# MECHANICS AND ENERGETICS OF ACTIVELY SHORTENING AND LENGTHENING AIRWAY SMOOTH MUSCLE

A Thesis
Submitted to the Faculty
of Graduate Studies, University of Manitoba
in partial fulfillment
of the requirements for the degree of
Master of Science

by
Brenda S.R. Hanks

April, 1979

# MECHANICS AND ENERGETICS OF ACTIVELY SHORTENING AND LENGTHENING AIRWAY SMOOTH MUSCLE

BY

BRENDA STUART ROULSTON HANKS

A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

MASTER OF SCIENCE

ABSTRACT

Muscles, in vivo are regularly stretched during stimulation, yet most studies have dealt with properties of the muscle during shortening and ignored the lengthening aspects. The present study investigated some of the mechanical and energetic properties of lengthening canine tracheal smooth muscle (TSM).

The first part of the study explored the often neglected side of the force-velocity relationship, during which active muscle is stretched. TSM was stimulated isometrically at optimum length ($l_o$) until $P_o$ (the maximum isometric tetanic tension) was reached, at which point a new load was instantly applied and the maximum velocity of either shortening (for loads less than $P_o$) or lengthening (for loads greater than $P_o$) measured. Data for loads less than $P_o$ could be fitted by Hill's hyperbolic equation (for the force-velocity relationship of the contractile element of muscle), with values of the constants consistent with those previously reported for the muscle. Between $P_o$ and 150% $P_o$, a linear relationship existed which deviated significantly from an extension of Hill's hyperbola. At loads greater than $P_o$ the velocity of lengthening was greater than predicted by the equation. However, canine TSM was able to resist these loads to such an extent that at 150% $P_o$ it lengthened at a velocity of only .02 $l_o$/sec., as compared to rat portal vein (Johansson et al, 1978), which at 140% $P_o$ lengthened at a velocity of .35 $l_o$/sec. TSM resembled skeletal muscle in its ability to resist stretch as greater loads were imposed.

In studies documenting the energetics of lengthening TSM, it

has been shown for skeletal muscle that the net rate of energy liberation of an elongating active muscle is less than that of a muscle contracting isometrically. There is no extra energy cost for the muscle while it is being stretched, even though the force exerted by it is much greater than during shortening at the same speed, as shown by the force-velocity studies.

The present study involved mechanical and biochemical methods to determine whether this energy saving property of actively stretched skeletal muscle exists in TSM. This phenomenon was demonstrated mechanically in experiments with muscles of different cross sectional areas contracting in opposition. It was shown biochemically by measuring the levels of high energy phosphates (adenosine triphosphate [ATP] and creatine phosphate [CP]) in actively stretching and actively shortening TSM. Results indicated that less ATP and CP were used in a muscle stretched during stimulation as compared to one shortening during stimulation.

Thus in both mechanical and biochemical experiments on TSM the present evidence showed that an active muscle which was stretched could maintain a much greater force and expend less energy than one which shortened.

ACKNOWLEDGEMENTS

I am profoundly grateful to my professor, Dr. N.L. Stephens, for giving me the opportunity to be his student, for his constant encouragement and support, and for the trust and confidence he placed in me. My sincere appreciation is extended to Dr. E.A. Kroeger for his sacrificial time and effort on my behalf and for his constructive criticism of this thesis. I am particularly indebeted to Richard Mitchell whose foresight and untiring efforts were alwasy forthcoming when the need arose. My gratitude is also extended to Uwe Kromer for his technical assistance and support. My thanks to Dr. Klaus Wrogemann, for having approved my thesis. And to Blanca, Robert, Lou, Virg, and all who have helped me to complete this work, my special thanks.

Finally, thank you all for being my friends.

This project was conducted with the aid of grants from the Medical Research Council of Canada and the Canadian Lung Association.

TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

# INTRODUCTION

## A. GENERAL INTRODUCTION

### Reasons for Studying Tracheal Smooth Muscle (TSM)

A thorough understanding of the mechanics and energetics of TSM is important in at least two respects:

1) in a pure sense: It is necessary to see whether smooth muscle resembles skeletal. TSM may be, within limits, an adequate model for other smooth muscles throughout the body, and it possesses the necessary mechanical properties for systematic studies of muscle function (Csapo, 1962; Hill, 1938-1939, 1948, 1964a, 1964b; Stephens, 1975). More information is required to determine how well the sliding filament model of contraction developed for skeletal muscle will apply to smooth muscle.

2) in an applied sense: TSM in itself may not be very important in controlling the distribution of ventilation. However, since it has been shown to be mechanically similar to smooth muscle of airways (ASM) to at least the 6th generation of bronchi (Hawkins & Schild, 1951; Nadel, 1973), it is a good model for ASM at this level where the distribution of ventilation becomes critical. Also, the pharmacology of ASM is qualitatively similar for the large airways and resistence units (Nadel, 1973; Permutt, 1973). Therefore a study of TSM could increase the understanding of this control.

## B. STATEMENT OF THE PROBLEM

The present study dealt with the mechanics and energetics of elongating active TSM, which until recently has been ignored. The force-velocity (F-V) relationship of canine TSM has previously been characterized (Stephens et al, 1969), however no study has yet been reported on the force-velocity relationship at loads which cause the

muscle to lengthen. There have been a few reports on the effects of stretching active skeletal muscle, and force-velocity curves during elongation have been reported for a few muscles (skeletal and smooth). It was therefore of interest to compare TSM to these.

Airway smooth muscle (ASM) is believed to be in a state of tonic contraction (Widdicombe, 1966). If this is so, then on inspiration the ASM in the lower airways is stretched and it is important to study what happens to the muscle during elongation.

Skeletal muscle studies have shown that there is a decrease in energy utilization during forcible lengthening of a contracted muscle (Abbott & Aubert, 1951; Hill, 1938). Therefore it was of interest to find out whether or not TSM showed the same phenomenom. Since adenosine triphosphate (ATP) and creatine phosphate (CP) are the most direct energy stores of the muscle, the energy costs of shortening versus lengthening TSM were determined by measuring concentrations of these high energy phosphates during the length changes.

## C. MUSCLE CONTRACTION

The basic mechanisms of contraction are common to all types of muscle. In skeletal muscle the four major contractile proteins are actin, myosin, tropomyosin and troponin. Actin and myosin are the only two proteins required for contraction; troponin and tropomyosin are involved in regulation.

Myosin, which forms the major component of the thick filaments is a 470,000 dalton protein with a bilobed head and rodlike tail. It consists of two heavy chains of 200,000 daltons, which exist mostly as helices wound about each other, and four light chains which

combine with the heavy chains in the head of the molecule. The adenosine triphosphatase activity and the actin-combining property of myosin are associated with the head of the myosin molecule.

Actin is composed of globular G-actin molecules which polymerize into long filaments, forming a double right-handed helix. In striated muscle, troponin and tropomyosin associate with actin to form the thin filaments. Whereas in skeletal muscle the contractile proteins are arranged into repeating sarcomeric units, which give the observable striation pattern, smooth muscle does not exist in such a state. Actin, myosin and adenosine triphosphate (ATP) can be extracted from smooth muscle and they interact in the same way as those extracted from skeletal muscle. It is not known whether troponin is present in all smooth muscles.

It is generally accepted that, in all muscles, contraction is initiated by depolarization of the cell membrane, brought about by either nervous activity, spontaneous activity, drugs or an increase in the external ion concentration. Similarly, calcium ions are involved in the excitation-contraction coupling process, the activation process which causes the fibre to contract. The term 'contraction' requires explanation. When a muscle is stimulated it contracts, the essential feature of contraction being a development of tension which results in a tendency to shorten. Whether or not the muscle actually shortens depends on the load imposed on it. If no change in muscle length is allowed the contraction is <u>isometric</u>. If the muscle shortens against a constant load the contraction is said to be <u>isotonic</u>. Muscles can also lengthen during contraction if the imposed load is great enough.

A nerve impulse reaching the neuromuscular junction or synapse between the nerve and the muscle fibre initiates the release of a transmitter. The transmitter then acts on the end plate membrane to increase the permeability of that membrane to $Na^+$ and $K^+$, thus resulting in depolarization of the end plate and triggering the impulse in the sarcolemma. This depolarization is transmitted to the interior via a transverse tubular system (T-tubules) which is continuous with the sarcolemma (Huxley & Taylor, 1958; Huxley, 1964(b)). This triggers calcium release from the associated sarcoplasmic reticulum and raises the intracellular calcium concentration to the threshold level needed for activation of contraction (Sandow, 1965). In skeletal muscle the calcium now binds to the troponin, which through a reaction mediated by tropomyosin, releases the inhibition previously effected by the troponin-tropomyosin complex and allows actin and myosin interaction to take place (Wakabayashi & Ebashi, 1968). Although the presence of troponin in certain types of smooth muscles has been reported (Carsten, 1971; Ebashi et al, 1966), it does not seem to be present in all smooth muscles. Therefore it is thought that in most smooth muscles the regulatory proteins do not belong to the troponin system and that calcium sensitivity of actomyosin ATPase activity is a property of the myosin molecule (Bremel, Sobieszek & Small, 1977). According to the classical sliding filament theory, (Huxley & Niedergerke, 1954; Huxley, 1957; Huxley, 1964(a)), the formation of physical cross-links (cross-bridges) between actin and myosin and the sliding of thin on thick filaments, produces tension development and shortening. Since the theory was proposed new findings of structural features have required it to be

updated. Huxley & Simmons (1971) proposed that the arm of the cross-bridge is compliant and that the myosin head, which rotates, can sustain tension. This will be discussed in more detail later. At present the theory of muscle contraction, whereby force is generated by physical cross-links, is widely accepted. Some of the major lines of evidence supporting the theory are as follows:

i) Gordon, Huxley & Julian (1966), using an experimental design which allowed them to study changes within individual sarcomeres, found that maximum tension was developed when there was maximal overlap of thin and thick filaments and that at lengths on either side of this region the maximum tension developed by the muscle was proportionately decreased. The amount of tension developed could therefore easily be correlated with the number of cross-bridges able to form at any given length.

ii) Rudy et al, (1965) demonstrated that the cross-bridges were detached from the I filament when ATP was present, and attached when ATP was absent. Marston et al, (1976) have demonstrated a third conformation of the cross-bridges in which they are attached and perpendicular to the axis. White (1978) speculates that the three conformational states observed structurally correspond to three states in a mechanical cycle of activity of the cross-bridges, involved in tension generation (see INTRODUCTION: Cross Bridge Theory)

iii) Huxley (1971) studied X-ray diffraction patterns of actively contracted muscle which indicated a change from the ordered, relaxed pattern found in unstimulated muscle, to a much more disordered pattern. This is predictable if in active muscle, the cross-bridges

undergo cycles of conformational changes in an asynchronous fashion. Carlson (1975) found similar results by intensity fluctuation Spectroscopy.

iv) Biochemical studies (Davies, 1964) have shown that the primary source of chemical energy for contraction is ATP. It seems that the cross-bridge is involved in the conversion of chemical energy to mechanical energy in muscle as the myosin head, which combines with the actin, acts as an ATPase.

Although there is substantial support for the mechanism of force generation by physical cross-links, there are phenomena which cannot be explained by it, and incontrovertable evidence for cross-bridge attachment during contraction in living muscle still does not exist. An electrostatic theory proposed by Iwazumi in 1970 is capable of explaining the data most frequently cited as lending strong support to the cross-bridge theory, and requires fewer assumptions. In this theory, force is generated by a dielectric rod suspended in an electric field between the plates of a capacitor. The rod is drawn into the field of the capacitor since the charge induced on the rod is believed to be the thin filament and the capacitor the cross-projection (cross-bridge of the classical theory). The separation of charge causes an electric field to be generated on each projection, thus forming a succession of force-generating sites along the thick filament. The hydrolysis of ATP creates the energy required to sustain charge separation. This has been shown to be of reasonable magnitude (Iwazumi, 1970).

That tension generation in muscle occurs by the formation of physical cross-links between actin and myosin is a supposition based

on different studies, a well-known one being rigor mortis. Here, electron micrographs have shown physical cross-links, thought to be a stage in the cycle of cross-bridge rotation, and representing an extreme example of the stiffness of contraction. However, the rigidity of rigor is induced at full overlap of thick and thin filaments where cross-linking is maximal, or at no overlap where it is absent (Haselgrove, 1975). A comparison of the two theories is given by Noble & Pollack, (1977). Both theories can adequately explain most actin-myosin interaction in in vitro systems. Fluorescence experiments fail to distinguish between the two theories. Oplatka et al, (1974), performed an experiment which can easily be explained by an electrostatic theory but cannot be explained by the cross-bridge theory. The sarcomere length versus tension studies of Gordon et al, (1968), lend strong support to the cross-bridge theory. However, the correlation between length and tension depends upon where the tension is measured; specifically the phenomenon of creep has been ignored in the classical theory. This phenomenon interested Iwazumi and the theory he proposed (1970) accounts for creep and many of the observations which have been claimed as conclusive evidence for cross-bridges. The major problem with this theory is that it rests heavily on the assumption that the ionic strength in the vicinity of each cross-bridge is extremely low. This is regarded by most as unlikely (Noble & Pollack, 1977). More ultrastructural and biochemical findings are needed before the theory could become more prominent.

Since the cross-bridge theory is still the most universally accapted, it will be the one discussed in this manuscript.

Cross Bridge Theory

That cross-bridges act as independent force generators is supported by the finding that the force produced by a muscle is proportional to filament overlap, and from the finding that the maximum velocity of contraction is independent of the degree of overlap (Gordon, Huxley & Julian, 1966). A complete cross-bridge cycle involves attachment of the myosin heads to the actin filaments, a change in angular conformation and production of force, detachment and recovery. The load on the muscle determines whether or not it shortens. If the collective action of all the cross-bridges active at any instant in time produces a force greater than the load on the muscle, then the filaments will slide past each other so as to produce shortening. After detachment from an actin molecule the cross-bridge can then attach to a new one further down the actin filament, since in the time before attachment occurs the filaments have moved. If the force production by the cross-bridges just balances the load on the muscle, then there will be no relative movement of the filaments. In this case a cross-bridge would attach to the same actin from which it previously detached. If the load on the muscle is greater than the cross-bridges can bear, then the filaments will slide so as to produce an extension of the sarcomere, distorting the cross-bridges in the opposite direction to that produced during shortening.

In the cross-bridge theory an assumption which as yet lacks adequate evidence, is that one or more ATP molecules is/are hydrolysed for each cycle of mechanical activity. Therefore, the cycle of activity undergone by the actomyosin acting as an ATPase is the same cycle of activity as the actomyosin acting as a force producer. This

being the case, it would be expected that the rate of energy utilization by the muscle would be proportional to the rate of cycling of the bridges. However, it has been observed that the rate of energy utilization by a muscle increases when the muscle shortens and decreases when the muscle lengthens. (This will be dealt with in more detail below: Section E. ENERGETICS OF STRETCH.) As a result of this observation it is necessary to suppose (White, 1978), that at least one of the rate constants in the cycle has a certain value during isometric contractions, increasing as the muscle shortens and decreasing as the muscle lengthens.

In the most simplistic explanation, the cross-bridge attaches to the actin in one state, and proceeds to a new state changing its shape and thereby generating force between its two attachment points on the two filaments. Huxley & Simmons (1971, 1973) and Huxley (1974) have proposed a model which agrees with the basic cross-bridge theory, but have expanded it so as to explain observations made since the original theory was proposed. They applied rapid step changes of length to the muscle, and by noting the time related tension changes, found that the initial rapid changes of tension were due to the activity of attached bridges before significant detachment had had time to take place. They concluded that there must be a rearrangement of the attached cross-bridge population between two, or probably more, attached states. Their suggested mechanism for force production was as follows: The cross-bridge consists of two components arranged in series; an instantaneous elastic element (the AB linkage) and a damped force-generating element, as seen in Fig. 1. The myosin head has several attachment sites through which it can bind

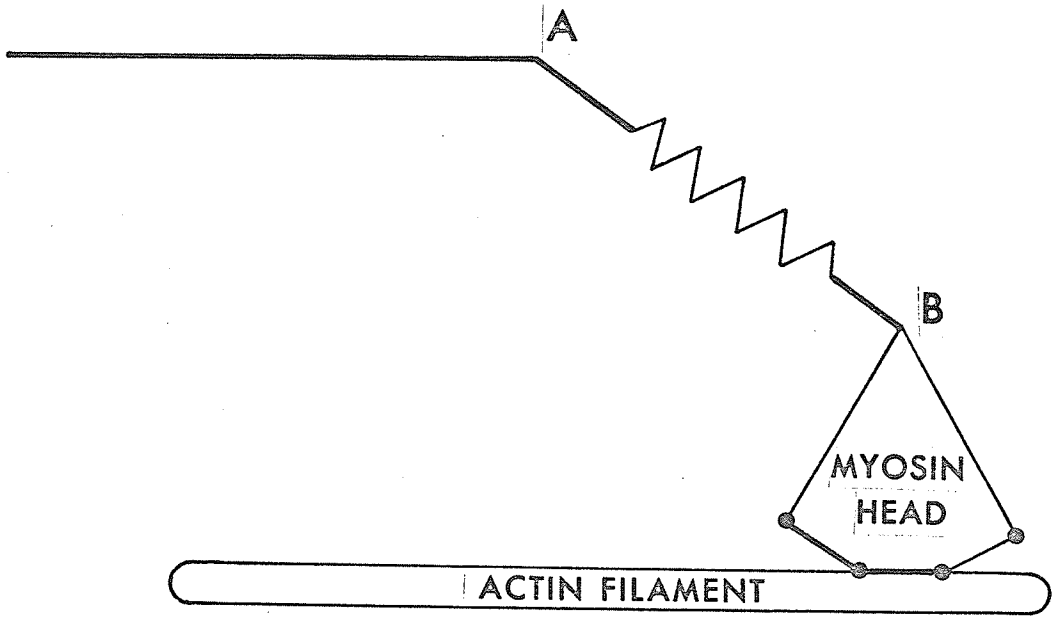



Fig. 1 Schematic representation of the model of Huxley and Simmons (1971, 1973) for force production by cross-bridges. The cross-bridge consists of two components; an instantaneous elastic element (the AB linkage) and a damped force-generating element. The myosin head is shown here bound by two of its attachment sites to an active actin region.

to corresponding sites on an actin active region. They postulate that the simultaneous attachment of the head at two consecutive sites constitutes a stable state and that force is generated by a stepwise movement along the actin filament, from one stable position to the next, each having a progressively lower potential energy than the preceding one. Huxley & Simmons consider that there are probably four attachment sites, allowing three stable states.

The diagram (Fig. 1) represents the attachment of a myosin head to an actin filament in the state favoured at the peak of a tetanus. Under tetanic conditions, when a head attaches initially it is able to move easily to the next position and thereby generate tension. As a result of the increasing tension during development of tetanus, the probability of the head making the next step is reduced. However, if it did, then the probability of it becoming detached by combining with ATP is increased. Therefore, at the peak of a tetanus the heads are energetically constrained to spend most of their time in an intermediate position.

If the muscle is stretched during a contraction the situation is altered. During the early part of a stretch tension is generated by extension of the AB link only, but at some point the attached head begins to be forced backwards, against its tendency to move to the next position of lower potential energy. Tension increases and the length change is now shared between backward rotation of the head and further extension of the elastic linkage. However, the filaments can only sustain this position up to a certain length and tension, and anything greater than this will cause the cross-bridge to be pulled off its site and move to the next attachment site in that direction.

This model has been supported further by Flitney & Hirst (1976) who studied the effects of single and double cycles of stretch and release on the tension response and the relative sliding movements of actin and myosin filaments in active frog muscle. The model also accounts for observations of Flitney & Hirst (1976), that a muscle subjected to stretch resisted initially by developing a high force, but when the relative sliding movement of the actin and myosin filaments exceeded 12 nm, the cross-bridges linking the two became forcibly detached.

Basically, then, this model explains force production by cross-bridges using a series of transitions between attached states which result in a sliding force between the two sets of filaments. The cross-bridge cycle is believed to agree with the biochemical cycle. Each cycle of hydrolysis of ATP by myosin in the presence of actin involves the association and dissociation of actin once. This is what is required to fit with the mechanical cycle.

## D. MUSCLE MECHANICS

Most studies on smooth muscle mechanics have followed the classical experimental techniques designed for skeletal muscle, and data from such studies have been easily explained using skeletal muscle models. The maximum tension development is a measure of the contractile function of muscle and is related to the number of cross-bridges acting within the muscle. The *a* constant (Hill, 1938) gives an index of the force-generating capacity of a muscle and an examination of the values of this constant reveals that most smooth muscles fall into the same range as skeletal (Stephens *et al*,

1969). Mechanical studies (Hellestrand & Johansson, 1974; Herlihy & Murphy, 1974; Meiss, 1971; Stephens et al, 1969; Stephens & Kromer, 1971) suggest that the smooth muscle model, like that of skeletal, must consist of a contractile element in series with a series elastic component. Therefore the skeletal muscle model probably fits smooth muscle also.

Information on contractile properties of muscle can be obtained from isometric studies, such as the maximum tension development of a muscle which gives a measure of the number of cross-bridges formed and represents the sum of the tensions developed at each bridge. The study of the length-tension relationship can give information on the elastic, viscous and plastic nature of muscle.

However, in measuring energy-utilizing reactions extrapolated from mechanical methods, measurements of tension developed or distances shortened are inadequate since it is the rates of change in the biochemical reactions that are important and therefore the rates of change in tension and length. These properties can be investigated isometrically by measuring the rate of tension development and the time to reach maximum tension. Another muscle parameter, the tension-time integral, has been shown to be an index of the stable component of maintenance energy rate (Mommaerts, 1969). However, muscle function has been shown to be most meaningfully studied in terms of its force-velocity relationship. This has been established for both skeletal (Hill, 1938) and smooth muscle (Csapo, 1962). Since force-velocity relationships give a measure of power production, they provide a better account of energy utilization than isometric studies. Once the force-velocity relationship is established, vari-

ation of velocity in contractions against combinations of forces, inertias and elasticities can be predicted by ordinary mechanics (Wilkie, 1949).

Before valid force-velocity curves can be obtained, the muscle under study must possess certain characteristics as noted by Brady (1965). These are 1) the individual muscle fibres must be parallel to each other 2) the major portion of the tissue must be muscle 3) the muscle should be capable of being tetanized and the maximum tension development be relatively independent of length over a usable range of muscle lengths, and 4) the resting tension at optimal length must be small. Stephens et al, (1969) have shown that the trachealis muscle is suitable for force-velocity measurement as it satisfies these criteria.

(i) Force-Velocity Relationship at Loads Less Than $P_o$

The force-velocity relationship at loads less than $P_o$ (the maximum isometric tension development) describes the ability of the muscle to shorten and move a load through a distance. When a muscle is maximally stimulated and a constant load is applied the muscle contracts at a velocity determined by the magnitude of the load and the intrinsic properties of the muscle. Thus the force-velocity relationship allows study of muscle mechanics under dynamic conditions of muscle shortening.

The quantitative relationship between force and velocity has been fitted by a number of equations, the most widely used being A. V. Hill's (1938, 1939) characteristic hyperbolic equation for muscle shortening,

$$V\ (P + \underline{a}) = \underline{b}\ (P_o - P) \qquad - \qquad \text{EQUATION 1}$$

in which P represents the external force or load applied to the muscle, $P_o$ the maximum isometric tension developed by the muscle, V the velocity of shortening, and a and b are constants with biological significance, whose values are different for different muscles. They define the asymptotes of the rectangular hyperbola. The constant a has units of force and reflects the number of force-generating sites, so that the more actomyosin cross-bridges, the greater the value of a. For most vertebrate muscles its value lies between 0.15 $P_o$ and 0.25 $P_o$ (White, 1978). The b constant is responsible for the rate that a muscle can contract. Skeletal muscle studies have shown its value to be an index of the energy-utilizing reactions during contraction, such as the rate of ATP hydrolysis during attachment and detachment of cross-bridges. The b constant depends upon environmental factors such as temperature and pH which affect enzymatic rates.

To prove that the force-velocity relationship of a muscle is hyperbolic, a linearized transform of Hill's characteristic equation (1) can be used,

$$\frac{P_o - P}{V} = \frac{P}{b} + \frac{a}{b} \qquad \text{EQUATION 2}$$

When $P_o - P / V$ is plotted against P the points should appear along a straight line. From this, Stephens et al, (1969) deduced that the force-velocity relation for TSM is hyperbolic and the Hill equation can be applied to it.

The theoretical maximum velocity of shortening (V max), which represents the velocity of an unloaded muscle, may be obtained by solving the equation,

$$V_{max} = \frac{P_o b}{a} \qquad \text{EQUATION 3}$$

which is derived from the Hill equation.

Hill (1938, 1939) has shown that $a$ and $P_o$ are functions of the thickness of the muscle; the thicker the muscle the greater these values. As a result, the value of $a / P_o$ is fairly constant for various skeletal muscles and TSM (Stephens et al, 1969). The value of $P_o$ is an index of strength of the muscle, as it measures the number of force-generating sites (actomyosin cross-bridges) formed during activation and therefore is a summation of the tension developed by each cross-bridge. However, it has not been conclusively demonstrated that there are discrete actin and myosin filaments with force-generating bridges between them in TSM. Biochemically it has been found that both actin and myosin exist in smooth muscle cells. Actin filaments have been demonstrated in many smooth muscles including TSM. Myosin filaments have been shown in a variety of smooth muscles (Choi, 1962; Conti et al, 1964; Needham & Shoenberg, 1964; Small, 1977; Somlyo et al, 1973; Sobiesjek, 1977; Yamauchi & Burnstock, 1969). Therefore, myosin filaments probably exist in all smooth muscle cells and it is possibly the preparation for microscopy which destroys them (Small, 1977).

Since $V_{max} = P_o b / a$ and $P_o / a$ is fairly constant between muscles, the significance of the $b$ constant with respect to the speed the muscle can contract can be seen. This relationship of $b$ to V max is obvious if frog skeletal muscle with a $b$ value of .331 $l_o$/sec. is compared to TSM with a $b$ value of .04 $l_o$/sec. ($l_o$ is the length at which maximum active tension is elicited). Using these values in EQUATION 3 shows that frog skeletal muscle contracts much

faster than TSM.

$$V_{max} \text{ (frog sk. } 0^{\circ} \text{ C)} = \frac{P_o}{a} (.331) = 1.29\ l_o/\text{sec.}$$

(Hill, 1938, 1939)

$$V_{max} \text{ (TSM } 37^{\circ} \text{ C)} = \frac{P_o}{a} (.04) = .17\ l_o/\text{sec.}$$

(Stephens et al, 1969)

This suggests that the slowness of TSM is due to the slow rate that energy-utilizing reactions occur in this muscle.

The ability to fit the Hill equation to the force-velocity relationship of TSM (Stephens et al, 1969) is of some interest since it may eventually permit analysis of the function of the trachealis in terms of a model similar to that proposed for skeletal muscle. To date, there is reasonable evidence (as mentioned above) of the existence of discrete myosin filaments in mammalian smooth muscle, but the theory of generation of force by actin-myosin bridge formation cannot be applied with confidence (vide supra). However, the strong qualitative similarity between the mechanical aspects of skeletal and smooth muscle, as shown in length-tension and force-velocity relationships (Stephens et al, 1969), series elastic component (Stephens & Kromer, 1971) and temperature studies (Stephens et al, 1977), suggests that force generation in TSM may have a mechanism similar to that in striated muscle. Therefore, the present studies on TSM will be discussed with respect to the skeletal muscle model, and using concepts derived from skeletal muscle mechanics to explain phenomena observed in this smooth muscle preparation.

(ii) Force-Velocity Relationship at Loads Greater Than $P_o$

In spite of the importance of understanding the capacity of muscles to resist stretch, there are few reports of the force-

velocity relationship at loads greater than $P_o$ in the literature.

As early as 1882, Fick found that at loads greater than $P_o$, an active muscle lengthens. A muscle subjected to a stimulus of constant strength and frequency can shorten more quickly with a smaller load. As the load increases the shortening velocity decreases until a load is reached ($P_o$) which just prevents the muscle from shortening at all. With a load greater than this the muscle is forced to lengthen and as the load increases further, the muscle lengthens more rapidly. In principle, Hill's hyperbolic equation for the force-velocity relation of active muscle (EQUATION 1) could apply to lengthening as well as to shortening, at least for loads immediately beyond $l_o$. For shortening, velocity is positive and P is less than $P_o$. In lengthening, velocity is negative and P is greater than $P_o$. After Fick (1882) it was Wyman (1926) and Levin & Wyman (1927) who examined further the fact that the tension exerted during lengthening was greater than for an isometric contraction, and that as the load increased, the lengthening velocity increased. Quantitative measurements to see whether a hyperbolic function still applied at loads greater than $P_o$ came later.

Katz (1939), working with frog sartorius muscles, found that the rate of lengthening for loads greater than $P_o$ was less than expected by an extension of Hill's hyperbola. Aubert (1956) confirmed this finding for loads up to 1.5 $P_o$.

Katz (1939) found that he could only use loads up to 1.7 $P_o$, since a load greater than this resulted in complete relaxation (a rapid extension down to the resting length). He also found that loads of this magnitude caused permanent after-effects indicating

a weakening or complete break of some contractile links. As Hill explained (1938), the immediate effects of subjecting a frog muscle to a load greater than $P_o$ is to cause it to lengthen, rapidly at first, and then, if the load is not too great, more slowly. If the load is too great, however, the muscle 'gives' or 'relaxes' or 'slips'. Thus in the study by Katz, when loads of less than 1.7 $P_o$ were applied there was a rapid initial stretch which may be ascribed to stretching of elastic elements, followed by a steady slower lengthening velocity attributed to the active contractile element. If loads greater than 1.7 $P_o$ were applied there was a very large initial 'give' and no steady slower lengthening velocity following it. The load at which this initial 'give' becomes too great varies between muscles. Katz did not do a force-velocity study at loads greater than $P_o$ with the retractor penis of the tortoise because he found that this muscle 'gives' even if the applied force is only slightly greater than $P_o$. He limited his study to frog sartorius muscles which gave very slow lengthening velocities unless the applied load was considerably greater than $P_o$. Aubert (1956) found that this 'give' occured at about 1.5 $P_o$.

From these studies it became clear that although Hill's equation provided a good description of the relationship between force and velocity for shortening velocities, experimental data on skeletal muscles did not support his equation when lengthening velocities were used. Polissar (1952) described a physicochemical model for the contractile process in muscle which agreed more closely with experimental results on the force-velocity curve at loads greater than $P_o$ than the hyperbolic curve. In his model, the contractile

units could exist in either a long state L or in a short state S, and that the reactions $L \rightleftharpoons S$ were coupled with reactions in the sarcoplasm. A steady state cycle was achieved in an isometric tetanus. The length of the muscle depended on the distribution of the units between the two states. An increase in tension shifted the distribution in favor of L causing the muscle to lengthen. The speed of shortening or lengthening in an isotonic contraction was a result of the net speed of the reaction $L \rightleftharpoons S$. The greater the load the faster the reactions $S \rightleftharpoons L$. This interpretation agreed with experimental results and also explained the apparent 'yielding' of muscle under large loads.

The discussion so far has dealt with force-velocity curves of skeletal muscle obtained by applying a constant load and measuring the resulting velocity of either shortening or lengthening. Curtin & Davies (1972) show a force-velocity curve for frog sartorius muscle obtained by stretching or shortening a muscle at a constant velocity, and measuring the tension produced. The observed tension reached a peak of about 1.4 $P_o$ at a velocity of 0.2 $l_o$/sec., and then as the velocity increased the tension started to decrease so that the highest tension studied was about 1.4 $P_o$. (A schematic diagram of the shape of this curve is shown in Fig. 2.) This decrease in the tension at high velocities did not appear to be due to fatigue or damage to the muscle.

A study at the same time by Chaplain (1972) showed similar results. He also worked with frog sartorius muscles and measured the velocities of lengthening while applying a constant load during a maintained tetanus. He found that as the muscles were stretched they developed greater tension up to a certain velocity of lengthening.

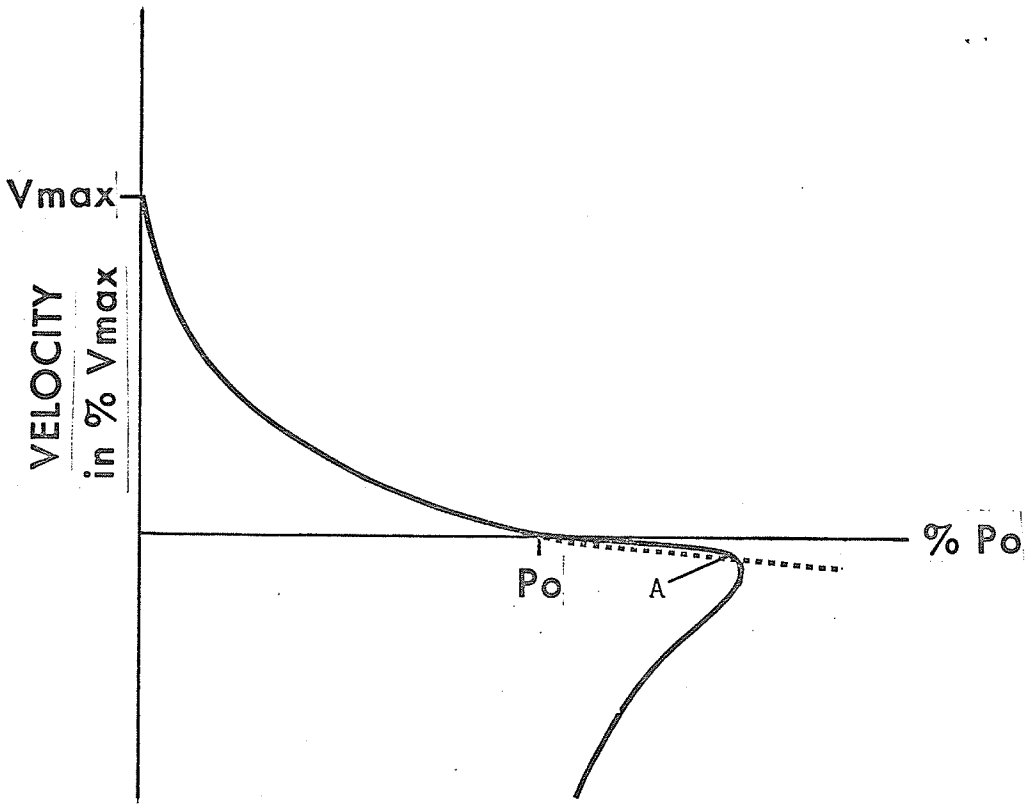



Fig. 2 Schematic diagram of the shape of the force-velocity curve found for skeletal muscle at loads less than and greater than $P_o$. The broken line shows the curve obtained by an extension of Hill's hyperbola. The solid line shows the relation obtained when a constant velocity of shortening or lengthening is applied and the tension is recorded. When the curve is determined by applying constant loads and measuring the resultant velocities, the curve obtained between $P_o$ and 150% $P_o$ is similar to the above tracing up to A, then it asymptotes vertically downward.

At velocities higher than this, the increase was always followed by an actual decrease in the muscle force, as shown schematically in Fig. 2.

As these results show, when force-velocity curves are determined by applying a constant velocity of stretching and measuring the force produced, there no longer exists a unique force-velocity relation for negative velocities since for different velocities of lengthening the same force is developed by the muscle.

When force-velocity curves were determined by applying a load and measuring the resulting velocities of shortening and lengthening, a given load would allow a single constant velocity as discussed above. Recently, Johansson et al, (1978) have reported force-velocity curves for smooth muscles obtained in this manner.

They performed quick-stretch and quick-release experiments on preparations of smooth muscle from rat portal vein and rabbit urinary bladder. In contrast to skeletal muscle, application of loads greater than $P_o$ to these two muscles produced rates of lengthening greater than expected from an extrapolation of Hill's hyperbola, particularly in portal vein (see Fig. 3). All skeletal muscle studies had shown the velocities of lengthening at loads up to at least 1.4 $P_o$ to be less than an extrapolation of Hill's hyperbola. Johansson also found for both muscles that at very great loads, the muscle 'gave', the load being about 1.4 $P_o$ for rat portal vein and greater than this for rabbit urinary bladder. The shape of the force-velocity curves beyond $P_o$ revealed that the smooth muscle of the bladder was better able to resist rapid stretch than the smooth muscle of the vein.

In the present study, the force-velocity relationship

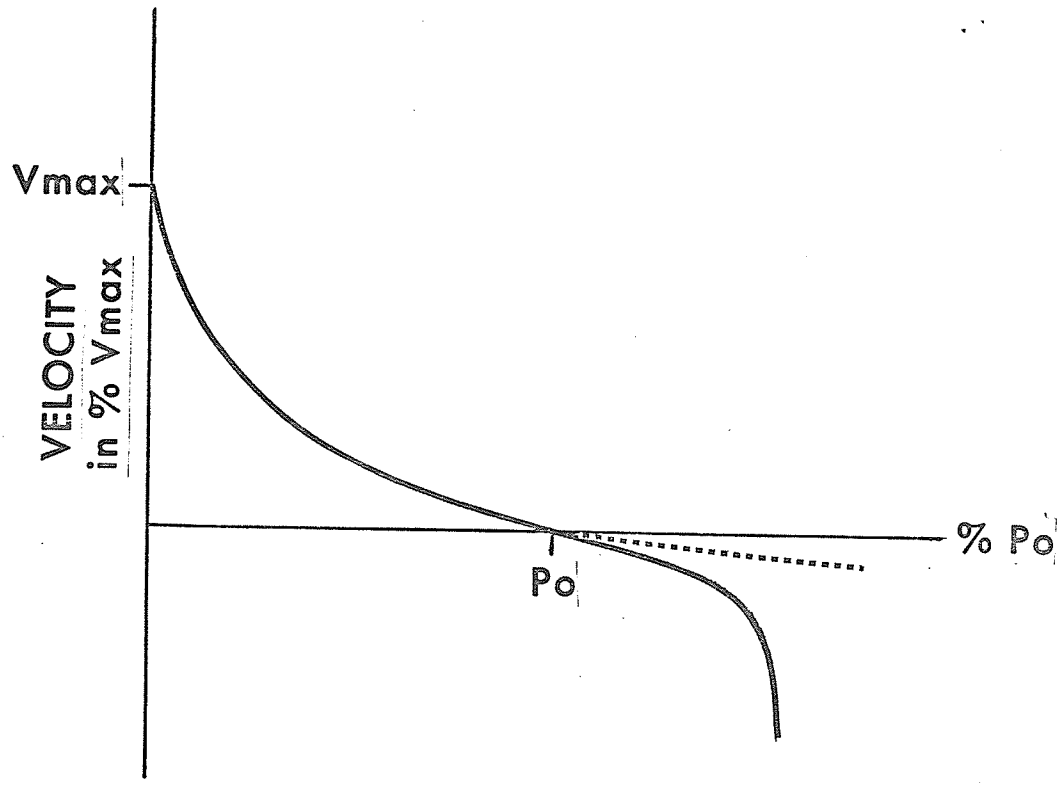



Fig. 3 Schematic diagram of the force-velocity relationship obtained for the smooth muscles of the rat portal vein (Johansson, 1978), rabbit urinary bladder (Johansson, 1978), and canine TSM (Hanks, 1979). At all tensions greater than $P_o$ the velocity of lengthening is greater than expected from an extrapolation of Hill's hyperbola (shown by the dotted line).

up to loads of 1.5 $P_o$ of canine TSM was determined, by applying a constant load and measuring the resultant velocity of lengthening. It was of interest to discover whether TSM was similar to either skeletal or smooth muscles studied to date in its ability to resist stretch.

## E. ENERGETICS OF STRETCH

In active muscle, chemical reactions are responsible for the production of force and movement. Within an interval of time the energy from these chemical reactions is either transformed into work or appears as production or absorption of heat,

i.e. ENERGY = HEAT + WORK

The heat plus work produced by muscle can be measured. The work during shortening and lengthening is determined as follows: When an active muscle exerts a force P and shortens a distance x it does an amount of positive work (Px). If the muscle is stretched a distance (-x) while exerting a force, it absorbs work (-Px). The energy from the chemical reactions believed to be responsible for the heat plus work can be calculated. In order to find out whether all the important chemical reactions have been accounted for, the values of the right and left hand side of the equation can be compared. If they are equal, the energy from the observed chemical reaction can explain the heat plus work, and all the chemical reactions that are contributing must have been accounted for. If the two sides are not equal, either an error has been made in determination of the values or an unidentified reaction occurred which was not accounted for.

There is a substantial amount of evidence (Curtin & Waledge, 1978) to indicate that more heat plus work is produced during con-

tractions than can be explained by the observed metabolic reactions.

From the work of several investigators (Abbott & Aubert, 1951; Fenn, 1923, 1924; Hill & Howarth, 1959) it has been established that the heat plus work in a complete cycle of contraction and relaxation during which a muscle is stretched is less than when the muscle contracts isometrically. This means that the cost of resisting stretch is much less than that of positive work. Possible explanations for a reduced heat plus work output during stretch are: a) work is stored mechanically during stretch and remains stored during relaxation b) an exothermic reaction is prevented by the stretch and c) an endothermic reaction is caused by the stretch. Possibilities b) and c) seem more likely than a).

The central reaction of the contractile process has been shown to be the hydrolysis of adenosine triphosphate (ATP) by myosin ATPase. Chemical measurements on actively stretched muscles have shown a decreased ATP utilization as compared to isometric contractions (Aubert & Marechal, 1963; Butler et al, 1972; Curtin & Davies, 1975; Infante et al, 1964; Marechal, 1964, 1965; Wilkie, 1968). A net synthesis of ATP during stretch has not been found (Curtin & Davies, 1975). It therefore seems possible that the prevention of ATP splitting by stretch explains the reduced heat plus work.

Early in vivo experiments used oxygen consumption to determine energy costs. A dramatic example of the principle of active resistance to stretch requiring less energy than positive work was demonstrated by Abbott et al (1952). Two bicycle ergometers were coupled in opposition so that when the pedals of one went forward those of the other went backward. One person pedalled forward on one bicycle and another

person on the other bicycle resisted. Both subjects exerted the same amount of force and the speed was kept constant with the use of a metronome. The only difference was that the subject pedalling forward was shortening active muscles and doing positive work while the other subject pedalling backward was having his active muscles stretched. The result was that within a short time and with very little effort the person providing resistance was able to reduce the other person to exhaustion. Oxygen consumption tests using the same apparatus were later performed and these confirmed that the process of providing active resistance to stretch required much less oxygen than positive work.

An in vitro method of showing this principle was designed using canine TSM. The intent was to find out whether a muscle being continually stimulated and stretched would retain energy longer than a muscle being stimulated and shortened. The actual experiment and results are described below.

As in skeletal muscle the ultimate energy source for smooth muscle contraction derives from the hydrolysis of ATP. In skeletal muscle the ATP that is hydrolysed is immediately rephosphorylated by creatine phosphate (CP) and it is difficult to show a decrease in ATP levels during contraction unless the reaction ADP + CP $\longrightarrow$ ATP + creatine is prevented. In smooth muscle, however, decreases in ATP levels during contraction can be easily demonstrated (Kroeger & Stephens, 1971). Therefore, in a study of high energy phosphate utilization of smooth muscle both ATP and CP must be considered.

Since the results from skeletal muscle had all shown decreased utilization of ATP during stretch, the next part of this study involved

finding out changes in ATP and CP levels during stretching of active TSM compared to during shortening. If smooth muscle was similar to skeletal with respect to energy utilization during stretch then a comparison of ATP and CP levels between shortening and lengthening should have revealed that less ATP and CP were used during lengthening.

## F. EXPERIMENTAL PLAN AND SPECIFIC AIMS

Tracheal smooth muscle (TSM) isolated from mongrel dogs was subjected to the following studies in order to gain an understanding of the mechanical and energetic aspects of shortening and lengthening:

1) the force-velocity relationship at loads less than $P_o$ using a quick-release method. This was done in order to compare the shape of the curve and value of the constants with previous reports using an after-load stop technique. If the quick-release method was adequate for loads less than $P_o$, then it would be reasonable to use the same method at loads greater than $P_o$.

2) the force-velocity relationship at loads greater than $P_o$. This would then be compared to reports obtained from skeletal muscle and one other study on smooth muscles to see whether TSM resembles other muscles studied in its ability to resist stretch.

3) an interesting *in vitro* demonstration to show that a muscle being continually stretched could use less energy than one allowed to shorten.

4) to determine whether less high energy phosphates (ATP & CP) were used by active TSM being stretched than by active TSM allowed to shorten.

5) analysis of these results in order to gain a better understanding of the mechanics and energetics of actively elongating TSM,

and to interpret the data in terms of the cross-bridge theory of muscle contraction which has been developed for skeletal muscle.

# METHODS

A. FORCE-VELOCITY STUDIES

Mongrel dogs were anaesthetized by intravenous injections of 30mg/Kg body weight sodium pentobarbitol. The trachea was dissected out rapidly via a midline cervical incision, and placed in a beaker of ice-cold Krebs-Henseleit solution. The dog was then sacrificed.

In the dog the cartilaginous rings of the trachea are incomplete dorsally. The gap is bridged by a fibromuscular membrane, the dorsal component being the tracheal smooth muscle (TSM) or trachealis as seen in Fig. 4. The muscle fibres run transversely. At the level of each ring the muscle bundles end in small tendons which fuse with the outer surface of the cartilage. A ring was cut away and cut in half ventrally. The cut ends were everted causing the externally located muscle bundle to separate from the membrane (Fig. 5). This was dissected out.

The lower end of the muscle strip was tightly attached with 5-0 braided surgical silk to a steel rod, which passed through a mercury seal in the bottom of the bath, to a Grass Ft. 03 force transducer mounted on a rack and pinion, allowing changes in muscle length to be made.

The upper end of the muscle strip was tied with a short piece of 5-0 braided surgical silk to a titanium lever mounted on jewelled bearings. The lever was held in place by a rotary solenoid, about 1 mm away from the thread which supported the muscle. The lever arm ratio was 20:1. Appropriate loads were randomly applied to the lever by a length of rubber band in order to avoid inertia.

The displacement of the lever during isotonic shortening and

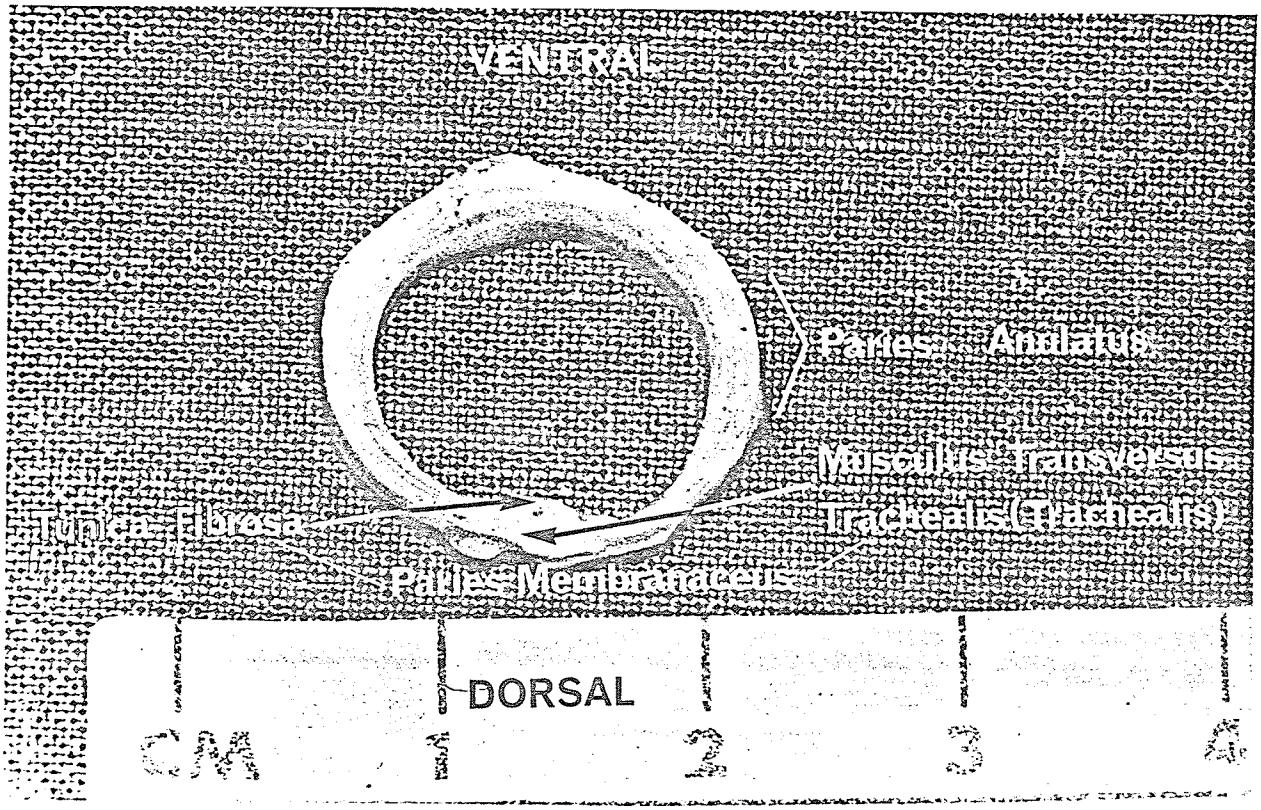



Fig. 4 A single intact canine tracheal ring showing the incomplete cartilaginous ring, closed by the paries membranaceous dorsally.

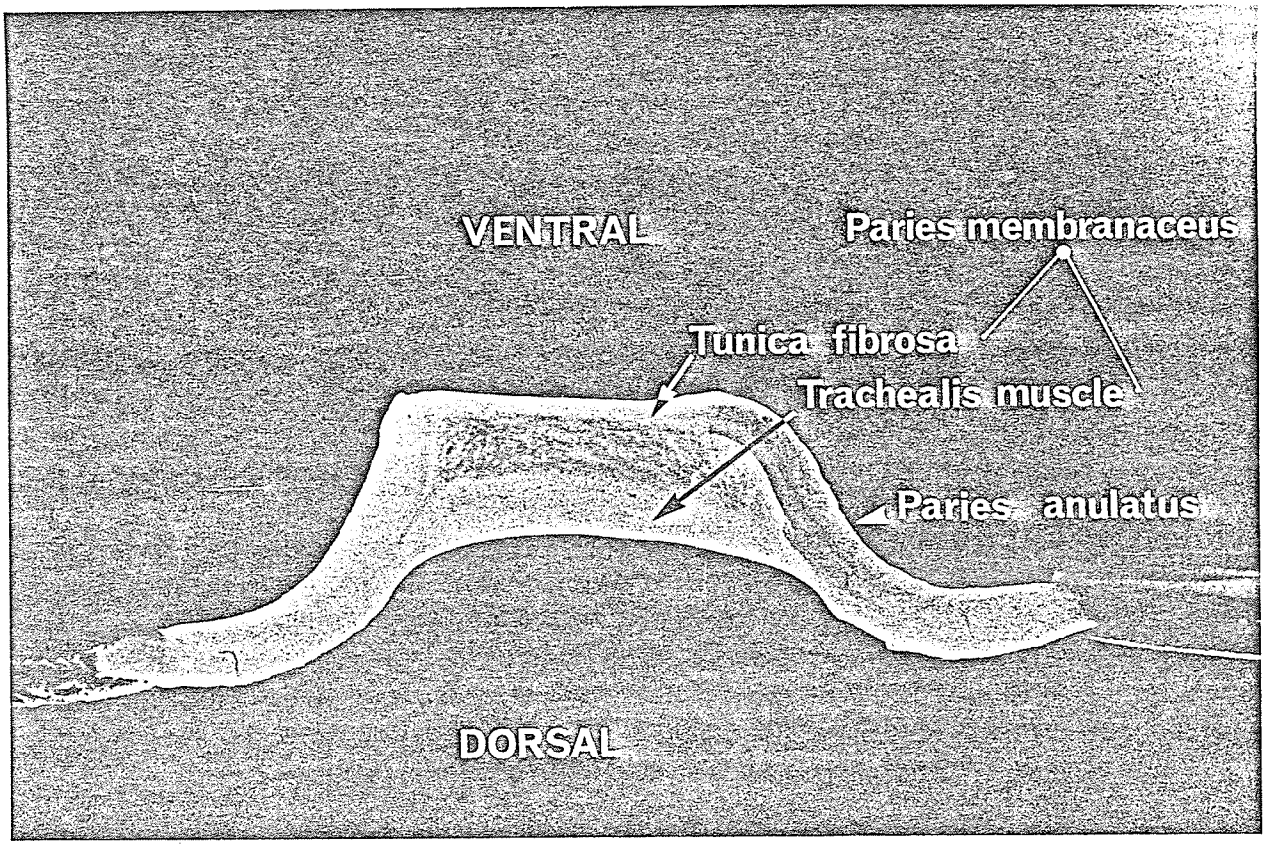



Fig. 5 Photograph of a single tracheal ring shown in the transverse plane. The cut everted tracheal ring is shown. Eversion causes the trachealis and the tunica to separate, and facilitate further dissection of the TSM.

lengthening was measured by a Packard 7-DCDT displacement gauge. The force and displacement gauges were connected to a Beckman multi-channel recorder (Type RP Dynograph) set at high sensitivity.

The muscle strip was mounted in a 40 ml cylindrical bath containing Krebs-Henseleit solution, (Table 1), changed frequently, and maintained at $37 \pm 0.5^{o}C$. A 95-5 $O_2$-$CO_2$ gas mixture was used to maintain a bath pH of 7.40, $PO_2$ of 60 mmHg, and $PCO_2$ of 40 mmHg.

The cross-sectional area of the muscle was estimated at the end of the experiment using the length of the tissue and its blotted wet weight, and assuming the density of the tissue to be one.

Supramaximal electrical stimulation was effected from a 60 Hz AC source via platinum plate electrodes placed close to the tissue and oriented to give a transverse field. Fig. 6 shows a diagram of the apparatus.

After mounting, the muscle was stretched to its approximate $l_o$-length at which maximum active tension per unit cross-sectional area is elicited - by placing a 0.8g resting tension on it (which has been shown to stretch the muscle to its approximate optimal length). It was then allowed to equilibrate in the bath for 1 hour prior to measurement of any physiological parameters.

It was necessary to carry out a stimulus-response and length-tension study after the equilibration period was complete to determine the supramaximal stimulus and $l_o$ for the force-velocity study.

Stimulus - Response

The voltage required to achieve maximal stimulation was determined by applying stimuli of increasing strength and recording the

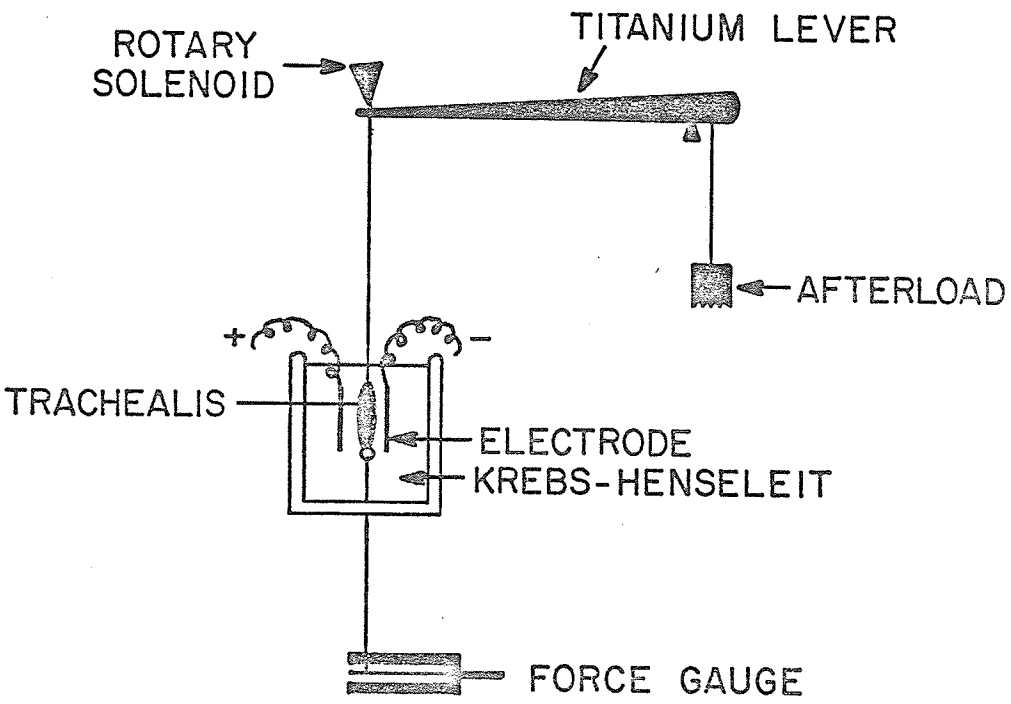
TABLE I

Composition of Krebs-Henseleit Solution

| | mM |
|---|---|
| NaCl | 115 |
| $NaHCO_3$ | 25 |
| $NaH_2PO_4$ | 1.38 |
| KCL | 2.51 |
| $MgSO_4 \cdot 7H_2O$ | 2.46 |
| $CaCl_2$ | 1.91 |
| dextrose | 5.56 |

osmolarity = 304 mOsM

Fig. 6




SCHEMATIC DIAGRAM OF THE APPARATUS FOR SIMULTANEOUS MEASUREMENT OF SHORTENING AND LENGTHENING VELOCITIES, AND RATE OF TENSION DEVELOPMENT

isometric tension developed. A rest period of 5 minutes was required between stimulations. The duration of the stimulus was the minimum necessary to elicit the maximum response, which for TSM was 10-12 seconds. Increasing voltages were applied until no further increases in response were seen. The graded response at higher voltages increased until the maximal stimulating voltage was attained. Beyond this, all voltages were supramaximal. The supramaximal voltage used for length-tension force-velocity studies was about two voltages greater than maximal.

Length - Tension

Length-tension studies were performed to determine $l_o$. Since an entire length-tension curve was unnecessary, the study could begin by adjusting the rack and pinion to set the length of the equilibrated muscle a millimeter or 2 shorter than the length during equilibration. The solenoid was left in place throughout. The muscle was stimulated with a supramaximal stimulus of optimal duration (the minimum time for obtaining maximum response). The total tension developed was recorded. The muscle was then stretched and stimulated every 5 minutes until the length was found which gave the maximum tension on stimulation.

Once the stimulus-response and length-tension studies were completed and the supramaximal tetanizing stimulus and $l_o$ had been found, the force-velocity study was begun. The solenoid was set to hold $l_o$ constant when afterloads were added.

Shortening Velocities

The muscle was stimulated and it developed tension isometrically. When the tension record reached a plateau, the solenoid was released

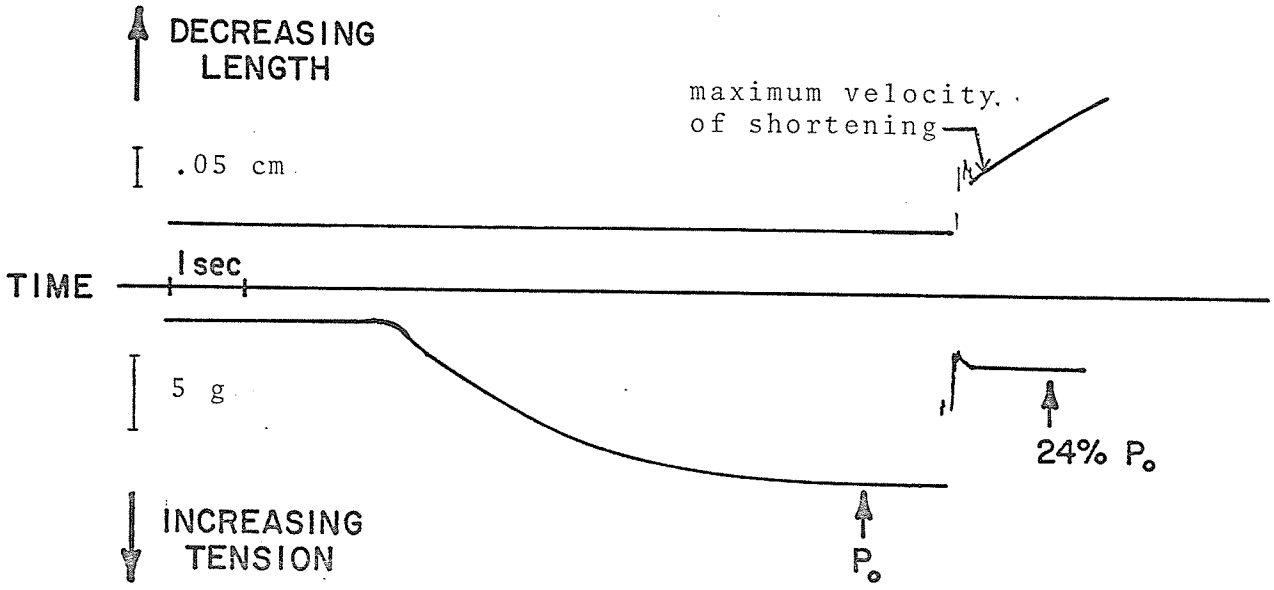
and the tension dropped rapidly to a value determined by the isotonic load. Afterloads were added in random sequence. Successive stimulations were 7 minutes apart. On stimulation, records of tension development and muscle shortening as functions of time were obtained, as shown in Fig. 7. The height of the tension trace above the baseline represented the load the muscle was bearing. The maximum slope of the shortening trace (following the series elastic component) was the maximum velocity of shortening of the muscle under that load.

Lengthening Velocities

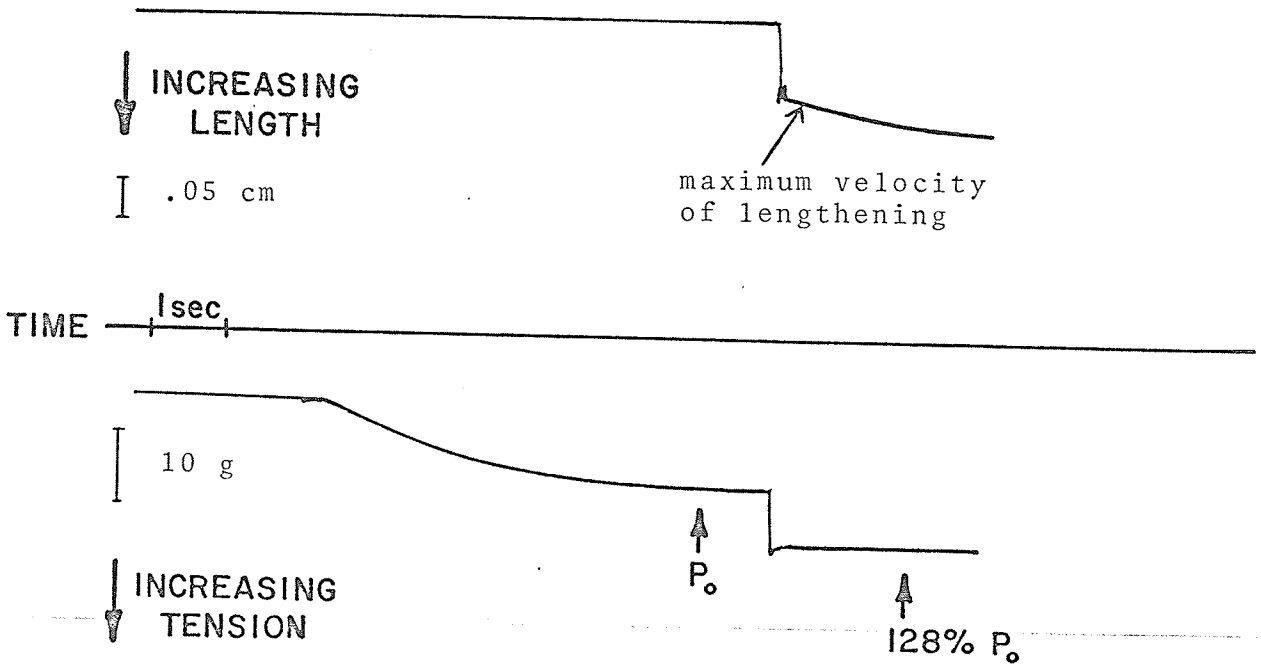
At loads close to and greater than $P_o$ (the tension obtained at $l_o$ using supramaximal stimulation) the muscle lengthened during stimulation. When the afterload approached $P_o$, the muscle was shortened by 1 mm, before lengthening studies were made. This was to ensure that the maximum elongating velocity was measured at a time when the muscles were at $l_o \pm 0.5$ mm. Within this range $P_o$ changed by less than 5%.

Afterloads between approximately 100 and 150% $P_o$ were added in random sequence. The muscle was stimulated and tension developed isometrically. When the tension record reached a plateau, the solenoid was released and the tension increased rapidly to a value determined by the isotonic load. After 5 minutes the muscle was stimulated to produce an isometric contraction and 7 minutes after this the next load was studied. The records of tension development and muscle lengthening as function of time were obtained, as shown in Fig. 7. The lengthening trace showed initial elastic change before settling into a steady lengthening trace, which was assumed to represent contractile element function. A lengthening trace is shown

Fig. 7 RECORD OF TENSION AND SHORTENING vs TIME USING QUICK RELEASE AT $P_o$




RECORD OF TENSION AND LENGTHENING vs TIME USING QUICK STRETCH AT $P_o$



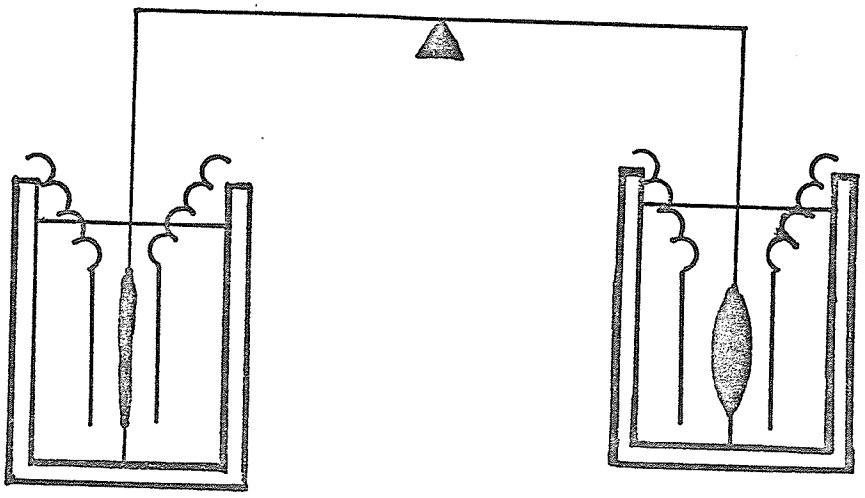
in Fig. 7. The first steady lengthening component was the tracing used for the lengthening velocities in the force-velocity curve.

B. ISOTONIC LEVER EXPERIMENTS

Two adjacent strips of TSM were dissected out as previously described. One strip was cut so that it was wider than the adjacent strip. Both ends of each muscle were tied with 5-0 braided surgical silk, the thinner muscle being tied so as to make it longer than the wider muscle. The length of each muscle was measured before being cut away from the tracheal ring. One end of each muscle strip was then tied to opposite ends of a lever (isotonic Harvard heart smooth muscle transducer) which recorded length changes as shown in Fig. 8. The lower end of one muscle strip was tied to the base of a muscle bath. The lower end of the other muscle strip was attached to a silver chain link. This link was attached to 3-0 braided surgical silk, which passed through a mercury seal in the bottom of the bath to a Grass FT.03 force transducer mounted on a rack and pinion, allowing changes in muscle length to be made.

Each muscle strip was mounted in a 25 ml cylindrical bath containing Krebs-Henseleit solution, changed frequently and maintained at 37± .05°C. A 95-5 $O_2$-$CO_2$ gas mixture was used to maintain a bath pH of 7.40, $PO_2$ of at least 600 mmHg and $PCO_2$ of 40 mmHg.

Once the muscles were in the baths and attached to the lever, the muscles were stretched to their approximate $l_o$, by adjusting the rack and pinion until a .8g resting tension was recorded. The length of each muscle was measured and the difference between the two was similar to the difference already measured in situ. The muscles were left to incubate in the baths for 1 hour. A schematic diagram of the apparatus is given in Fig. 8.



1.2 cm
8 mg
CSA = .007 cm2

1.0 cm
10 mg
CSA = .010 cm2

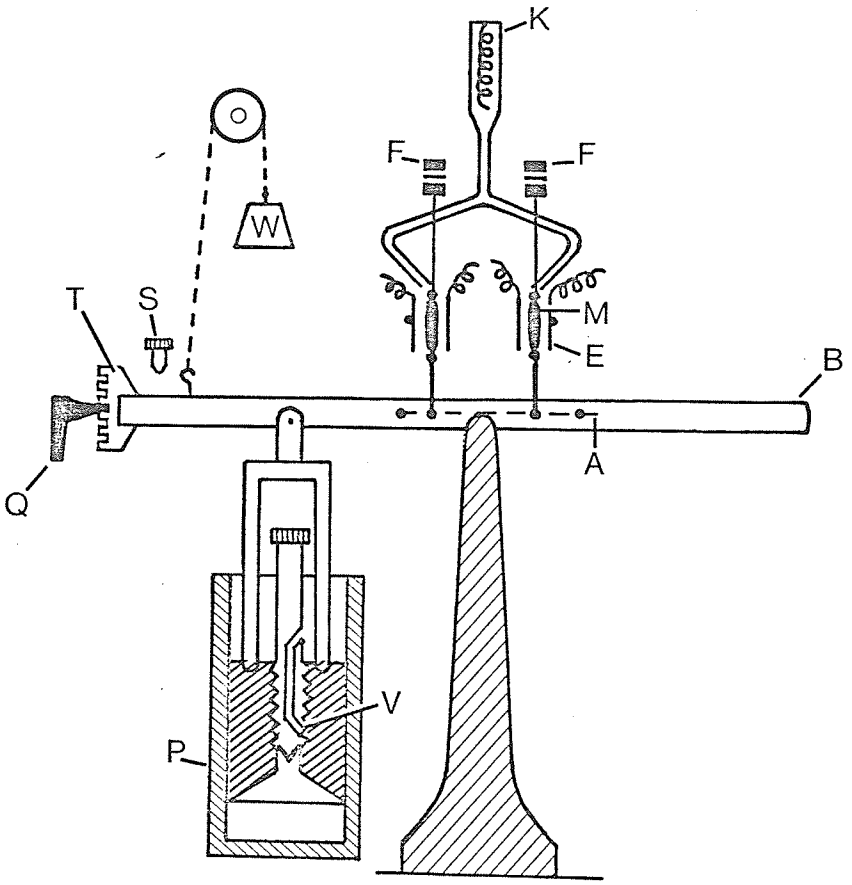
Fig. 8 Schematic diagram of the apparatus used in the isotonic lever experiments.
CSA = Cross Sectional Area
The muscles are attached by a lever and are stimulated electrically.

Supramaximal electrical stimulation was effected from a 60 Hz AC source via platinum plate electrodes placed close to the tissue and oriented to give a transverse field. The electrodes were arranged so that the current flowed through both muscles at the same time. The muscles were stimulated supramaximally at 5 minute intervals until a constant tension development was reached. The voltage chosen remained constant for all succeeding stimulations. After a 5 minute rest, the muscles were stimulated until the tension development began to decline after reaching a plateau. The length changes were recorded continuously and once the muscles returned to their original lengths, they were again stimulated. Upon each stimulation, one muscle shortened and the other lengthened. The procedure was repeated until the muscle which shortened on the first stimulation lengthened and the other muscle shortened. Then the muscles were left for 30 minutes before a final stimulation was given.

## C. HIGH ENERGY PHOSPHATE STUDIES

### 1) Mechanical Apparatus for Muscle Lengthening and Shortening

Two strips of TSM were cut out as previously described. They were cut from adjacent rings and every effort was made to make them the same size. The lower end of each muscle was tied by 5-0 braided surgical silk to hooks set equidistant on either side of the fulcrum of a Levin-Wyman ergometer. A schematic diagram of the apparatus is shown in Fig. 9. The upper end of each muscle strip was attached with 5-0 braided surgical silk to a Statham UC2 force transducer mounted on a rack and pinion. The force gauges were connected to a Tetronix RM S64, two-channel storage oscilloscope which enabled simultaneous measurements of active tension produced by both muscles.




A Axis of rotation of lever B
E Electrode
F Isometric force gauge
K Krebs-Henseleit solution
M Tracheal smooth muscle strip
P Dashpot containing mineral oil
Q Release arm
S Adjustable stop
T Tooth gripping notched plate
V Needle valve in piston of dashpot
W Suspended weight

Fig. 9 Schematic diagram of the Levin-Wyman ergometer as used in the experiment to determine high energy phosphate levels of active muscles shortening and lengthening at identical velocities.

Supramaximal field electrical stimulation was effected from a 60-Hz AC source via platinum plate electrodes placed as close as possible to the tissue. Current density of the plates was approximately 400 mamp/cm. The muscles were perfused by a continuous drip system with Krebs-Henseleit solution, which flowed around the muscles whilst passing alongside the electrode.

Following an equilibration period of 1 hour, electrical stimulus response and length-tension studies were performed to determine the supramaximal tetanizing stimulus and $l_o$ of the TSM. The muscle lengths were then adjusted so that the muscle to be lengthened was set 1 mm shorter than $l_o$ and the muscle to be shortened was set 1 mm greater than $l_o$. Following this, the muscles were electrically stimulated until $P_o$ was reached, at which time the release arm was detached and the lever allowed to rotate at a pre-set velocity of .2 mm/sec. The stop (S in Fig. 9) was placed so as to stop the lever after 10 seconds, 2 mm from the starting point of rotation. The stimulus was then turned off and the lever set back to its original position. This procedure of stimulation and lever release was carried out at 2 minute intervals for 4 trials. On the 5th stimulation, 2 minutes after the preceding one, the electrodes were removed and $10^{-8}$M Carbachol in Krebs-Henseleit solution was dripped over the muscles to stimulate them. As the tension plateaued, the release arm was removed and the lever allowed to rotate. After 10 seconds, the tissues were instantly frozen using a pair of Wollenberger clamps which had been pre-cooled in liquid nitrogen (b.p. - 196$^{o}$C). The tissues were then freeze-dried and stored in this state until ready for ATP and CP determination.

2) ATP and CP Determination

a) Perchloric Acid Extraction

The method was modified from that described by Cain & Davies (1962) and Kroeger & Stephens (1971). The freeze-dried strips of TSM were weighed using a Perkin Elmer AD-2 electronic micro-balance. The tissues were placed in test-tubes, and 500 ul of 0.5M perchloric acid added to each. The samples were placed in the lyo-philizer until the perchloric acid just began to boil. The vaccuum was immediately released and the test-tubes tapped lightly until all tissue samples sank to the bottom. They were then equilibrated by being vigorously shaken in a $3^{o}C$ water bath for 1 hour. The samples were kept on ice for as much of the procedure as possible. Following equilibration, 400 ul was extracted from each and put into separate 1 ml centrifuge tubes, and 15 ul TraKCl buffer (0.33M triethanolamine and 1.1 NKOH brought to pH 7.5 with HClO) added to each. Neutralization was achieved by the addition of 30% KOH to bring the solution to pH 7.0. The $KClO_4$ precipitate was centrifuged out at 3000 RPM for 2 minutes. From the supernatant, 400 ul was extracted and freeze-dried.

b) Enzymatic Determinations of ATP and CP

The reactions:

(1) Glucose + ATP $\xrightarrow{\text{Hexokinase}}$ Glucose-6-Phosphate + ADP

(2) Glucose-6-Phosphate + NADP + $H^{+}$ $\xrightarrow[\text{Phosphate Dehydrogenase}]{\text{Glucose-6}}$ 6-Phosphogluconic acid + NADPH

can be coupled to measure limiting amounts of ATP through the increase in optical density of NADPH at 340 mu as NADPH is produced in the reactions outlined.

The subsequent determination of CP can be made by the addition of creatine phosphokinase (CPK) which catalyzes the reaction:

$$\text{Creatine Phosphate} + \text{ADP} \xrightarrow{\text{CPK}} \text{Creatine} + \text{ATP}$$

The ATP thus formed is immediately measured through reactions (1) and (2) above, and since all reactions proceed stoichiometrically and the molar extinction coefficient is known, the concentrations of ATP and CP in the extracts can be determined.

The method was modified from that of Lamprecht and coworkers (1974). The ATP and CP contents were assayed sequentially in a medium containing: 100 mM Tris-HCl at a pH of 7.5, 5 mM $MgCl_2$, 3 mM glucose, 5 uM dithiothreotol (DTT), 2.5 mM NADP. The freeze-dried extracts of TSM along with these reagents were pipetted into a semi-micro cuvetto (light path 1 cm, volume 1 ml). After mixing, the cuvettes were placed in a Pye Unicam SP1800 uv spectrophotometer, and read at 340 nm. The change in absorbance following the addition of hexokinase and glucose-6-phosphate dehydrogenase to the assay medium permitted ATP quantitation. When there was no further change in absorbence, CPK (dissolved in DTT with an equal weight of bovine plasma albumine) was added and the increase in absorbance due to the reaction with CP was also recorded.

Since the reactions proceed stoichiometrically, the amounts of ATP and CP originally in the muscle extract were calculated from the increase in optical density ($\Delta E$) and the millimolar extinction coefficient of NADPH (6.22). Appropriate standards were also measured as a check on the method. The equation used for the estimation of ATP and CP content was:

$$\frac{\text{umoles ATP (or CP)}}{\text{g dry weight}} = \frac{E}{6.22} \times \frac{1}{\text{tissue wt. (mg.)}} \times 1000 \quad \text{EQUATION 5}$$

# RESULTS

## A. FORCE-VELOCITY RELATIONSHIP AT LOADS LESS THAN $P_o$

The force-velocity relationship at loads less than $P_o$ demonstrated the muscle's capacity to shorten under various loads. Using the apparatus described in Fig. 6, twelve force-velocity experiments were conducted and an average force-velocity curve calculated from their mean. Fig. 7 shows a typical record of tension and shortening used to determine the points for a force-velocity curve. The resulting curve (Fig. 10) was a rectangular hyperbola as demonstrated by a goodness of fit test, and therefore Hill's (1938) equation was applied. As stated in the Introduction, the equation is,

$$(P + \underline{a})\ (V + \underline{b}) = (P_o + \underline{a})\ \underline{b} \qquad - \qquad \text{EQUATION 1}$$

where P represents load, V the velocity of shortening, $P_o$ the maximum tension which the muscle is able to produce, $\underline{a}$ a constant with units of force, and $\underline{b}$ a constant with units of velocity. A linearized transform of the equation, equation 2, was applied to each experiment to prove that the relationship was hyperbolic. When $P_o - P / V$ was plotted against P, the data points fell on a straight line (Natrella, 1963; Ostle, 1956). Fig. 10 shows the resulting straight line of $P_o - P / V$ plotted against P, derived from the mean of 12 experiments.

Calculation of the regression equation (Snedecor, 1946) based on equation 2 gave the slope $1/\underline{b}$, and therefore the velocity constant $\underline{b}$. From the value of $\underline{b}$, and the intercept $\underline{a}/\underline{b}$, a was determined. The theoretical maximum velocity at zero load (V max) was obtained from equation 3, which is derived from the Hill equation. Table 2 gives the various constants obtained for TSM. All values have been normalized for purposes of comparison. Force constants are therefore expressed as grams/$cm^2$ and velocity constants are expressed as

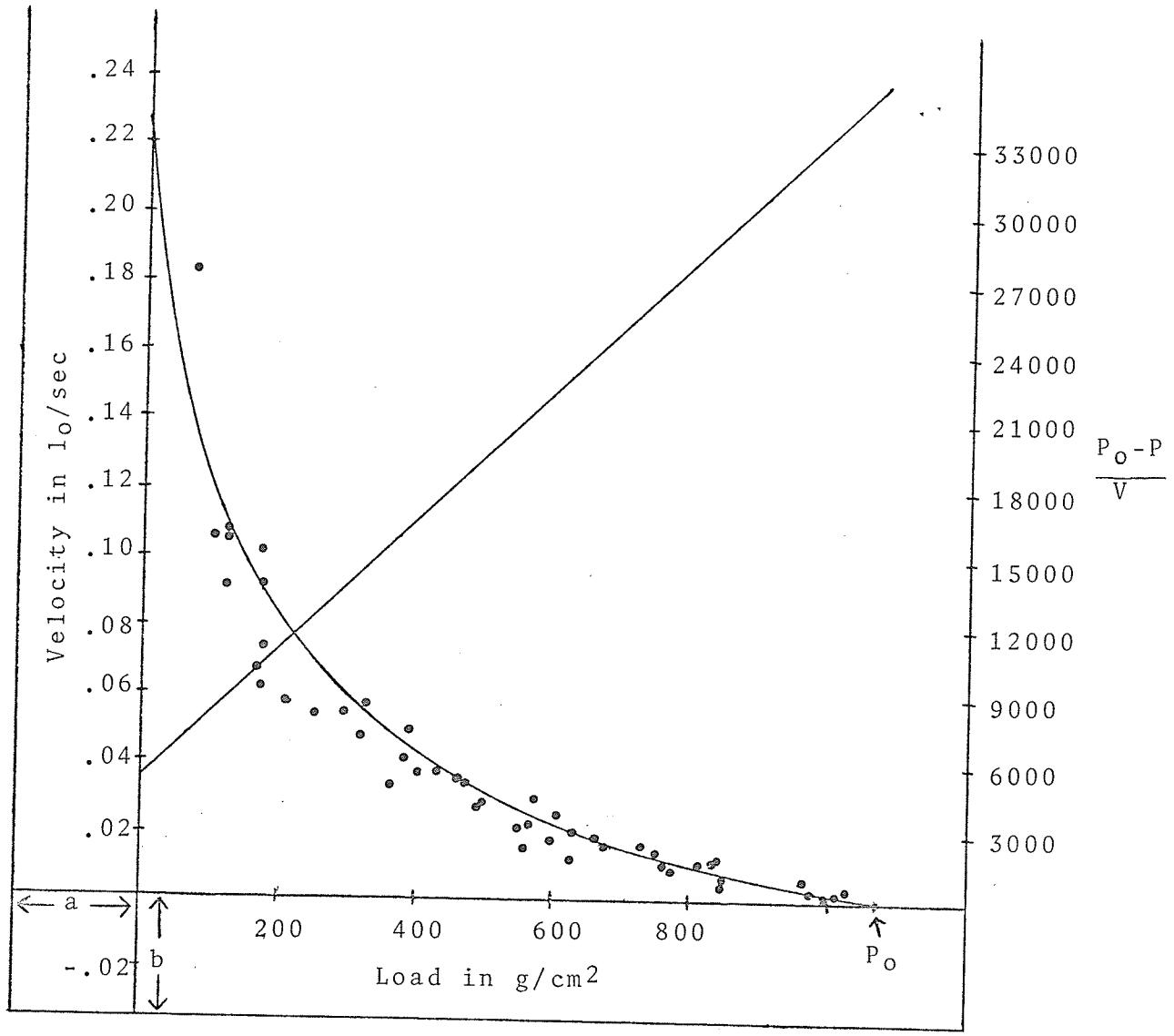



Fig. 10. Mean force-velocity curve for canine TSM derived from 12 experiments. The curve was drawn using the mean value of the constants from Hill's hyperbolic equation. The straight line of $P_o$-P/V was also drawn using the constants. The values of the constants and their standard errors are given in Table 2. For each of the 12 experiments a goodness of fit test yielded a correlation coefficient $r^2 > .95$, thereby proving statistically that the relationship was hyperbolic.

$l_o$ (optimal length)/sec.

B. FORCE-VELOCITY RELATIONSHIP AT LOADS GREATER THAN $P_o$

At loads greater than $P_o$, the experimental force-velocity relationship was found to deviate from an extension of Hill's hyperbola. This is shown in Fig. 11, where the force-velocity curve has been extended to 150% $P_o$. The constants obtained from the curve at loads less than $P_o$ were substituted into Hill's force-velocity equation to obtain a predicted curve for elongation. This extension of the hyperbola is shown by the broken line in Fig. 11. The experimental result obtained from an average of 9 experiments is shown by the solid line between $P_o$ and 150% $P_o$. A linear function fitted the results, the constants of which are given in Table 2. A statistical test was performed at 140% $P_o$, which showed that the curve obtained experimentally deviated significantly from an extension of Hill's hyperbola.

C. ISOTONIC LEVER EXPERIMENTS

In experiments where two muscles of different size were attached to either end of a lever, the observations were quite interesting. In a typical experiment, on the first stimulation the muscle with the larger cross-sectional area shortened, as shown by the first tracing in Fig. 12. After 20 minutes of being stimulated every 2 minutes, the larger muscle still shortened but to a lesser extent. On the next stimulation, the smaller muscle shortened. If a 30 minute rest without stimulation was then given the muscles, the stimulation after this time period yielded a response similar to the initial one in which the larger muscle once again shortened.

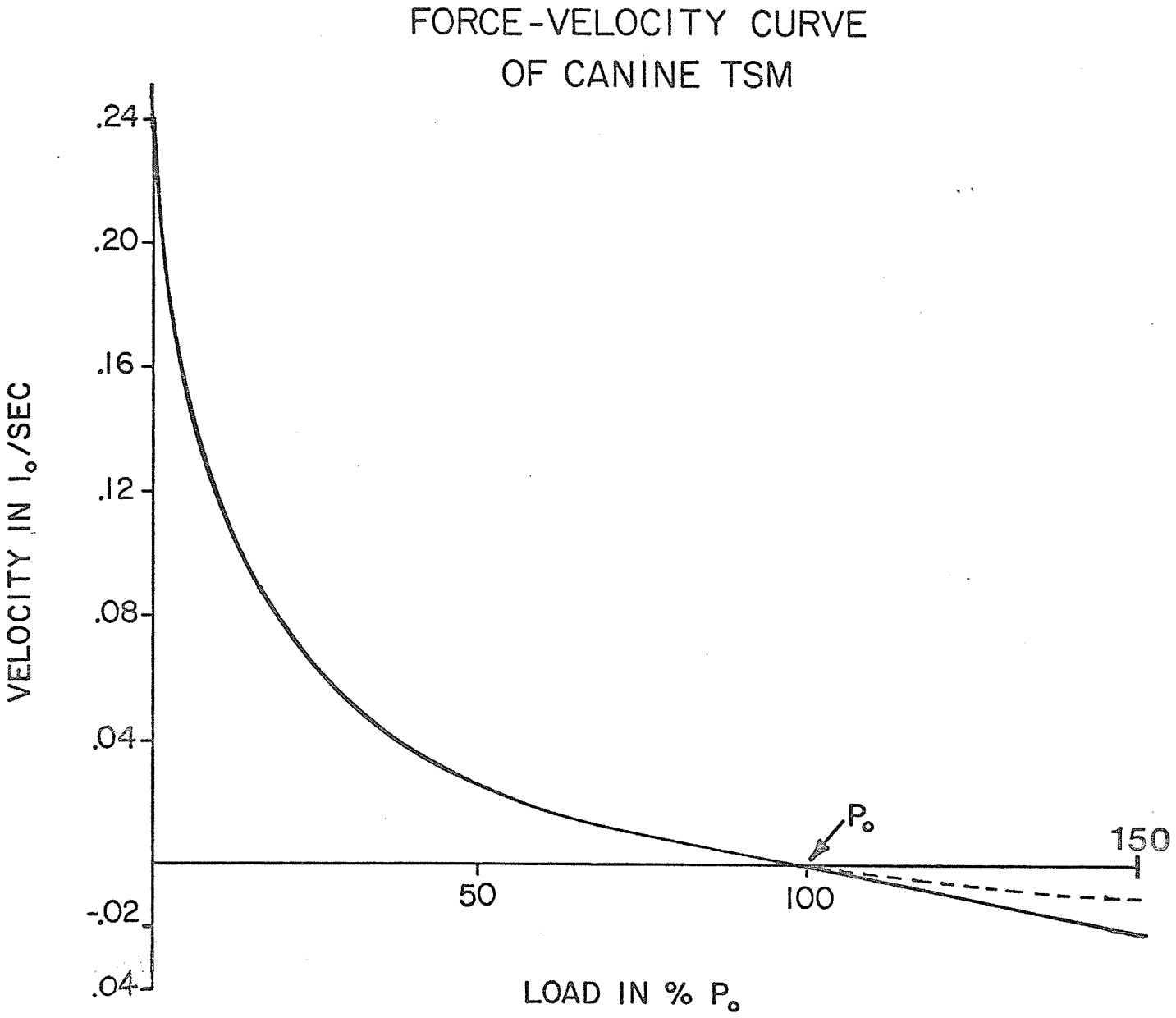



Fig. 11 The hyperbolic curve shown up to $P_o$ is as shown in Fig. 10. The experimental results obtained from a mean of 9 experiments for loads between $P_o$ and 150% $P_o$ are shown by the solid line, and as can be seen, it deviates from an extension of Hill's hyperbola (the dotted line). Data points have been excluded for clarity. Linear constants for loads greater than $P_o$ are given in Table 2.

TABLE 2

HYPERBOLIC FORCE-VELOCITY PARAMETERS
FOR LOADS LESS THAN $P_o$
(N = 12)

| | |
|---|---|
| $P_o$ ($g/cm^2$) | 1083 ± 106 |
| Vmax ($l_o$/sec) | .23 ± .03 |
| b ($l_o$/sec) | .035 ± .005 |
| a ($g/cm^2$) | 182 ± 33 |
| $a/P_o$ | .18 ± .03 |

LINEAR FORCE-VELOCITY CONSTANTS
FOR LOADS GREATER THAN $P_o$
(N = 9)

| | |
|---|---|
| Intercept | .0287 ± .0038 |
| Slope | $3.19 \times 10^{-4} \pm .38 \times 10^{-4}$ |

All values include standard errors of the means.

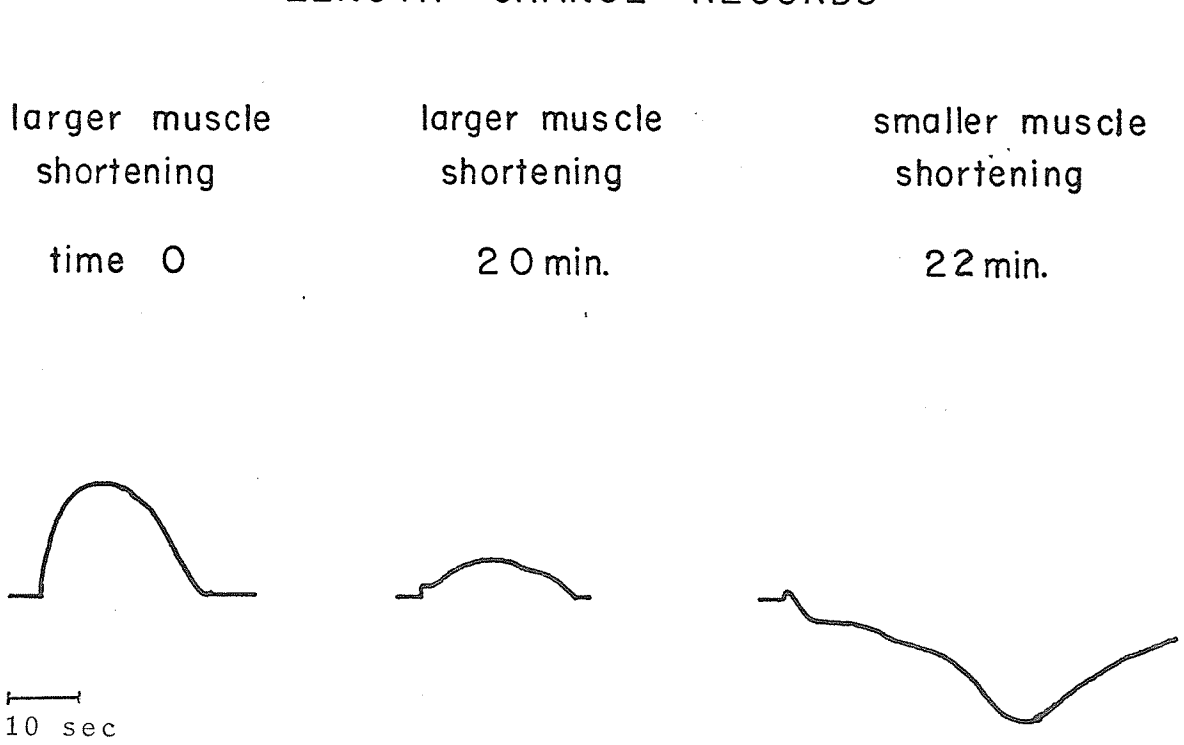
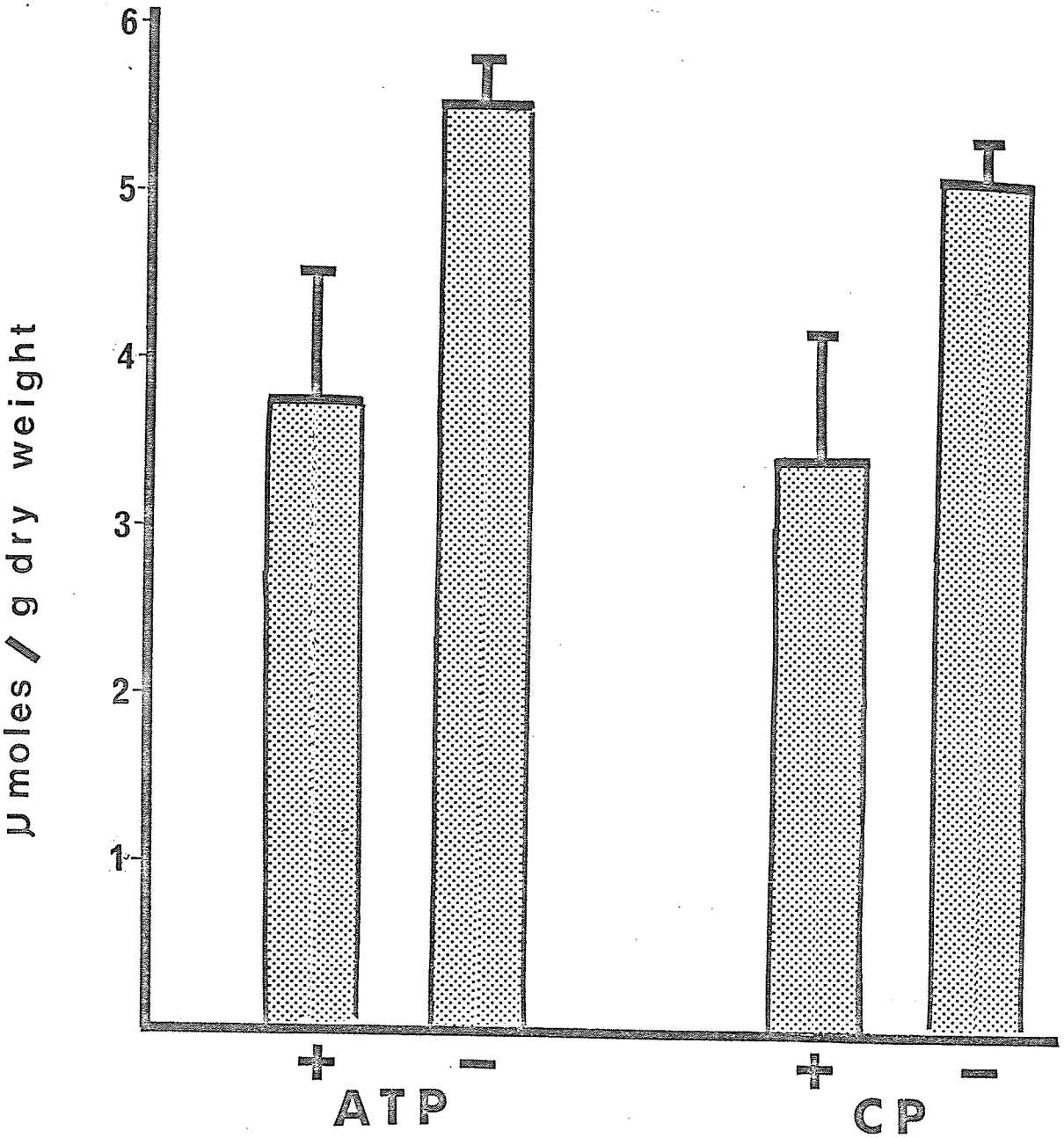



Fig. 12 Length change records for the isotonic lever experiments.
On successive stimulations at 2 minute intervals the larger muscle shortened initially. After 20 minutes, it still shortened but to a much lesser extent. On the next stimulation the smaller muscle shortened as shown by the tracing at 22 minutes. This prevented the larger muscle from shortening at all; in fact the larger muscle was now forced to lengthen. If the muscles were left unstimulated for half an hour, the next stimulation resulted in a tracing similar to that at time zero.

## D. HIGH ENERGY PHOSPHATE DETERMINATIONS

The adenosine triphosphate (ATP) and creatine phosphate (CP) content of TSM samples undergoing active shortening and lengthening were determined as described in the Methods section. The muscles were instantaneously frozen at a moment when both muscles had been stimulated for identical time periods, but one had been shortening at a given velocity and the other had been lengthening at the same velocity. The mean and standard error for the ATP and CP contents calculated from 5 pairs of muscles are shown by a bar graph in Fig. 13, with the actual numerical values printed underneath. It can be seen that the muscles which were actively stretched had a higher content of both ATP and CP than actively shortening muscles.




| | | | | | |
|---|---|---|---|---|---|
| ATP | + | 3.78 ± .74 | CP | + | 3.43 ± .77 |
| | - | 5.55 ± .24 | | - | 5.09 ± .25 |

Fig. 13 High energy phosphate levels in shortening (+) and lengthening (-) TSM.
Two muscle strips of similar size were instantaneously frozen while one was shortening at about .02 $l_o$/sec and the other lengthening at the same speed. At the time of freezing both muscles were at approximately $l_o$. The levels of ATP and CP in each muscle were determined and are shown above for 5 pairs of muscles. In the lengthening muscles more ATP and CP remained.

# DISCUSSION

## A. FORCE-VELOCITY RELATIONSHIP AT LOADS LESS THAN $P_o$

Consideration of both tension developed and the velocity of shortening is required in any study of the contractile properties of muscle. According to Hill (1938) and Csapo (1962), determination of the force-velocity relationship of muscle provides the most meaningful study of muscle function. As shown by Fig. 10, the force-velocity curve obtained for canine TSM using the Q-R method fitted a hyperbolic function, as when the linear transform (Equation 2) of Hill's equation (Equation 1) was used, the data points fitted a straight line.

If the values of the force-velocity constants of canine TSM reported by Antonissen (1977) and those of the present study (Table 3) are compared, it can be seen that $P_o$ (the maximum isometric tetanic tension) and V max (the maximum velocity of shortening at zero load) are similar but that the values of the constants a and b differ.

The differences could be due to the age differences of the dogs. In the study by Antonissen, young pups were used, whereas in the present study the majority of the dogs were much older, and age-related differences in the muscle could exist.

A second possibility is that the observed differences in the values of the constants resulted from the use of different techniques to determine the force-velocity curve. In the study by Antonissen, an afterload stop technique was employed, whereas in the present study a quick-release method was preferred, as it allowed the lengthening velocities to be performed also. The afterload stop method utilizes a stop which is positioned so as to hold the muscle at $l_o$ when afterloads are added. The muscle is stimulated isometrically

TABLE 3

HYPERBOLIC FORCE-VELOCITY PARAMETERS

| Constants | Canine TSM 1 | Canine TSM 2 |
|---|---|---|
| $P_o$ ($g/cm^2$) | 1083 ± 106 | 1211 ± 71 |
| Vmax ($l_o$/sec) | .23 ± .03 | .23 ± .02 |
| b ($l_o$/sec) | .035 ± .005 | .047 ± .003 |
| a ($g/cm^2$) | 182 ± 33 | 263 ± 32 |
| a x b | 6.37 | 12.36 |
| $a/P_o$ | .18 ± .03 | .23 ± .04 |

Canine TSM 1 - Present studies using Quick-Release Method

Canine TSM 2 - Antonissen (1977) using Afterload Stop Method

Force-velocity parameters calculated (using Hill's analysis) from data obtained in force-velocity experiments performed by different techniques. Note that whilst values of $P_o$ and Vmax are similar, the values of a and b differ. All values include standard errors of the means.

till the developed tension just exceeds the afterload, at which time the muscle shortens and the shortening velocity is measured.

The quick-release method, as described in the methods section, allows the muscle to be stimulated isometrically at $l_o$ until $P_o$ is reached. At this point, the lever is released and the muscle undergoes a sudden elastic recoil before the shortening of the contractile element can be measured. The quick-release method allows the muscle to reach a fully-activated state before shortening velocities are measured. However, it is possible that the sudden elastic recoil immediately before the shortening of the contractile element could affect the contractile apparatus and cause the altered values of the a and b constants found in the present study. Using Hill's interpretation of the functional significance of a and b (1938), the decrease in the value of a suggests a functional loss of force-generating sites in the muscle. The reduction in the value of b suggests that the rate at which energy-liberating reactions for contractile purposes is decreased. Barany (1967) confirmed that the process involved is the hydrolysis of ATP by myosin ATPase.

As pointed out by Hill (1938) and confirmed by Woledge (1968), the product of the a and b constants (a x b) of the force-velocity equation is equal to the maintenance energy rate. This value is lower for the present study than in the previous study reported by Antonissen (1977). It is reassuring that the differences between the two studies seem to be due to the efficiencies of the muscles, and that although the energy costs may differ, V max (the maximum shortening velocity at zero load) and $P_o$ (the maximum isometric tension developed) remain similar (see Table 2 & 3).

Since in the present study the force-velocity relationship determined by the quick-release method fitted a hyperbolic function, it seemed reasonable to analyze the data in terms of Hill's force-velocity equation. Comparison of the $\wedge$ quick-release method of this study with the afterload stop technique performed previously on TSM revealed that certain differences existed with respect to the values of the constants a and b, but that the values of $P_o$ and V max were similar. Also, the a values for both studies were within the range found for the majority of vertebrate muscles, between .15 and .25 (White, 1978).

The quick-release method therefore seemed adequate for the study of the force-velocity characteristics of TSM at loads both below and above $P_o$.

## B. FORCE-VELOCITY RELATIONSHIP AT LOADS GREATER THAN $P_o$

The elongating side of the force-velocity relationship of canine TSM was determined up to loads of 150% $P_o$, as shown in Fig. 11. The records from one typical experiment (Fig. 7) reveal a rapid initial stretch, which is believed to be due to elastic elements, followed by a slower lengthening component resulting from the contractile element. The elastic length change may be due in part from the cross-bridges themselves (Huxley & Simmons, 1971; Johansson et al, 1978; Julian & Sollins, 1975). However, the magnitude of the length change, especially at large load applications, suggests that the major portion of the response originates in more distensible structures such as extracellular elastic fibres. In the present study on TSM, loads exceeding 150% $P_o$ were not studied since loads of these magnitudes resulted in an initial stretch of the muscle beyond 110% $l_o$. In order to ensure that the lengthening velocities were recorded

at $l_o$, all studies between 100 and 150% $P_o$ started with the muscle at approximately 90% of $l_o$. At this length the muscle still develops maximum isometric tension, yet the resulting stretch brings it to approximately $l_o$ and the lengthening velocity can be measured without interference from increased resting tension developing at longer lengths. Another reason why loads in excess of 150% $P_o$ were not studied in TSM was that they caused permanent damage to the muscle tissue, and the initial value of $P_o$ could not be attained again.

The mean force-velocity curve obtained from the study is shown in Fig. 11. The force-velocity constants from the shortening curve were used to extend the hyperbola up to 150% $P_o$, as shown by the dotted line in Fig. 11, and the curve derived experimentally compared to this predicted curve. Lengthening velocities of TSM increased faster than expected from the hyperbolic curve.

Reports in the literature on the elongating side of the force-velocity relationship are rare, especially for smooth muscle. However, Johansson (1978) has performed quick-stretch experiments (similar to those used for TSM studies) on smooth muscle from the rat portal vein and rabbit urinary bladder. In both types of smooth muscle, the application of loads greater than $P_o$ resulted in lengthening velocities greater than expected from an extrapolation of Hill's hyperbola. The major difference is that the rabbit urinary bladder is able to resist being stretched to a much greater extent than rat portal vein. Canine TSM resembles Johansson's bladder preparation more closely. In fact, canine TSM can resist stretch to such an extent that at 150% $P_o$ it lengthened at a velocity of only .02 $l_o$/sec, compared to rat portal vein, which at 140% $P_o$ is already lengthening at

.35 $l_o$/sec. Since elastic components have already been taken into consideration through the use of the quick-stretch method, and the study occurring close to $l_o$, the difference between the two muscles' abilities to resist stretch could reside in the compliance of the cross-bridges, or in their reattachment capacities, which may be inherently different for the two types of smooth muscle.

There have been a few force-velocity studies at loads greater than $P_o$ on skeletal muscle. The lengthening velocities at loads greater than $P_o$ have in all cases been found to be less than predicted by an extension of Hill's hyperbola, up to 140% $P_o$ (Aubert, 1956; Chaplain, 1972; Curtin & Davies, 1972; Katz, 1939). This differs from the smooth muscle studies which all had lengthening velocities greater than predicted. Skeletal muscle seems better suited to resist stretch than smooth muscle. Similar results have been reported for skeletal muscle even though different techniques have been employed for the force-velocity studies. Katz (1939) and Aubert (1956) determined the force-velocity relationship of elongating frog sartorius muscles by applying a constant load and measuring the resultant velocity. The lengthening velocities deviated from Hill's hyperbola towards relatively lower lengthening velocities at moderate increases of P above $P_o$. At still greater loads the muscle lengthened rapidly, this 'give' occurring at 150% $P_o$ (Aubert, 1956). An alternative method for studying the force-velocity relationship at loads greater than $P_o$ is to apply a constant velocity of lengthening and measure the resulting force produced by the muscle. This has been done for skeletal muscle. Curtin & Davies (1972) and Chaplain (1972), working with frog sartorius muscle, determined the force-velocity

curve by stretching the muscles at constant velocities and recording the tension produced at 100% $l_o$ during movement. A schematic diagram of their results is given in Fig. 2. As the velocity of stretching increased, so did the tension up to 140% $P_o$ at 0.2 $l_o$/sec. However, as the velocities of stretching increased beyond 0.2 $l_o$/sec., the tension produced by the muscle decreased. These studies showed that the same force could be developed by the muscle at two different velocities of lengthening. Chaplain (1972) suggested an explanation for this observation as follows: the shape of the force-velocity curve reflects the numbers of active myosin cross-bridges. Since the muscles are stimulated tetanically, the high calcium levels would provide maximal activation. The postulate is that the tension rise at very low velocities of lengthening results from an actual compression of the cross-bridges attached to the actin filaments as the two sets of filaments are displaced against each other contrary to their normal sliding direction. As velocities increase this effect will be masked as the number of cross-bridge links decreases. This theory is not a complete explanation of the application of the cross-bridge theory to actively elongating muscles.

The theory put forward by Huxley & Simmons (1971, 1973) which describes a possible mechanism of force production by muscle, can be used to explain the tension increase noted as lengthening velocity increases up to a certain point, and can also possibly explain why the tension decreases if the lengthening velocity is great enough. The theory, as described in the introduction, envisages the myosin head as having a small number of attachment sites through which it can bind to corresponding active sites on an active region (Fig. 1).

Flitney & Hirst (1976) have performed studies on actively stretching skeletal muscles and have suggested an explanation of the cross-bridge mechanism during stretch, based on the theory of Huxley & Simmons (1971, 1973). This can be used to explain the force-velocity relationship at loads greater than $P_o$.

If the active muscle is being slowly stretched, actomyosin cross-bridges would be able to form but would not be able to rotate in the forward direction as in shortening. The cross-bridges would remain attached initially and tension would be generated by extension of the AB links (Fig. 1). At some point, some of the heads would begin to be forced backwards, and further increase the tension. The tension produced would depend upon the backward rotation of the head and the extension of the elastic linkage. The muscle would lengthen, since after a certain tension is reached by any particular cross-bridge, the myosin head would be forced away from the actin filament and spring back until it could attach to another actin site, repeating the process. If the tension placed on the muscle or the velocity of lengthening is too great, the cross-bridges would remain in their most extended positions with the AB links fully extended and the myosin heads attached to the actin filaments in their farthest backward position, thereby sliding from one active site to the next without being given a chance to develop further tension. This could explain why at loads greater than 150% $P_o$ in the studies (by Aubert, 1956; Johansson, 1978; and the present ones) (see Fig. 3) the velocity increases disproportionately, and at velocities greater than a certain value (Chaplain, 1972; Curtin & Davies, 1972) (Fig. 2), the tension decreases disproportionately.

## C. ENERGY COST OF ELONGATING ACTIVE MUSCLES

These experiments on the force-velocity relationships of elongating muscles have all shown that tension was higher than $P_o$ during stretch and depended on the velocity of stretch (Aubert, 1956; Chaplain, 1972; Curtin & Davies, 1972; Katz, 1939; present study). Edman et al, (1977) have confirmed this finding using simple skeletal muscle fibres. This increased force production during stretch seems to be associated with no extra energy cost. In fact, a decrease in energy utilization by active muscles which are forcibly stretched has been shown by many investigators using a variety of methods, such as oxygen consumption measurements, heat studies, and biochemical tests (Abbott & Aubert, 1951; Abbott, Aubert & Hill, 1951; Chauveau, 1901; Fenn, 1924; Hill & Howarth, 1959).

An active muscle which is forcibly stretched has been said to do negative work. Although in a strict sense a muscle cannot perform 'negative' work, this term has been used to describe the occurence of an elongating active muscle. Therefore, out of convenience, the term 'negative' work will be used in this discussion. The early studies on the physiological cost of negative work were based on oxygen consumption. Chauveau (1901) and Abbott et al, (1952), using oxygen consumption tests on human subjects, showed that the physiological cost of negative work was much less than that of positive work.

Heat measurements on active stretching muscles have been made and have supported the oxygen consumption studies. Within an interval of time, the energy from chemical reactions is either transformed into work or it appears as a production or absorption of heat; ENERGY = HEAT + WORK. It has been reported by Fenn (1923, 1924), Abbott &

Aubert (1951), and Hill & Howarth (1959) that heat + work in a complete cycle of contraction and relaxation during which a muscle is stretched is less than when it contracts isometrically. It may even be reduced to zero (Hill, 1960; Hill & Howarth, 1959), but a negative heat + work over a complete cycle has never been observed.

Chemical measurements on stretching active muscles have shown that less ATP usage occurs during contractions with stretch than during isometric contractions (Aubert & Marechal, 1963; Butler et al, 1972; Curtin & Davies, 1975; Infante et al, 1964; Marechal, 1964, 1965; Wilkie, 1968).

The studies discussed so far have all dealt with skeletal muscle, and all reports from them have concluded that less energy is utilized when an active muscle is stretched compared to isometric or shortening contractions, even though in all cases the muscles are being tetanically stimulated. It was of interest to determine whether the same phenomenon exists for smooth muscle, and specifically for TSM since the degree of contraction of airway smooth muscle is important in the control of distribution of ventilation. An in vitro experiment was therefore designed using canine TSM which parallelled the in vivo studies on human subjects of Abbott et al, (1952). The methods and results of this experiment have been given under the appropriate sections. The two muscles were stimulated identically throughout the procedures. The only difference was that one muscle lengthened and the other shortened. After several stimulations the muscle that had previously lengthened during each stimulation, was now able to shorten. If smooth muscle is similar to skeletal with respect to its elongating properties (and this has already been discussed for its

force-velocity relation), then the explanation could be that the muscle being stretched did not use as much energy during each stimulation as the shortening muscle. It would then follow that after a certain amount of time the shortening muscle, (A) although larger, would have less energy than the smaller muscle (B) which had been stretched continually. Therefore, upon stimulation, muscle (B) would now be able to shorten, since it would have been able to conserve more of its energy stores. It also follows that if a long enough rest period was then given the muscles, so that they would have time to re-equilibrate, a stimulation following the rest would produce length changes identical to the first stimulation. This was shown to be the case for TSM. If the muscles were left unstimulated for 30 minutes, the next stimulation showed the larger muscle once again shortening, as seen in Fig. 12.

This experiment on smooth muscle, although supporting the concept developed for skeletal muscle of decreased energy utilization by an elongating muscle, gave no insight into the energy stores involved. As it had been shown for skeletal muscle that less ATP usage occurs during contraction with stretch, an experiment (previously described) was conducted to determine the amounts of CP and ATP present in lengthening <u>versus</u> shortening TSM. The concentrations of both ATP and CP were found to be greater in the muscles which had been stretched (Fig. 13). It seems then that TSM resembles skeletal muscle in that less ATP usage occurs in muscles that are stretched. Indeed, in view of the observation that stretched skeletal muscle uses less energy than isometrically contracting muscle, an additional experiment comparing these two conditions in smooth muscle is indicated.

From the various types of experiments on both skeletal and smooth muscle, an overall conclusion which can be derived is that there is a diminished energy utilization in active muscles which are stretched. The heat studies, which showed that heat + work for elongating muscles was less than for isometrically contracting ones, suggest that either an exothermic process is prevented by the stretch or an endothermic reaction (such as a reversal of ATP splitting) is caused by the stretch. The chemical measurements on stretched skeletal muscles all showed a decrease in ATP usage. A net synthesis of ATP during stretch has not been found. Curtin & Davies (1975) found that at velocities of lengthening which gave the lowest mean ATP-splitting rate, the rate was about 25% of that during an isometric contraction. From these results, it therefore seems the prevention of ATP splitting by stretch explains the reduced output of heat + work.

Whether the reduction in net ATP usage is due to a lower rate of the ATP-splitting reaction, or an increased rate of an ATP synthesizing reaction was a question which Gillis & Marechal (1974) investigated. They compared the incorporation of $^{32}Pi$ into ATP by glycerol-extracted rabbit muscle during isometric contraction and during elongation. Some incorporation of $^{32}Pi$ was found, both with and without stretches, but the amount was insignificant, suggesting that the rate of the forward reaction is 100-1000 times faster than the backward rate. Therefore, the resynthesis of ATP is probably not of quantitative importance in the energetics of stretches.

Although many observations on stretching active muscle support the hypothesis that stretch prevents most or all of the ATP-splitting by actomyosin, Hill & Howarth (1959) believe that this explanation

is incomplete. They have reported a number of observations of total heat + work during stretch to be much less than 25% of the isometric controls. About 25% of the heat + work during isometric contraction is believed to be associated with "activation" processes rather than actomyosin activity, so the finding that less than this amount is involved for a stretched muscle requires elucidation.

Hill & Howarth (1959) have also observed that the heat + work output during a stretch became negative in experiments with short tetanuses in which the start of the stretch was delayed by several tenths of a second. Since prevention of ATP-splitting alone would not cause this, a net endothermic process is apparently occurring. Infante et al, (1964) have shown that this process is not ATP synthesis. The heat absorption is unlikely to be due to the thermoelastic effect, since this is much smaller. Curtin & Woledge (1978) suggest the intriguing possibility that the endothermic process is the reverse of the process producing the unexplained heat + work during contraction. This needs further investigation.

It still seems, however, that stretch prevents most or all of the ATP-splitting reaction. The paradox that must be explained, then, is how the force enhancement during stretch can occur without a concomitant increase in the activation of the contractile system. The molecular mechanism of negative work is not fully understood, but any theory of muscle contraction must be able to account for the ability of the muscle to develop tendons greater than $P_o$ and maintain them during elongation with little breakdown of ATP. The model proposed by Huxley & Simmons (1971, 1973) based on the cross-bridge

theory, can be used to explain the active stretching muscle in terms of energetics, as will now be discussed. As described earlier, this model accounts for the extra force generated during stretch by the pulling out of the cross-bridges. The AB link (Fig. 1) acts like an elastic body, so that extra tension arises as this is forcibly extended and as the myosin head is forced to rotate in a backwards direction, against its tendency to move to the position of lower potential energy. If the degree of extension of the muscle is enough to pull the cross-bridges off their original sites before they have gone through the complete cycle, then they will still have the ability to combine with actin without requiring further ATP. The myosin heads could conceivably slide from one active site to the next, developing tension at each site until being forced to the next.

## D. GENERAL CONCLUSIONS

Although much remains to be learned about the molecular mechanism of negative work, it seems fortunate in considering skeletal muscles during locomotion, that muscle can develop extra tension when stretched in the activated state and that the energy cost of the tension produced during negative work is slight. Advantages can also be seen for airway smooth muscle. Since the smooth muscle of the lower airways probably possesses resting tone, then on inspiration, active muscle may be forcibly stretched. This could result in increased stiffness of the muscle and prevent any tendency of it to bulge outward and increase dead space. The added benefit would be that the increased stiffness involves no extra energy cost.

It is also significant that the present studies on the stretching of active smooth muscle qualitatively resemble studies on skeletal

muscle. As mentioned in the Introduction, the theory of generation of force by actin-myosin bridge formation (Huxley, 1964) cannot be applied to smooth muscle with confidence. However, the observations on TSM with respect to the stimulus response (Stephens et al, 1968), length-tension and force-velocity relationships (Stephens et al, 1969) and the present studies on the elongating side of the force-velocity relationship and energy changes during stretching, are qualitatively similar to such studies on skeletal muscle. These similarities suggest that force generation in smooth muscle may reside in a mechanism similar to that in striated muscle.

In conclusion, both mechanical and biochemical experiments on TSM give evidence that an active muscle which is stretched can exert much greater force and yet expend less energy than a muscle which shortens, and that these phenomena may play an important role in maintaining airway patency during normal breathing and maximum ventilation during exercise.

# REFERENCES

Abbott, B.C. and X.M. Aubert (1951). Changes of energy in a muscle during very slow stretches. Proc. Roy. Soc. B. 104-117.

Abbott, B.C., Bigland, B. and J.M. Ritchie (1952). The physiological cost of negative work. J. Physiol., Lond. 117, 380-390.

Antonissen, L.A. (1977). Studies of airway smooth muscle in a canine model of allergic asthma, MSc Thesis, University of Manitoba.

Aubert, X.M. (1956). Le couplage énergétique de la contraction musculaire. Editions Arsica, Bruxelles.

Aubert, X. and G. Maréchal (1963). La fraction labile de la thermogenèse associée au maintien du la contraction isometrique. Arch. Intern. Physiol. Biochem. 71:282-283.

Barany, M. (1967). ATPase activity of myosin correlated with speed of muscle shortening. J. Gen. Physiol. 50: 197.

Brady, A.J. (1965). Time and displacement dependence of cardiac contractility: problems in defining the active state and force-velocity relations. Fed. Proc. 24: 1410-1420.

Bremel, R.D., Sobieszek, A., and J.V. Small (1977) In: The Biochemistry of Smooth Muscle, Ed. by N.L. Stephens, Publ.: Univ. Park Press, Baltimore, Maryland, pp.553-550.

Butler, T.M., N.A. Curtin, and R.E. Davies (1972). Comparison of ATP usage in muscle during isometric tetanus and activated isovelocity stretch. Fed. Proc. 31:337.

Cain, D.F. and R.E. Davies (1962). Breakdown of adenosine triphosphate during a single contraction of working muscle. Biochem. Biophys. Res. Com. 8:361-366.

Carlson, F.D. (1975). Structural fluctuations in the steady state of muscular contraction. Biophys. J. 15:633-649.

Carlson, F.D. and D.R. Wilkie (1974). Muscle Physiology, Prentice Hall Inc., Englewood Cliffs, N.J.

Carsten, M.E. (1971). Uterine smooth muscle: troponin. Arch. Biochem. Biophys. 147:353-357.

Chaplain, R.A. (1972). The force-velocity relation of frog sartorius muscle at constant velocities of lengthening. Experientia 28:292.

Chauveau, A. (1901). C.R. Acad. Sci., Paris, 182, 194.

Choi, J.K. (1962). Fine structure of the smooth muscle of the chicken's gizzard. In: Electron Microscopy 5th Int. Congress for Electron Microscopy. Vol. 2, M-9, Ed. Breese, S.S., New York: Academic Press.

Conti, G., Haenni, B., Laszt, L., and C.H. Rouiller (1964). Structure et ultrastructure de la cellule musculaire lisse de paroi carotidienne a l'état de repos et a l'état de contraction. Angiologica 1:119-140.

Csapo, A. (1962). Smooth muscle as a contractile unit. Physiol. Rev. 42(5):7-33.

Curtin, N.A. and R.E. Davies (1972). Chemical and mechanical changes during stretching of activated frog skeletal muscle. Cold Spring Harb. Symp. Quant. Biol. p. 619-626.

Curtin, N.A. and R.E. Davies (1975). Very high tension with very little ATP breakdown by active skeletal muscle. J. Mechanochem. Cell Motility 3:147-154.

Curtin, N.A. and R.C. Woledge (1978). Energy changes and muscular contraction. Physiological Reviews 58(3).

Davies, R.E. (1964). Adenosine triphosphate breakdown during single muscle contractions. Proc. R. Soc.,B., 160:480-484.

Ebashi, S., Iwakura, H., Nakajima, M., Nakamura, R., and Y. Ooi (1966). New structural proteins from dog heart and chicken gizzard. Biochem. Z. 345:201-244.

Edman, K.A.P., Elzinga, G., and M.I.M. Noble (1978). Enhancement of mechanical performance by stretch during tetanic contractions of vertebrate skeletal muscle fibres. J. Physiol., 281:139-155.

Fenn, W.O. (1923). A quantitative comparison between the energy liberated and the work performed by the isolated sartorius muscle of the frog. J. Physiol. Lond. 58: 175-203.

Fenn, W.O. (1924). The relation between work performed and the energy liberated in muscular contraction. J. Phys. Lond. 58:373-395.

Fick, A. (1882). Mechanische arbeit und wärmeentwicklung b.d. Muskelthätigkeit. Leipzig.

Flitney, F.W. and D.G. Hirst (1976). Filament sliding and energy absorbed by the cross-bridges in active muscle subjected to cyclical length changes. J. Physiol. 276: 467-479.

Goldspink, G. (1978). Muscle Energetics In: Mechanics and Energetics of Animal Locomotion. Ed. by Alexander, R.M. and G. Goldspink. London, Chapman and Hall, pp. 57-79.

Gordon, A.M., Huxley, A.F., and F.J. Julian (1966). Tension development in highly stretched vertebrate muscle fibres. J. Physiol. 184:143-169.

Gordon, A.M., Huxley, A.F. and F.J. Julian (1966). The variation in isometric tension with sarcomere length in vertebrate muscle fibres. J. Physiol. 184:170-192.

Haselgrove, J.C. (1975). X-ray evidence for conformational changes in the myosin filaments of vertebrate striated muscle. J. Mol. Biol. 92:113-143.

Hawkins, D.F. and H.O. Schild (1951). The actions of drugs in isolated human bronchial chains. Brit. J. Pharmacol. 6:682-690.

Hayek, H.V. (1960). The Human Lung. Publ.: Hafner Publishing Co., New York, pp. 127-226.

Hellstrand, P. and B. Johansson (1974). The force-velocity relation in phasic contractions of venous smooth muscle. Acta. Physiol. Scand. 93:157-166.

Herlihy, J.T. and R.A. Murphy (1974). Force-velocity and series elastic characteristics of smooth muscle from the hog carotid artery. Circ. Res. 34:461-466.

Hill, A.V. (1938-1939). The heat of shortening and the dynamic constants of muscle. Proc. R. Soc. Lond. B. 126:136-195.

Hill, A.V. (1948). On the time required for diffusion and its relation to processes in muscle. Proc. R. Soc. B. 135:446-453.

Hill, A.V. (1949). The heat of activation and the heat of shortening in a muscle twitch. Proc. R. Soc. Lond. B. 136:195-211.

Hill, A.V. (1964a). The effect of load on the heat of shortening of muscle. Proc. R. Soc. B. 159:297-318.

Hill, A.V. (1964b). The effect of tension in prolonging the active state in a twitch. Proc. R. Soc. B. 159: 589-595.

Hill, A.V. and J.V. Howarth (1959). The reversal of chemical reactions in contracting muscles during an applied stretch. Proc. Roy. Soc. Lond. B. 151:169-193.

Huxley, A.F. (1957). Muscle structure and theories of contraction. Prog. in Biophys. 7:257-318.

Huxley, A.F. (1974). Muscular Contraction. J. Physiol. 243:1-43.

Huxley, A.F. and R. Niedergerke (1954). Structural changes in muscle during contraction. Nature 173:971-973.

Huxley, A.F., and R.M. Simmons (1971). Proposed mechanism of force generation in striated muscle. Nature 233: 533-538

Huxley, A.F. and R.M. Simmons (1973). Mechanical transients and the origin of muscular force. Cold Spring Harbor Symp. Quant. Biol. 37:669-680.

Huxley, A.F. and R.E. Taylor (1958). Local activation of striated muscle fibres. J. Physiol., Lond. 144:426-441.

Huxley, H.E. (1964a). Structural arrangements and the contraction mechanism in striated muscle. Proc. Roy. Soc. Lond. B. 160:442-448.

Huxley, H.E. (1964b). Evidence for continuity between the central elements of the triads and extracellular space in frog sartorius muscle. Nature, Lond. 202:1067-71.

Infante, A.A., Klaupiks, D., and R.E. Davies (1964). Adenosine triphosphate changes in muscles doing negative work. Science 144:1577-1578.

Iwazumi, T. (1970). A new field theory of muscle contraction. Thesis, University of Pennsylvania.

Johansson, B., Hellstrand, P., and B. Uvelius (1978). Responses of smooth muscle to quick load changes studied at high time resolution. Blood Vessels 15:65-82.

Julian, F.J., Sollins, M.M. (1975). Variation of muscle stiffness with force at increasing speeds of shortening. J. Gen. Physiol. 66(3):287-302.

Katz, B. (1939). The relation between force and speed in muscular contraction. J. Physiol. (Lond.) 96:45-64.

Kroeger, E.A. and N.L. Stephens (1971). Effect of hypoxia on energy and calcium metabolism in airway smooth muscle. Am. J. Physiol. 220:1199-1204.

Lamprecht, W., Stein, P., Heinz, F., and H. Weisser (1974). In: Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) Vol. 4, pp. 1777-1779, Academic Press, New York.

Lamprecht, W., and I. Trautschold (1974). In: Methods of Enzymatic Analysis (Bergmeyer, H.U., ed.) Vol. 4, pp. 2101-2110, Academic Press, New York.

Levin, A., and J. Wyman (1927). Proc. Roy. Soc. London, B. 101:218.

Marechal, G., (1964). Phosphorylcreatine and ATP changes during shortening and lengthening of stimulated muscle. Arch. Intern. Physiol. Biochem. 72:306-309.

Marechal, G. and G. Beckers-Bleukx (1965). La phosphoryl-creatine et les nucleotides adénylique d'un muscle strié à la fin d'un étirement. J. Physiol., Paris 57: 652-653.

Marston, S.B., Rodger, C.D., and Tregear, R.T. (1976). Changes in muscle cross bridges when $\beta,\gamma$ -imido ATP binds to myosin. J. Mol. Biol. 104:263-276.

Meiss, R.A. (1971). Some mechanical properties of cat intestinal muscle. Am. J. Physiol. 220:2000-2007.

Mommaerts, W.F.H.M. (1969). Energetics of muscular contraction. Physiol. Rev. 49:427-508.

Murphy, R.A. and J. Megerman (1977). Protein interactions in the contractile system of vertebrate smooth muscle. In: The Biochemistry of Smooth Muscle Ed. N.L. Stephens, Univ. Park Press, Baltimore, Md., pp. 398-473.

Nadel, J.A. (1973). In: Asthma: Physiology, Immunopharmacology and Treatment, Ed. Austen, K.F., and L.M. Lichenstein, Academic Press, New York, and London, pp. 324.

Natrella, M.G. (1963). Experimental Statistics, Washington, D.C., U.S. Gov't. Printing Off., Dept. of Commerce, Nat'l. Bur. of Standards, Handbook 91, pp. 5-22, 5-23.

Needham, D.M. and C.F. Shoenberg (1964). Proteins of the contractile mechanism of mammalian smooth muscle and their possible location in the cell. Proc. R. Soc. B. 160:517-522.

Noble, M.I.M. and G.H. Pollack (1977). Molecular Mechanism of Contraction. Circ. Res. 40:333-342.

Oplatka, A., Gadasi, H., and Borejdo (1974). The contraction of "ghost" myofibrils and glycerinated muscle fibres irrigated with heavy meromyosin subfragment-1. Biochem. Biophys. Res. Commun. 58:905-912.

Ostle, B. (1956). Regression analysis-I. One independent variable. In: Statistics in Research, Iowa State College Press, Annes, Iowa, pp. 117-201.

Polissar, M.J. (1952). Physical chemistry of contractile process in muscle. Amer. J. Physiol. 168:766-781.

Reedy, M.K., Holmes, K.C., and R.T. Tregear (1965). Induced changes in orientation of the cross bridges of glycerinated insect flight muscle. Nature 207:1276-1280.

Sandow, A. (1965). Excitation-contraction-coupling in skeletal muscle. Pharmacol. Rev. 17:265-320.

Small, J.V. (1977). The contractile apparatus of the smooth muscle cells: structure and composition. In: The Biochemistry of Smooth Muscle Ed. N.L. Stephens, Univ. Park Press, Baltimore, Md., pp. 379-441.

Snedecor, G.W. (1946). Statistical Methods Applied to Experiments in Agriculture and Biology. 4th Ed., The Collegiate Press, Inc., Ames, Iowa.

Sobieszek, A. (1977). Vertebrate smooth muscle myosin. Enzymatic and structural properties. In: The Biochemistry of Smooth Muscle Ed. N.L. Stephens, Univ. Park Press, Baltimore, Maryland, pp. 413-433.

Somlyo, A.P., Devine, C.E., Somlyo, A.V., and R.V. Rice (1973) Filament organization in vertebrate smooth muscle. Phil. Trans. R. Soc. B. 265:223-229.

Sonnenblik, E.H. (1962). Force-velocity relations in mammalian heart muscle. Am. J. Physiol. 202: 931-939.

Stephens, N.L. (1965). The effect of $CO_2$ on length-tension relationships of pulmonary artery smooth muscle. Physiologist 8:280.

Stephens, N.L. (1975). Physical properties of contractile systems. In: Methods in Pharmacology Vol. 3, Smooth Muscle Ed. E.E. Daniel and D.M. Paton, Publ: Plenum Press, New York, pp. 265-296.

Stephens, N.L., R. Cardinal, and B. Simmons (1977). Mechanical properties of tracheal smooth muscle: effects of temperature. Am. J. Physiol. 223(3): C92-C98.

Stephens, N.L., E.A. Kroeger, and J.A. Mehta (1969). Force-velocity characteristics of respiratory airway smooth muscle. J. Applied Physiol. 26:685-692.

Stephens, N.L. and U. Kromer (1971). Series elastic component of tracheal smooth muscle. Am. J. Physiol. 220: 1890-1895.

Wakabayashi, T., and S. Ebashi (1968). Reversible change in physical state of troponin induced by calcium ion. J. Biochem. (Tokyo) 64:731-732.

White, D.C.S. (1978) Muscle Mechanics In: Mechanics and Energetics of Animal Locomotion Ed. Alexander, R.M. and G. Goldspink, London, Chapman and Hall, pp. 23-55.

Widdicombe, J.G. (1966). The regulation of Bronchial calibre. In: Advances in Respiratory Physiology Ed. Caro. London: Edward Arnold (Pub.) Ltd., pp. 48-82.

Wilkie, D.R. (1950). The relation between force and velocity in human muscle. J. Physiol. 110:249-280.

Wilkie, D.R. (1968). Heat, work and phosphorylcreatine breakdown in muscle. J. Physiol., London 195:157-183.

Wyman, J. (1926). Studies on the relation of work and heat in tortoise muscle. J. Physiol. 61:337-352.

Yamauchi, A., and G. Burnstock (1969). Post-natal development of smooth muscle cells in the mouse vas deferens. A fine structural study. J. Anat. 104: 1-5.