was then added and vortexed immediately. After 30 minutes at room temperature, the OD was determined in a spectrophotometer at a wavelength of 660 nm. Determinations were made in triplicate and standards containing 0.5ml of 0.05, 0.1 and 0.2 mg/ml bovine serum albumin were also employed. Protein concentration was determined as follows:

\[
[\text{Protein}] = \frac{\text{OD}_{\text{sample}}}{\text{OD}_{\text{standard}}} \times [\text{standard}] \times DF
\]

Where DF is the dilution factor and was 10 for myosin and 25 for actin.
D. CALCIUM ACTIVATED ATPase;

Fifty ul of myosin solution (2mg.myosin/ml in 500mM KCl, 50mM Tris, pH 7.4) was added to 850ul of either buffer A (58.82mM Tris-HCl, 5.88mM KCl, 11.76mM CaCl₂, pH 7.4) or buffer B (58.82mM Glycine, 5.88mM KCl, 11.76mM CaCl₂, pH 9.8) and the solutions vortexed and pre-incubated for 5 minutes at 37°C in a water bath. The reaction was initiated by the addition of 100ul of 30mM ATP (sodium salt) at pH 7.4 and terminated after 5 minutes by the addition of 1ml 2% SDS in 50mM Tris-HCl, pH 7.2. The solutions were then placed in an ice bath to slow the spontaneous hydrolysis of ATP. Assays were run in triplicate with a myosin blank, the OD of which was subtracted from that of the experimental tubes to allow for ATP hydrolysis occurring in the absence of myosin. Inorganic phosphate liberation was determined according to the method of Rockstein and Herron (65), which consisted of adding 3ml of an acid molybdate-iron sulphate solution (11.74g (NH₄)₂Mo₇O₄.4H₂O, 38ml conc. H₂SO₄, 10g FeSO₄.7H₂O, 15ml 1N H₂SO₄, bought to 600ml with distilled water), vortexing, and reading OD after 10 minutes at a wavelength of 720 nm. Standards of 0.1, 0.3 and 0.6 mM PO were also assayed.

The velocity of ATP hydrolysis was calculated using the following equation:

v = (ODsamp-ODbl)/ODst x [stand] x l/.05 x 1/[M] x 1/time.

The results being expressed in units of umoles of Pi liberated/mg.myosin/min.
E. ACTIN AND MAGNESIUM ACTIVATED ATPase

Fifty microliters of myosin solution (approx. 2mg myosin/ml in 500mM KCl, 50mM Tris, pH 7.4) was added to 750ul of buffer containing 50mM Tris-HCl, 3.33mM MgCl₂, 0.133mM CaCl₂, and either no KCl (I=0.075), 20mM NaOH (I=0.095), or 50mM KCl (I=0.120) all at pH 7.6. One-tenth of one ml (100ul) of actin solution ([actin] = 0.3-12mM in 50mM KCl) was added, vortexed and the solution pre-incubated for 5 minutes at 37°C in a water bath. The reaction was initiated with the addition of 100ul of 30mM ATP solution and terminated at either 5 minutes (I=0.075) or 10 minutes (I=0.095 and 0.120) with 1ml 2% SDS 50mM Tris, pH 7.2 after which they were placed in an ice bath. The rest of the procedure followed the Ca⁺⁺ activated protocol, including the same standards and blanks.
Vmax and Km were determined for each of the myosins isolated from animals 8-15. They were determined at two ionic strengths as it has been found that differences which occur between fast myosins at low ionic strength after light chain exchange are eliminated at higher ionic strengths (57). The same observations have been made on the myosin subfragments (55).

Linear regression analysis of the Eadie-Scatchard plots were used to determine Vmax and Km. The Eadie-Scatchard plot of v/[A] versus v has a gradient of -1/Km with the intercept at the v axis giving Vmax. Several advantages were found in using this plot as opposed to the commonly used Lineweaver-Burke plot of 1/v versus 1/[A]. Firstly, the Eadie-Scatchard plot gives values which are fairly evenly spaced (see Fig.3) whereas the Lineweaver-Burke plot has values concentrated toward the origin of the line (see Fig.2). The problem with this is that when applying linear regression to unevenly spaced data, the most extreme values have the greatest effect on the results. In the case of the Lineweaver-Burke plot it is the values which are obtained at low [A] which effect the plot the most, and it is these values which are of the least significance as they approach first order kinetics. Finally, it has been suggested that the Eadie-Scatchard plot is better to use when errors in v are significant as it does not involve reciprocals of v and
hence will not amplify the error (66). Analysis of these plots yielded mean correlation coefficients of $-0.8776$ for $I = 0.075$, and $-0.8861$ for $I = 0.095$ (N=16).

Statistical comparison between the control and hypertrophied groups for the various assays were made using the paired t-test. The 0.05 confidence level was selected for significance, but when appropriate, higher confidence levels are reported.
RESULTS

The average weight of the hypertrophied plantaris was approximately 83% greater than its contralateral control (Table I), this value being almost identical to those of Ianuzzo et al. (39) and Noble et al. (47), who obtained 80% and 88% respectively, employing the same surgical procedure.

The yields of myosin for the control and hypertrophied plantaris were not significantly different (Table I), indicating that the protein content of the muscle has increased in proportion to its weight (51). This discounted the possibility of selective extraction of one type of myosin. The yields are also in accord with those of Bárány (28) revealing that about 60% of the total myosin in the muscle was extracted for biochemical assays.

Myosin isolated from the hypertrophied plantaris exhibited a 7% reduction in its calcium activated ATPase value when assayed at both pH 7.4 and pH 9.8, when compared to its contralateral control (Table II).

Actin activated myosin ATPase activities were determined on myosin isolated from the plantaris muscles of animals 8-15. Actin concentrations from 0.03 to 10.8μM were used in the assays. The assays were run in duplicate and averaged to determine reaction velocity. Regression analysis of the Eadie-Scatchard plots yielded correlation coefficients between 0.68 and 0.99 (mean = 0.89, n = 32). The Km and
Vmax determined for each myosin were averaged, and the results shown in Table III. Hypertrophy resulted in a significant decrease in the Vmax and Km values at both ionic strengths. The decreases were 28%, 36%, 31% and 34% for Vmax (I=0.075), Km (I=0.075) Vmax (I=0.095) and Km (I=0.095) respectively. Increasing I resulted in a large increase in Km (P < .005) as has been previously demonstrated (67,68). This fact makes it increasingly difficult to determine Km and Vmax as I approaches the physiological ionic strength of 0.125. Figure 1. shows the V versus [A] plots for the actin activated ATPase assays.

The typical Michaelis-Menton type curves' yield linear Lineweaver-Burk plots (Fig. 2). Regression analysis of these plots yielded a mean correlation coefficient of r = .97 (n = 32). The Eadie-Scatchard plots are of the type used to determine Vmax and Km for each myosin sample.

Figure 4 is a graph constructed to show the % reduction in the actin activated ATPase values for any given actin concentration. The equations for these graphs were determined from Vmax and Km as shown in appendix D.
### TABLE I

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>HYPERTROPHY</th>
</tr>
</thead>
<tbody>
<tr>
<td>MUSCLE WET WEIGHT (g)</td>
<td>0.297 ± .006</td>
<td>0.530 ± .020*** (16)</td>
</tr>
<tr>
<td>MYOSIN YIELD (mg./g)</td>
<td>37.83 ± 1.13</td>
<td>36.35 ± 1.02 (16)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., significant at *** P < .0005. Numbers in parenthesis indicate n.

### TABLE II

**CALCIUM ACTIVATED MYOSIN ATPase (umoles Pi liberated/mg.myosin/min.)**

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>HYPERTROPHY</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH 7.4</td>
<td>0.900 ± .017</td>
<td>0.834 ± .025*** (16)</td>
</tr>
<tr>
<td>pH 9.8</td>
<td>2.082 ± .066</td>
<td>1.936 ± .069** (16)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., significant at *** P < .005, ** P < .025. Numbers in parenthesis indicate n.

### TABLE III

**ACTIN ACTIVATED ATPase**

<table>
<thead>
<tr>
<th>I = .075</th>
<th>CONTROL</th>
<th>HYPERTROPHY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vmax.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.375 ± .095</td>
<td>0.986 ± .048*** (8)</td>
</tr>
<tr>
<td></td>
<td>Km</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3.04 ± 0.28</td>
<td>1.96 ± 0.24*** (8)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>I = .095</th>
<th>CONTROL</th>
<th>HYPERTROPHY</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Vmax.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.076 ± .089</td>
<td>0.738 ± .035*** (8)</td>
</tr>
<tr>
<td></td>
<td>Km</td>
<td></td>
</tr>
<tr>
<td></td>
<td>8.83 ± 1.59</td>
<td>5.80 ± 0.57** (8)</td>
</tr>
</tbody>
</table>

Values are mean ± S.E.M., significant at *** P < .005, ** P < .05. Numbers in parenthesis indicate n. Vmax. in units of umol./mg./min. Km in units of uM.
FIGURES 1a and 1b. Reaction velocity versus actin concentration at I = .075 (top) and I = .095 (bottom). Points are mean ± S.E.M. —△— Control, —— Hypertrophy.
FIGURES 2a and 2b. Lineweaver-Burk plots for I = .075 (top) and I = .095 (bottom). Points are mean ± S.E.M. —— Control, —— Hypertrophy.
FIGURES 3a and 3b. Eadie-Scatchard plots for $I = .075$ (top) and $I = .095$ (bottom). Points are mean ± S.E.M. —— Control, —— Hypertrophy.
FIGURE 4. Percent Decrease In Velocity at Given Actin Concentrations. Equations for these graphs are derived as shown in appendix D. Dotted lines indicate the actin concentration at which hypertrophy results in a 7% reduction in actin activated ATPase.
DISCUSSION

In accord with prior investigations, the hypertrophied plantaris had increased in size by an average 83%. It is anticipated, as has previously been demonstrated (47,51), that a change in the histochemical profile of the hypertrophied muscle had occurred.

As with previous studies employing the same model (47,51), myosin isolated from the hypertrophied plantaris exhibited a significant 7% reduction in its calcium activated ATPase when assayed at both pH 7.4 and pH 9.8, when compared to its contralateral control (Table II). Assuming a threefold increase in the number of histochemically determined alkaline labile fibers (from 8% to 24%), we would have anticipated a greater depression in the ATPase of the hypertrophied muscle myosin at pH 9.8.

Sréter et al. (69) showed that incubation at alkaline pH resulted in a loss of ATPase activity in the myosin isolated from slow-twitch muscle, with minimal reduction in fast-twitch muscle myosin activity. Jablecki and Kaufman (70) have since quantified this somewhat to indicate that around pH 9.8 a 65% inactivation of slow myosin would be expected. This, combined with the observation of Bárány et al. (28), that fast muscle myosin exhibits a 300% activation at this pH, would indicate the extent of ATPase depression anticipated following hypertrophy would be approximately 17% at pH 9.8 (see Appendix B for
calculation). The reason for the discrepancy between the anticipated and actual depression is unclear at the present time, but has been reported by others (47, 51).

A possible reason for the discrepancy between the histochemical alterations and the biochemical activity can be explained with regard to the findings of Maxwell et al. (71). It is known that the histochemical procedure for the determination of alkaline lability is reliant upon the use of Ca$^{++}$ ions for trapping inorganic phosphate (72). When the amount of calcium phosphate exceeds its solubility product, it precipitates resulting in a stain. This is therefore a threshold response. If we consider that there is a range of ATPase values exhibited in fibers of a particular type, and that some of these values are close to the threshold, then a slight shift in the values of these ATPase may cause an apparent major shift in fiber type. Thus a complete transformation may be inferred histochemically, when in fact only a small shift had occurred in a biochemical sense.

To examine the possible physiological consequence of the muscle adaptation associated with compensatory hypertrophy, the actin activated myosin ATPase activity of control and hypotrophied muscle was examined. In contrast to Ca$^{++}$ activation, actin activation of the myosin ATPase, at both ionic strengths examined, resulted in a 30% reduction in Vmax following hypertrophy (Table III). The reduction in actin activated ATPase activity following hypertrophy is
dependent on actin concentration, however. Our findings suggest that any concentration of actin above 1.7uM at I = .075, or 2.7uM at I = .095 will result in a greater depression in ATPase activity than was observed with calcium activation (Figure 4.). In order to assess the significance of these observations one needs to know the approximate actin concentration in muscle. In attempting to determine the approximate in vivo concentration of actin relative to myosin, it is difficult to account for the effects of steric blocking and molecular orientation factors bought about due to the highly organised structure of the myofibrillar system. It is likely that this structural organization optimises the "effective" concentration of actin by increasing the probability of an operative collision. If the Km value does in fact represent the approximate intracellular level of actin (66), then at the Km value for the control plantaris a decrease of 13% and 17% in actin activated ATPase would be expected following hypertrophy at ionic strengths of .075 and .095 respectively. These values are somewhat greater than those observed using calcium activation. It is tempting to speculate that the increased depression of the actin activated ATPase over the Ca activated values are a result of the influence of the light chains. At the present time however, the only conclusion which can be drawn is that there is an alteration in the binding capacity of the myosin to actin which results in a decreased catalytic function.
In addition to determining the difference between both Ca$^{++}$ and actin activation of control and hypertrophied muscle myosin, the kinetic properties of the actin activated myosin were examined. Although these kinetic properties have never been reported for hypertrophied muscle myosin, the values for Vmax and Km for the control plantaris are in accord with those found for rabbit fast-twitch muscle myosin (57,68), when taking ionic strength into account. At I = .075 the Vmax is reduced 28%, from 1.375 to 0.986 umoles/mg./min. following hypertrophy, whilst Km is reduced 36%, from 3.04 to 1.96uM. At I = .095 the Vmax is reduced 31%, from 1.076 to 0.738 umoles/mg./min., whilst the Km declines 34%, from 8.83 to 5.80uM, all these alteration being statistically significant. The alterations are in the direction expected in view of the light chain changes which accompany hypertrophy. It is tempting to hypothesize that it is in fact the light chains which are responsible for the altered catalytic function of the hypertrophied muscle myosin. Many investigators have attempted to determine whether or not the light chains effect the catalytic function of myosin. To control the phenotype of the heavy chain, light chain exchange in the presence of ammonium chloride is often used (57,68), therefore any subsequent difference in catalytic function is a result of either the new light chain present, or the treatment employed. Unfortunately, the deleterious effect of the ammonium chloride treatment on catalytic function, obscures any
alteration which may have occurred due to the presence of the light chains. An example of this can be seen in the experiments of Wagner (68), who showed a 40% decrease in Km (from 18uM to 10uM) following the replacement of the native fast light chains with cardiac light chains. Although the alteration was pronounced, it could not be attributable to the cardiac light chains present, as a control fast myosin which underwent ammonium chloride treatment had a Km of 3uM. The model used in the present experiments affords the benefit of being able to extract a "naturally" hybridized myosin directly from the hypertrophied muscle, no loss of ATPase activity can then confound the interpretation of the results. The effect of the light chains on the function of myosin still remains in doubt, however, the fact that in the present experiments, the actin activated ATPase gave a substantially greater decrement in the reaction velocity following hypertrophy than did calcium activation, would tend to support the hypothesis that the light chains are implicated in the actin-myosin interaction in a regulatory capacity. A definitive conclusion however, is not possible in the absence of data on the heavy chain alterations which may accompany hypertrophy.

It has been previously demonstrated that differences observed between the A1-A1 and A2-A2 fast myosin homodimers at low ionic strengths are not apparent at higher I (57), these observations have also been made on the subfragments S1(A1) and S1(A2) (55), and also on the
hybrid S1 containing cardiac light chain I (68). Knowing this effect of ionic strength, it was interesting to view its effect on the myosin isolated here. As is apparent from table III, the magnitude of the trends is preserved with increasing I, suggesting that they will in fact be of similar magnitude at the physiological ionic strength of I = .125. The inhibitory effect of ionic strengths this high on ATPase activity has precluded the possibility of obtaining reliable kinetic data here. Even using the highly sensitive electrotitrimetric pH-stat method for determining V, other researchers have expressed difficulty in obtaining reliable extrapolations to Vmax and Km (57). If these trends are significant at I = .125, and are therefore operational in the working muscle, the question arises as to their functional significance.

In a simplified version of the interaction between actin and myosin, the following sequence is thought to occur (see 20 for review); A + M* ⇌ AM* ⇌ AM ⇌ A + M, where A denotes actin, M denotes myosin, and * denotes the energized conformation of myosin. The transition of myosin from the energized state to the ground state results in cross-bridge movement, and hence contraction. Vmax is by definition, the maximal velocity that would be observed when all of the enzyme is present in the ES form, or as shown here, the AM form. Vmax is therefore intimately related to the rate of product release, or the rate of dissociation of actin from myosin. Km, the Michaelis constant, is a combination of the
rate constants involved in all steps of the conversion of A+M* to A+M, thus its physical significance cannot be stated with certainty in the absence of other kinetic data. The greatest significance of Km here lies in the fact that it is inversely proportional to the affinity of actin for myosin, thus the decrease in Km accompanying hypertrophy is interpreted as an increase in the affinity of the two proteins for each other. Whereas Vmax is associated with the rate of breakdown of the enzyme complex, Km is known to be associated with the rate of formation of this complex. Thus the decrease in Vmax and Km following hypertrophy would result in a faster formation of AM* with a slower dissociation of AM to A+M. Both these observations would suggest that the time spent in the phase of the actin-myosin interaction is longer following compensatory hypertrophy. This observation can be related to the efficiency of the various muscle types in different types of movement.

Goldspink et al. (50), have shown that the slow soleus muscle of the hamster is about five times more efficient in maintaining isometric tension than fast muscles of the same animal. They concluded that the energetic efficiency of slow muscle during isometric work was due to the longer crossbridge engagement time. The cycling of each crossbridge is known to cost an ATP, if tension can be stored in the form of the crossbridge for a longer period of time, the ramifications as far as ATP turnover and
tension maintenance are obvious.

Awan et al. (73); in a similar series of experiments, found fast muscle more efficient during dynamic work than slow muscle. This is presumably because myosin crossbridges which are pulling are doing so against those still in the transiently inactive bridged conformation. The underlying suggestion is that fast muscles have evolved for efficiency in performing dynamic work whilst slow muscles have evolved for efficiency in maintaining isometric tension (50).

The hypertrophying plantaris muscle can be thought of as taking on a role of postural maintenance as it is isometrically active for the duration of the overload. In this respect, the advantage in increasing the apparent affinity of the actin for the myosin and decreasing the Vmax would be realised.
SUMMARY AND CONCLUSIONS

Whole myosin isolated from hypertrophied muscle exhibits a significant 7\% reduction in its calcium activated ATPase activity at both pH 7.4 and pH 9.8. The fact that the reduction in ATPase activity was not of the 17\% magnitude expected on the basis of the increased number of alkaline labile fibers, is thought to be due to either the inability of Ca\(^{++}\) to reflect the light chain changes in the hypertrophied myosin molecule, or an incomplete transformation in the myosin phenotype. The actin activated ATPase data show significant reductions of approximately 30\% in Vmax and Km following hypertrophy at both ionic strengths. The persistence of these trends probably indicate an altered in vivo function. Although these data support the idea that the changes observed are attributable to the altered light chain pattern in the hypertrophied plantaris myosin, confirmation of this hypothesis is not possible in the absence of data on the alterations in phenotype of the myosin heavy chain.

The significance of these trends in a muscle with an altered functional demand can be explained by the fact that the increase in the affinity of the hypertrophied myosin for actin and decreased rate of product release, as indicated by the decrease in Vmax, would result in a longer engagement time between the actin and myosin. This in turn would aid in maintaining isometric tension whilst conserving ATP.
In summary, it seems the adaptations which take place in the myosin molecule in response to compensatory hypertrophy are of a definite advantage to the functioning of the muscle in its new role of maintenance of isometric tension.
### APPENDIX A

#### RAW DATA

<table>
<thead>
<tr>
<th>ANIMAL No.</th>
<th>WEIGHT (g)</th>
<th>YIELD (mg/g)</th>
<th>[MYOSIN] (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP</td>
<td>HP</td>
<td>CP</td>
</tr>
<tr>
<td>1</td>
<td>.255</td>
<td>.528</td>
<td>34.6</td>
</tr>
<tr>
<td>2</td>
<td>.322</td>
<td>.610</td>
<td>41.3</td>
</tr>
<tr>
<td>3</td>
<td>.278</td>
<td>.581</td>
<td>37.8</td>
</tr>
<tr>
<td>4</td>
<td>.269</td>
<td>.380</td>
<td>39.7</td>
</tr>
<tr>
<td>5</td>
<td>.300</td>
<td>.550</td>
<td>43.3</td>
</tr>
<tr>
<td>6</td>
<td>.305</td>
<td>.490</td>
<td>41.6</td>
</tr>
<tr>
<td>7</td>
<td>.328</td>
<td>.645</td>
<td>43.0</td>
</tr>
<tr>
<td>8</td>
<td>.303</td>
<td>.607</td>
<td>42.9</td>
</tr>
<tr>
<td>9</td>
<td>.280</td>
<td>.465</td>
<td>41.6</td>
</tr>
<tr>
<td>10</td>
<td>.342</td>
<td>.535</td>
<td>35.1</td>
</tr>
<tr>
<td>11</td>
<td>.315</td>
<td>.557</td>
<td>34.4</td>
</tr>
<tr>
<td>12</td>
<td>.302</td>
<td>.640</td>
<td>34.4</td>
</tr>
<tr>
<td>13</td>
<td>.291</td>
<td>.465</td>
<td>31.2</td>
</tr>
<tr>
<td>14</td>
<td>.266</td>
<td>.484</td>
<td>36.1</td>
</tr>
<tr>
<td>15</td>
<td>.293</td>
<td>.611</td>
<td>30.4</td>
</tr>
<tr>
<td>SOLEUS 16</td>
<td>.150</td>
<td>.150</td>
<td>23.5</td>
</tr>
</tbody>
</table>

**Note:** Both soleus muscles are control.

#### CALCIUM ACTIVATED ATPases (umoles/mg myosin/min.)

<table>
<thead>
<tr>
<th>ANIMAL No.</th>
<th>pH 7.4</th>
<th>pH 9.8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CP</td>
<td>HP</td>
</tr>
<tr>
<td>1</td>
<td>813</td>
<td>739</td>
</tr>
<tr>
<td>2</td>
<td>806</td>
<td>647</td>
</tr>
<tr>
<td>3</td>
<td>850</td>
<td>717</td>
</tr>
<tr>
<td>4</td>
<td>938</td>
<td>872</td>
</tr>
<tr>
<td>5</td>
<td>855</td>
<td>808</td>
</tr>
<tr>
<td>6</td>
<td>906</td>
<td>945</td>
</tr>
<tr>
<td>7</td>
<td>870</td>
<td>780</td>
</tr>
<tr>
<td>8</td>
<td>850</td>
<td>755</td>
</tr>
<tr>
<td>9</td>
<td>902</td>
<td>861</td>
</tr>
<tr>
<td>10</td>
<td>996</td>
<td>877</td>
</tr>
<tr>
<td>11</td>
<td>1015</td>
<td>1022</td>
</tr>
<tr>
<td>12</td>
<td>952</td>
<td>829</td>
</tr>
<tr>
<td>13</td>
<td>972</td>
<td>913</td>
</tr>
<tr>
<td>14</td>
<td>906</td>
<td>902</td>
</tr>
<tr>
<td>15</td>
<td>877</td>
<td>851</td>
</tr>
</tbody>
</table>

43
### ACTIN ACTIVATED ATPase (umoles/mg-myosin/min.)

\[ I = 0.075 \]

<table>
<thead>
<tr>
<th>ANIMAL No.</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ACTIN] (uM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.6</td>
<td>893</td>
<td>879</td>
<td>987</td>
<td>898</td>
<td>770</td>
<td>1053</td>
<td>1007</td>
<td></td>
</tr>
<tr>
<td>8.0</td>
<td>659</td>
<td>699</td>
<td>958</td>
<td>807</td>
<td>770</td>
<td>829</td>
<td>782</td>
<td></td>
</tr>
<tr>
<td>5.3</td>
<td>452</td>
<td>472</td>
<td>628</td>
<td>685</td>
<td>560</td>
<td>609</td>
<td>652</td>
<td>537</td>
</tr>
<tr>
<td>4.0</td>
<td>1.4</td>
<td>1.1</td>
<td>0.9</td>
<td>0.7</td>
<td>0.5</td>
<td>207</td>
<td>187</td>
<td></td>
</tr>
<tr>
<td>2.7</td>
<td>222</td>
<td>276</td>
<td>316</td>
<td>221</td>
<td>272</td>
<td>196</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.1</td>
<td>301</td>
<td>338</td>
<td>481</td>
<td>390</td>
<td>300</td>
<td>427</td>
<td>290</td>
<td>282</td>
</tr>
<tr>
<td>1.4</td>
<td>417</td>
<td>309</td>
<td>272</td>
<td>287</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.1</td>
<td>316</td>
<td>221</td>
<td>272</td>
<td>196</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.9</td>
<td>222</td>
<td>276</td>
<td>316</td>
<td>221</td>
<td>272</td>
<td>196</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.7</td>
<td>301</td>
<td>338</td>
<td>481</td>
<td>390</td>
<td>300</td>
<td>427</td>
<td>290</td>
<td>282</td>
</tr>
<tr>
<td>0.5</td>
<td>417</td>
<td>309</td>
<td>272</td>
<td>287</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>207</td>
<td>187</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

### HYPERTROPHY

| 10.6 | 790 | 866 |   |   | 961 | 925 |
| 8.0  | 584 | 710 | 759| 694| 772 | 804 | 761|
| 5.3  | 437 | 465 | 632| 593| 643 | 467 |   |
| 4.0  | 314 | 380 | 555| 355| 412 | 434 |   |
| 2.7  | 450 | 261 | 417| 520| 409 | 583 | 514|
| 2.1  | 359 | 268 | 244| 282|   |   |   |
| 1.4  | 322 | 187 | 217| 254|   |   |   |
| 1.1  | 247 | 258 |   |   |   |   |   |
| 0.9  | 245 | 247 |   |   |   |   |   |
| 0.7  | 245 | 247 |   |   |   |   |   |
| 0.5  | 245 | 247 |   |   |   |   |   |
**ACTIN ACTIVATED ATPases (umoles/mg. myosin/min.)**

\[ I = 0.095 \]

<table>
<thead>
<tr>
<th>ANIMAL No. 8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>.15</th>
</tr>
</thead>
<tbody>
<tr>
<td>[ACTIN] (uM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.9</td>
<td>522</td>
<td>535</td>
<td>488</td>
<td>566</td>
<td>448</td>
<td>523</td>
<td>522</td>
</tr>
<tr>
<td>5.4</td>
<td>453</td>
<td>435</td>
<td>394</td>
<td>477</td>
<td>393</td>
<td>429</td>
<td>445</td>
</tr>
<tr>
<td>3.9</td>
<td>423</td>
<td>395</td>
<td>351</td>
<td>446</td>
<td>358</td>
<td>375</td>
<td>390</td>
</tr>
<tr>
<td>2.2</td>
<td>251</td>
<td>268</td>
<td>212</td>
<td>297</td>
<td>225</td>
<td>227</td>
<td>245</td>
</tr>
<tr>
<td>1.1</td>
<td>135</td>
<td>130</td>
<td>103</td>
<td>139</td>
<td>105</td>
<td>116</td>
<td>132</td>
</tr>
<tr>
<td>0.4</td>
<td>62</td>
<td>72</td>
<td>54</td>
<td>60</td>
<td>38</td>
<td>49</td>
<td>48</td>
</tr>
<tr>
<td>0.3</td>
<td>44</td>
<td>47</td>
<td>29</td>
<td>45</td>
<td>29</td>
<td>39</td>
<td>45</td>
</tr>
</tbody>
</table>

**HYPERTROPHY**

<table>
<thead>
<tr>
<th>[ACTIN] (uM)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>10.9</td>
<td>478</td>
<td>444</td>
<td>410</td>
<td>436</td>
<td>501</td>
<td>489</td>
<td>469</td>
</tr>
<tr>
<td>5.4</td>
<td>392</td>
<td>381</td>
<td>328</td>
<td>340</td>
<td>385</td>
<td>350</td>
<td>354</td>
</tr>
<tr>
<td>3.9</td>
<td>386</td>
<td>297</td>
<td>304</td>
<td>321</td>
<td>297</td>
<td>348</td>
<td>262</td>
</tr>
<tr>
<td>2.2</td>
<td>219</td>
<td>214</td>
<td>177</td>
<td>207</td>
<td>222</td>
<td>211</td>
<td>175</td>
</tr>
<tr>
<td>1.1</td>
<td>119</td>
<td>130</td>
<td>97</td>
<td>106</td>
<td>120</td>
<td>108</td>
<td>98</td>
</tr>
<tr>
<td>0.4</td>
<td>64</td>
<td>63</td>
<td>50</td>
<td>53</td>
<td>58</td>
<td>43</td>
<td>37</td>
</tr>
<tr>
<td>0.3</td>
<td>48</td>
<td>53</td>
<td>32</td>
<td>38</td>
<td>40</td>
<td>37</td>
<td>34</td>
</tr>
</tbody>
</table>
APPENDIX B

CALCULATION OF THE EXPECTED REDUCTION IN ATPase AT pH 7.4 & 9.8

Assuming no hyperplasia occurs in the hypertrophied muscle the following calculations can be made.

<table>
<thead>
<tr>
<th></th>
<th>CONTROL</th>
<th>HYPERTROPHY</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Fast</td>
<td>92</td>
<td>% Fast</td>
</tr>
<tr>
<td>% Slow</td>
<td>8</td>
<td>% Slow</td>
</tr>
</tbody>
</table>

Assuming the activity of the fast fibres is 1, then that of the slow is 0.4.

\[
92 + (8 \times 0.4) = 95.2 \\
76 + (24 \times 0.4) = 86.2
\]

Thus a 9.5% decrease in activity is expected without inactivation.

Assuming a 65% inactivation in the slow fibre population (75) at pH 9.8, and a 300% activation of fast myosin;

\[
300 \times 92 + (8 \times 0.4 \times 0.35) = 27,601 \\
300 \times 76 + (24 \times 0.4 \times 0.35) = 22,803
\]

Thus a 17% reduction in ATPase is expected at pH 9.8.
APPENDIX C

CALCULATION OF IONIC STRENGTH

The reaction mixes for the actin activated ATPases contained the following;

<table>
<thead>
<tr>
<th>LOW I</th>
<th>MEDIUM I</th>
<th>HIGH I</th>
</tr>
</thead>
<tbody>
<tr>
<td>30mM KCl</td>
<td>30mM KCl</td>
<td>80mM KCl</td>
</tr>
<tr>
<td>50mM Tris</td>
<td>50mM Tris</td>
<td>50mM Tris</td>
</tr>
<tr>
<td>2.5mM MgCl</td>
<td>2.5mM MgCl</td>
<td>2.5mM MgCl</td>
</tr>
<tr>
<td>0.1mM CaCl</td>
<td>0.1mM CaCl</td>
<td>0.1mM CaCl</td>
</tr>
<tr>
<td></td>
<td>20mM NaOH</td>
<td></td>
</tr>
</tbody>
</table>

\[
I = \frac{1}{2} \sum M_i z_i^2, \\
\text{where } z = \text{the net charge of the ion,} \\
\text{and } M = \text{the molarity of the ion.}
\]

In determining the ionic strength Tris is assumed to hydrolize to Tris\(^+\) and Tris\(^0\). The Tris\(^0\) has no effect on ionic strength. Tris is also assumed to have an activity coefficient of 0.9. To determine \([\text{Tris}^+]\) we use the following equation:

\[
\text{pH} = \text{pK}_a + \log(\frac{[\text{Tris}^0]}{0.9[\text{Tris}^+]})
\]

We know the pH of the solution is 7.6 and the pK\(_a\) of Tris is 8.1.

\[
7.6 = 8.1 + \log(\frac{[\text{Tris}^0]}{0.9[\text{Tris}^+]})
\]

\[
0.316 = \frac{[\text{Tris}^0]}{0.9[\text{Tris}^+]} \\
0.2884 = \frac{[\text{Tris}^0]}{[\text{Tris}^+]} \\
[\text{Tris}^+] = 10,000/12,844 \times 0.05 \\
= 0.039
\]

\[
\text{LOW I} = \frac{1}{2}[0.03 + 0.03 + 0.0025 + (0.0025 \times 4) + 0.0001 \\
+ (0.0001 \times 4)] + 0.039 \\
= 1/2 \times [0.073] + 0.039 \\
= 0.075
\]

\[
\text{MED I} = \frac{1}{2}[0.073 + 0.02 + 0.02] + 0.039 \\
= 1/2 \times [0.113] + 0.039 \\
= 0.095
\]

\[
\text{HIGH I} = \frac{1}{2}[0.08 + 0.08 + 0.013] + 0.039 \\
= 0.125
\]
APPENDIX D

DETERMINATION OF THE EQUATION FOR THE EXPECTED % DECREASE IN V AT A GIVEN ACTIN CONCENTRATION.

Using the formula \( v = \frac{V_{\text{max}} x [A]}{(K_m + [A])} \), we find the % decline in ATPase activity given by the formula,

\[
100 - \left( \frac{V_{\text{max}}(H)/V_{\text{max}}(C) x 100}{(K_m(H) + [A]) x 100} \right)
\]

\[
\% \text{ DECLINE} = 100 - \left( \frac{V_{\text{max}}(H)/V_{\text{max}}(C) x (K_m(C) + [A])}{(K_m(H) + [A]) x 100} \right)
\]

For \( I = 0.075 \) this becomes,

\[
\% \text{ DECLINE} = 100 - \left(71.71 x (3.04 + [A])/(1.96 + [A])\right)
\]

For \( I = 0.095 \) this becomes,

\[
\% \text{ DECLINE} = 100 - \left(68.6 x (8.83 + [A])/(5.80 + [A])\right)
\]

These equations are graphed in figure 4.
REFERENCES


VITA AUCTORIS

GRAEME JOHN LANG was born in 1957, he received a B.Ed. degree from SCV Rusden, Australia in 1978. He was accepted at the University of Windsor in the Human Kinetics faculty in the fall of 1981 and received his M.H.K. from there in the fall of 1983.