The visco-elastic properties of skeletal muscle

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THE VISCO-ELASTIC PROPERTIES OF SKELETAL MUSCLE

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by

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CONTENTS

I SUMMARY

1. The visco-elastic constants of soleus and extensor carpi radialis longus (ECRL) muscles of the rat, gastrocnemius muscles of normal and dystrophic hamsters and gastrocnemius muscles of tortoise have been studied.
2. The transfer function between length and tension for a three component Maxwell model is developed.
3. The response of this model to a ramp stretch is found. During the static phase of the ramp, this is a single exponential decay.
4. Skeletal muscle is compared to the Maxwell model.
5. The model applies only to linear systems. All five muscles are linear, in both relaxed and tetanised states, within the range 0.0mm to 0.26mm stretch.
6. Stress relexation in muscle is shown to be reasonably well represented by the three component model.
7. The constants E_1 , E_2 and V_2 of the model all increase upon stimulation.
8. No differences can be shown between rat soleus and ECRL muscles in respect of their visco-elastic constants.
9. The values of E_1 for normal and dystrophic hamster gastrocnemius muscle, in both the relaxed and tetanised states, are significantly different. The values of E_2 are also different, but not those of V_2 . The isometric tensions and contraction times are significantly different in tetanised muscle.

10. Two other pairs of muscles are compared and differences shown between the values of E_1 and E_2 of tetanised muscle.

11. The values of E_1 , E_2 and V_2 of tortoise gastrocnemius muscle in the relaxed state decreases as the temperature increases, in the range 5°C to 30°C. In tetanised muscle E_1 and V_2 increase with temperature, while E_2 increases in the range 5°C to 20°C then decreases slightly. The isometric tension has a similar relationship to temperature as E_2 .

12. A five component Maxwell model is shown to give a better fit, to the stress relaxation, than the three component model.

13. The findings of the thesis are discussed in relation to current theories of muscle contraction.

II INTRODUCTION

The aim of this thesis is to examine some of the rheological properties of muscle. Although these properties should be considered from both a thermodynamic and mechanical point of view, it must be remembered that one of the functions of muscle is to control a mechanical system. It is necessary to be able to describe the mechanical properties of muscle before attempting a description of the wider, more complicated systems, such as limbs, of which muscles form a part. It is therefore justifiable to set up and examine mechanical models of muscle which may not be thermodynamically acceptable. In the introduction, some historical background is given, and this is followed by a detailed plan of the work. In subsequent sections, skeletal muscle is compared to a three component Maxwell model consisting of one viscous and two elastic elements (see Fig.3.1a). In the theory section the transfer function between length and tension, for imposed ramp length changes, is derived. In the next section the results obtained from the application of ramp stretches to muscle are compared with the model. Different muscle types are then compared in the relaxed and tetanised states and the effects of temperature on the visco-elastic constants of the model are examined. The application of a five component model to muscle is briefly discussed.

Historical background

Several reviews of the properties of skeletal muscle have been written. The biochemical basis of muscle action is well covered by Needham (1971) and the dynamic

properties have been recently reviewed by Close (1972). In 1960 Pringle presented a paper on modelling of muscle. However, the immediate historical background to the present research is presented here.

It has been known for a long time that muscles possess elastic properties. Muller (1837-40) in the early nineteenth century pointed out that muscle obeyed the laws governing elastic bodies, and in 1846 Weber wrote that excited muscle could be looked on as a new elastic body, with new stiffness and new natural length. Heidenhain (1864) and Fick (1882), on the other hand, put forward the view that stimulated muscle was not just a new elastic body, the result of a change in elasticity, but that the force and heat production properties of active muscle were the result of some inner change in the muscle.

However, in 1913 A.V. Hill reverted to the idea that stimulated muscle could be looked upon as a new elastic body, and that the energy of this new elastic body could be liberated either as tension or as heat (see also A.V. Hill, 1913-14). Later, in 1922, A.V. Hill introduced the concept of visco-elasticity playing a large part in determining some of the properties of muscle, for example the speed of contraction. To some extent this overcame the problems of non-reversibility of muscle elasticity. By 1922, then, views were again held that some at least of the properties of muscle could be explained in terms of elasticity and viscosity.

Since then several models have been put forward which attempt to simulate muscle behaviour.

Models of muscle

Levin and Wyman (1927) postulated a model consisting of three components, an undamped elastic element in series with a damped elastic component. This model, it was suggested, gave a better representation of the behaviour of muscle than the A.V. Hill 1922 model, which consisted of a single damped elastic component.

A paper was later published by A.V. Hill (1938) in which he found that the total energy liberation (work + heat of shortening) was a linear function of the load, decreasing as the load increased. The relationship between load, velocity of shortening, work and heat of shortening could be written in the form:

$$(P + a)(v + b) = b(P_0 + a) = \text{constant},$$

where P = actual load on the muscle,

P_0 = isometric tension,

a = shortening heat,

b = increase in total energy,

and v = velocity of shortening.

This equation (Hill's characteristic equation) gave a hyperbolic curve when plotted using values of a and b derived from energy measurements. The same hyperbolic curve could also be obtained by plotting the falling off of the velocity of shortening, v, as the load, P, on the muscle increased.

These findings led A.V. Hill to the conclusion that stimulated muscle could once more no longer be regarded as a new elastic body, that shortening and heat production of stimulated muscle were not due only to viscous and elastic

properties but were the result of the regulation of reactions providing the energy for contraction.

Here, then, is a situation where muscle is seen to have properties which are apparently visco-elastic in nature but may be biochemical in origin. A.V. Hill therefore postulated a model consisting of a contractile component (the damped elastic component of Levin and Wyman) in series with an undamped elastic component.

In 1957 A.F. Huxley published a paper in which he put forward the idea that force generation in a muscle could be the result of two sets of protein filaments, actin and myosin, sliding over one another, with chemical bonds being formed and broken as they pass. This hypothesis has since been substantiated and further developed (e.g. A.F. Huxley & Peachey, 1961; H.E. Huxley, 1964 & 1965). Any subsequent modelling of muscle must take into consideration the work of A.F. Huxley.

Bahler (1968), for example, has postulated a model of muscle which consists of a contractile element, or force generator, having viscous-like properties, in series with an undamped elastic element and with a parallel elastic element associated with the relaxed muscle. This model has been used to simulate isotonic force/velocity relationships and the isometric generation of force by the muscle, but the modelling of force generation is complex.

Study of the three component model

It has been suggested (Crowe, 1970; Apter & Graessly, 1970) that muscle may be represented by a three

component visco-elastic model. Such a model is the simplest which can represent stress relaxation in muscle. Changes in muscle upon stimulation may be simulated by changes in the visco-elastic coefficients, and in the unstretched length of the elastic elements of the model.

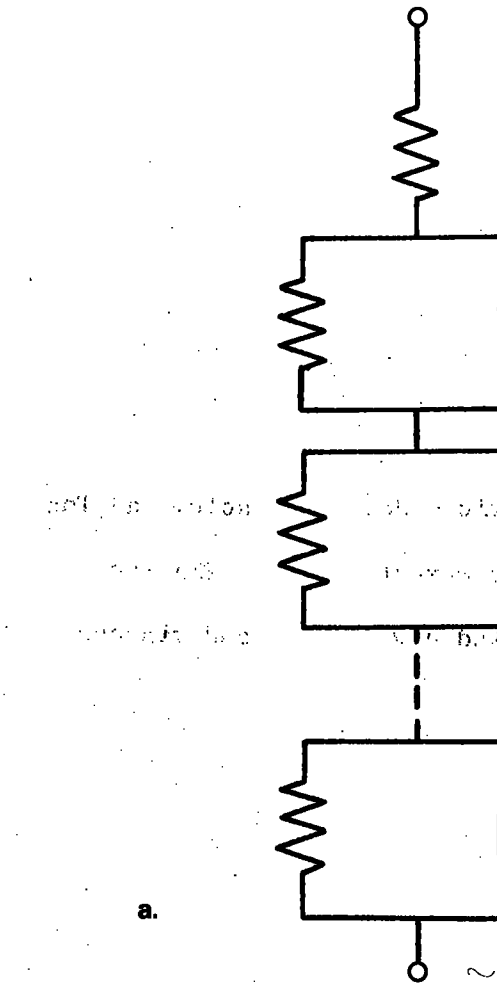
In this thesis such a model has been examined, using stress relaxation techniques, in order to determine whether differences in mechanical properties of muscle can be predicted by changes in the visco-elastic coefficients of the model.

Visco-elastic models of muscle can be represented in two ways (Pringle, 1960), either as a Maxwell model or as a Voigt model (see Fig.2.1). Such models are mathematically equivalent and are not logically distinguishable. Any properties of muscle which can be described by one can also be described by the other. However, in the analysis of stress relaxation, where tension is recorded in response to imposed length changes, the analysis becomes simpler if the Maxwell model is used. All data given in the later sections of the thesis refer to a three component Maxwell model as shown in Fig.3.1a.

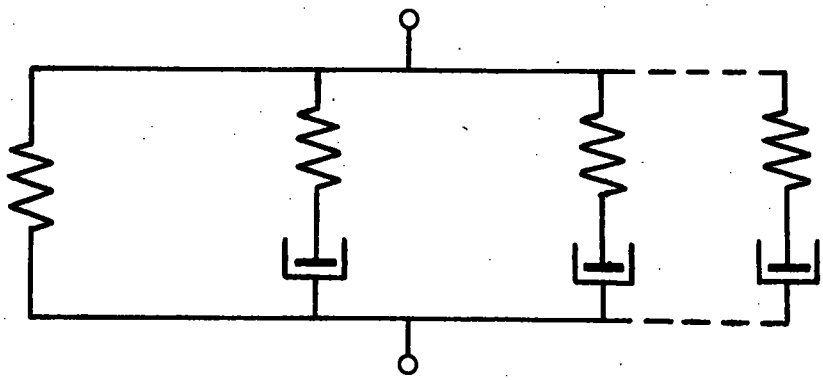
In the Theory section the transfer function of the three component Maxwell model is derived. This transfer function relates tension in the system to changes in length with the form of a ramp stretch (see Fig.3.2), in terms of the viscous and elastic elements of the model. A similar transfer function for a five component model is also derived.

Using the transfer function of the three component model, the limits of linearity have been determined within

Fig. 2.1 Visco-elastic models of muscle. a) The Voigt model. b) The Maxwell model. Elastic elements are represented by  , and viscous elements by  .



a.



b.

which the model can be applied to skeletal muscle. Working within these limits, differences in visco-elasticity between relaxed and tetanised muscle are examined. As already noted, earlier authors have attempted to explain muscle activity simply as a change in elasticity (Weber, 1846; A.V. Hill, 1913). This view has subsequently been shown to be wrong (Fenn, 1924; Wilkie, 1954). However, although activity in muscle is not purely the result of setting up a new elastic body, elasticity of muscle, as measured by stress relaxation, does change upon stimulation, and these changes are discussed in relation to later theories of muscle contraction.

The next topic to be investigated was whether or not differences in contraction properties of muscle (maximum isometric tension and the time taken to contract) are accompanied by differences in the visco-elastic constants of the model. Since A.V. Hill (1922) introduced the idea that the speed of contraction of a muscle is related to the visco-elastic properties of that muscle, several workers have looked for some correlation between speed of contraction and elastic properties (Buller et.al., 1960a & b). Wells (1965) and Close (e.g. 1965), in particular, have done much work on the comparison of fast and slow muscles of the rat. Two muscles of the rat, the soleus and the extensor carpi radialis longus (ECRL), have been compared in this thesis. In addition the gastrocnemii muscles from normal and dystrophic hamsters have also been compared.

If the visco-elastic constants of muscle are related to the contractile properties, how then will these constants relate to temperature? Several authors have looked at the effects of temperature on passive and active muscle (e.g.

Washington et al., 1955; Close & Hoh, 1968; Yeatman et.al., 1969; D.K. Hill, 1970). The effects of temperature on the properties of tortoise gastrocnemius muscle have been examined and these results are discussed in relation to the other findings of this thesis and to previously published work.

Finally, the results are discussed in relation to current theories of muscle contraction and possible improvements to the model are suggested.

III THEORY

1. The three component model

A Maxwell model consisting of two elastic elements, E_1 and E_2, and a viscous element, V_2, as shown in Fig. 3.1a, was used.

If this model is regarded as a linear system, then the transfer function, G, can be derived for tension changes, F, in terms of imposed length changes, L.

This model contains a time-dependent viscous element. The derivation of the transfer function therefore includes first order differential equations.

In terms of the notation of the Laplace Transform,

$$G(S) = \frac{F(S)}{L(S)}. \qquad 3.1$$

The tension generated is the sum of the tensions generated by the elastic element, E_1, and by the visco-elastic element containing E_2 and V_2. This can be expressed as

$$F(S) = F_1(S) + F_2(S). \qquad 3.2$$

1.1 The parallel elastic element

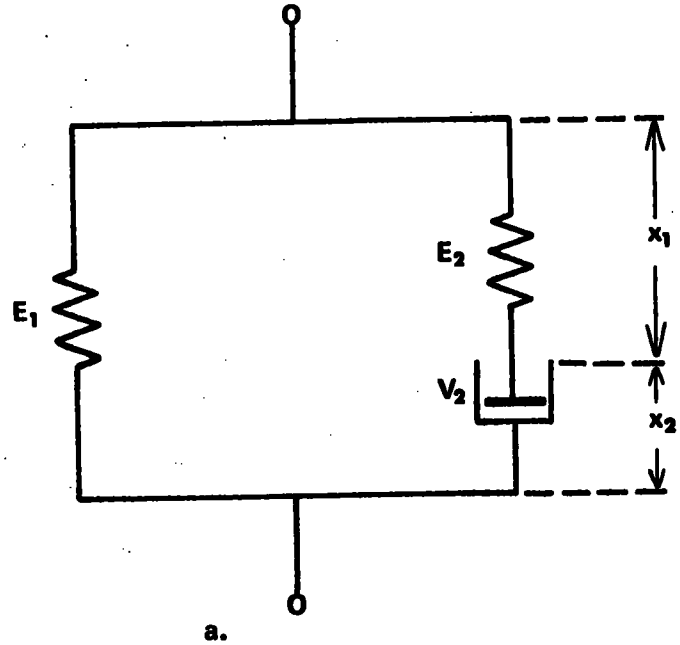
The tension generated by this element is the product of the modulus of elasticity and the imposed length change.

$$F_1(S) = E_1 L(S). \qquad 3.3$$

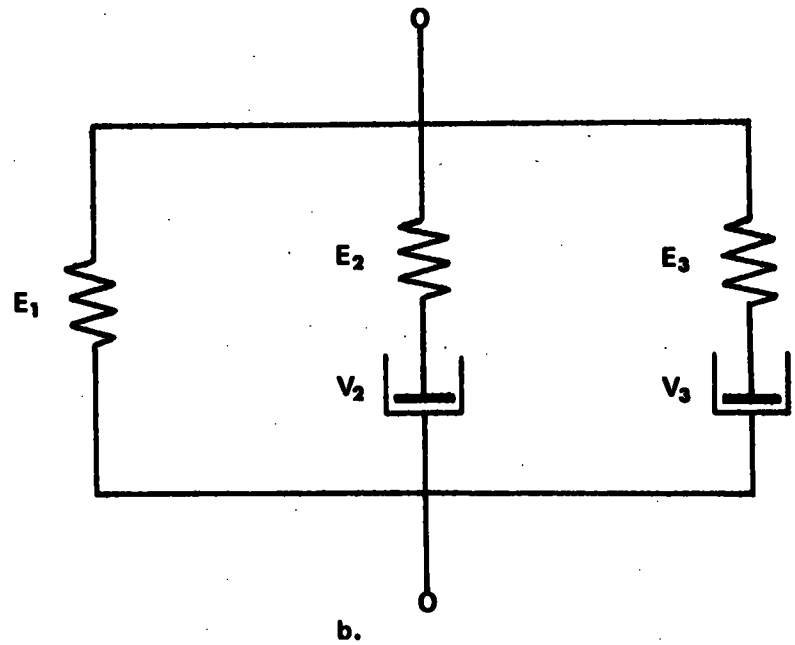
Fig. 3.1a The three component Maxwell model, consisting of a parallel elastic element, E_1 , and an elastic element, E_2 , in series with a viscous component, V_2 . In this system the overall length, L, is equal to the sum of the lengths of the series elastic element and the viscous element, that is

$$L = X_1 + X_2$$

Fig. 3.1b The five component Maxwell model, consisting of a parallel elastic element, E_1 , and two visco-elastic elements (E_2 , V_2 and E_3 , V_3).



a.



b.

1.2. The visco-elastic element

The change in length, L, consists of two components, the change in length (X_1) applied to E_2 and the change in length (X_2) applied to V_2 (see Fig. 3.1), thus:

$$L(S) = X_1(S) + X_2(S) \qquad 3.4$$

and $$F_2(S) = E_2 X_1(S) = V_2 S X_2(S). \qquad 3.5$$

From equations 3.4 and 3.5, the tension change, $F_2(S)$, can be found in terms of the length change, L (S).

$$F_2(S) = E_2 \{L(S) - X_2(S)\}$$

and $$F_2(S) = V_2 S X_2(S).$$

When $X_2(S)$ is eliminated, this gives

$$F_2(S) = E_2 L(S) - \frac{E_2 F_2(S)}{V_2 S}$$

or $$F_2(S) = \frac{E_2 V_2 S}{E_2 + V_2 S} \cdot L(S). \qquad 3.6$$

1.3 The transfer function

The transfer function of the three component Maxwell model can now be obtained by substituting equations 3.3 and 3.6 in equation 3.2, giving

$$F(S) = E_1 L(S) + \frac{E_2 V_2 S}{E_2 + V_2 S} \cdot L(S),$$

which in turn gives

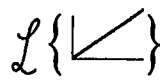
$$G(S) = \frac{F(S)}{L(S)} = \left\{E_1 + \frac{E_2 V_2 S}{E_2 + V_2 S}\right\}. \qquad 3.7$$

1.4 Imposed length changes

If the applied mechanical stretch is in the form of a ramp of height H, and of dynamic phase duration, T, as shown in Fig. 3.2, then the Laplace transform for the length change, L(S), can be derived.

1.41 Dynamic phase (0 < t < T)

The Laplace transform of a ramp starting at time t = 0, of unit height and of slope $\frac{1}{T}$ is as follows:

$$L(S) = \mathcal{L}\left\{\text{  }\right\} = \frac{1}{TS^2}. \qquad 3.8$$

1.42 Static phase (T < t)

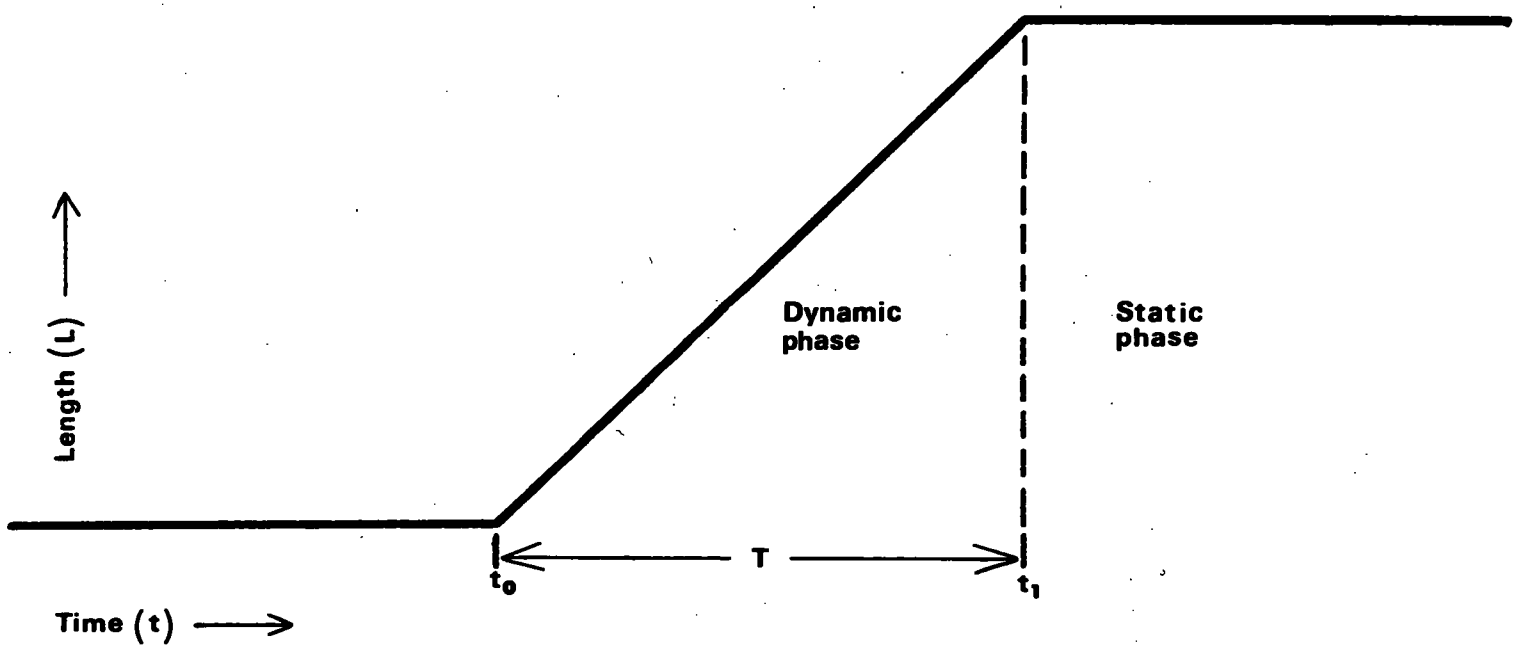
The Laplace transform of a second ramp, similar to that of equation 3.8 but starting at time t = T, can be derived:

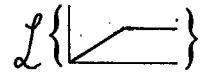
$$\mathcal{L}\left\{\text{  }\right\} = \frac{1}{TS^2} \cdot e^{-ST}. \qquad 3.9$$

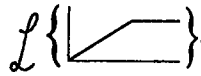
1.43 Ramp stretch

Equation 3.9 can be subtracted from equation 3.8 to give the Laplace transform of a ramp of the form given in Fig. 3.2,

Fig. 3.2 The ramp length change (L) applied to skeletal muscle. The ramp applied was of height, H, and consisted of two phases, a dynamic phase, of T seconds duration, where the length changed with constant velocity, and a static phase, where the length remained constant at the new value.


Length (L)
Dynamic phase
Static phase
T
t_0
t_1
Time (t)

$$\mathcal{L}\left\{\right.\text{  }\left.\right\} = \frac{1}{TS^2} - \frac{1}{TS^2}\cdot e^{-ST}$$

or $$L(S) = \mathcal{L}\left\{\right.\text{  }\left.\right\} = \frac{1}{TS^2}\cdot(1 - e^{-ST}). \qquad 3.10$$

1.5 Tension changes as a result of an imposed ramp stretch.

It is now possible to derive the transfer function for changes in tension, F, in the model, in terms of an imposed ramp stretch of height, H.

1.51 During the dynamic phase

From equations 3.7 and 3.8,

$$F(S) = \frac{1}{TS^2}\cdot\left\{E_1 + \frac{E_2V_2S}{E_2 + V_2S}\right\}.$$

Taking the inverse Laplace transform, the tension resultant from an applied ramp stretch of height, H, would be

$$F(t) = \frac{E_1H}{T}\cdot t + \frac{V_2H}{T}\cdot\left\{1 - \exp\left[-\frac{E_2}{V_2}\cdot t\right]\right\}. \qquad 3.11$$

1.52 During the static phase

From equations 3.7 and 3.10

$$F(S) = \frac{1 - e^{-ST}}{TS^2}\cdot\left\{E_1 + \frac{E_2V_2S}{E_2 + V_2S}\right\}.$$

Taking the inverse Laplace transform, the tension resultant from an applied ramp stretch of height, H, would be

$$F(t) = E_1 H + \frac{V_2 H}{T}\left\{\exp\left[-\frac{E_2}{V_2}\cdot t\right]\right\}\left\{\exp\left[\frac{E_2}{V_2}\cdot T\right] - 1\right\},$$

or, if t is measured from the start of the static phase (t_1 of Fig. 3.2), then

$$F(t) = E_1 H + \frac{V_2 H}{T}\left\{\exp\left[-\frac{E_2}{V_2}\cdot t\right]\right\}\left\{1 - \exp\left[-\frac{E_2}{V_2}\cdot T\right]\right\}. \qquad 3.12$$

Equation 3.12 can be expressed in the simplified form

$$F(t) = A + B.\exp\left[-Ct\right], \qquad 3.13$$

where $A = E_1 H,$

$$B = \frac{V_2 H}{T}\left\{1 - \exp\left[-\frac{E_2}{V_2}\cdot T\right]\right\},$$

and $$C = \frac{E_2}{V_2}. \qquad 3.14$$

2. The five component model

Consider a Maxwell model, similar to that of Fig. 3.1a but consisting of three elastic and two viscous elements, arranged as in Fig. 3.1b. In terms of the Laplace transform the transfer function relating length to tension, G(S) of equation 3.1, can be derived.

The tension, F(S), generated in the model is the sum of the forces in the elastic element and the two visco-elastic elements,

$$F(S) = F_1(S) + F_2(S) + F_3(S) \qquad 3.15$$

The relationship between the tension generated, F(S), and the imposed length change, L(S), is the same for both visco-elastic elements,

$$F_2(S) = \frac{E_2 V_2 S}{E_2 + V_2 S} \cdot L(S) \qquad 3.6$$

and

$$F_3(S) = \frac{E_3 V_3 S}{E_3 + V_3 S} \cdot L(S) \qquad 3.16$$

2.1 The transfer function

The transfer function of the five component Maxwell model can be obtained by substituting equations 3.3, 3.6 and 3.16 in equation 3.15 to give

$$F(S) = E_1 L(S) + \frac{E_2 V_2 S}{E_2 + V_2 S} \cdot L(S) + \frac{E_3 V_3 S}{E_3 + V_3 S} \cdot L(S).$$

This equation can be rearranged to give

$$G(S) = \frac{F(S)}{L(S)} = E_1 + \frac{E_2 V_2 S}{E_2 + V_2 S} + \frac{E_3 V_3 S}{E_3 + V_3 S} \qquad 3.17$$

2.2 Changes in tension as a result of an imposed ramp stretch

The transfer function for changes in tension, F, in terms of an imposed ramp stretch can be derived.

2.21 During the dynamic phase

From equations 2.8 and 2.17,

$$F(S) = \frac{1}{TS^2}\left\{E_1 + \frac{E_2V_2S}{E_2 + V_2S} + \frac{E_3V_3S}{E_3 + V_3S}\right\}.$$

Taking the inverse Laplace transform, the tension resultant from an applied ramp stretch of height H, would be

$$F(t) = \frac{1}{TS^2}\cdot t + \frac{V_2H}{T}\left\{1 - \exp\left[-\frac{E_2}{V_2}\cdot t\right]\right\} + \frac{V_3H}{T}\left\{1 - \exp\left[-\frac{E_3}{V_3}\cdot t\right]\right\}. \qquad 3.18$$

2.22 During the static phase

From equations 3.10 and 3.17,

$$F(S) = \frac{1 - e^{-ST}}{TS^2}\left\{E_1 + \frac{E_2V_2S}{E_2 + V_2S} + \frac{E_3V_3S}{E_3 + V_3S}\right\}.$$

Taking the inverse Laplace transform, the tension resultant from an applied ramp stretch of height H, would be

$$F(t) = E_1H + \frac{V_2H}{T}\left\{\exp\left[-\frac{E_2}{V_2}\cdot t\right]\right\}\left\{\exp\left[\frac{E_2}{V_2}\cdot T\right] - 1\right\}$$

$$+ \frac{V_3H}{T}\left\{\exp\left[-\frac{E_3}{V_3}\cdot t\right]\right\}\left\{\exp\left[\frac{E_3}{V_3}\cdot T\right] - 1\right\},$$

or, if t is measured from the start of the static phase (point t_1 of Fig. 3.2) then

$$F(t) = E_1H + \frac{V_2H}{T}\left\{\exp\left[-\frac{E_2}{V_2}\cdot t\right]\right\}\left\{1 - \exp\left[-\frac{E_2}{V_2}\cdot T\right]\right\} \qquad 3.19$$

$$+ \frac{V_3H}{T}\left\{\exp\left[-\frac{E_3}{V_3}\cdot t\right]\right\}\left\{1 - \exp\left[-\frac{E_3}{V_3}\cdot T\right]\right\}.$$

Equation 3.19 can be put into the simplified form

$$F(t) = A + B.\exp[-Ct] + D.\exp[-Et] \qquad 3.20$$

where

$$A = E_1H$$

$$B = \frac{V_2H}{T}\left\{1 - \exp\left[-\frac{E_2}{V_2}\cdot T\right]\right\}$$

$$C = \frac{E_2}{V_2}$$

$$D = \frac{V_3H}{T}\left\{1 - \exp\left[-\frac{E_3}{V_3}\cdot T\right]\right\}$$

and

$$E = \frac{E_3}{V_3}. \qquad 3.21$$

IV MATERIALS AND METHODS

1. Animal preparations.

1.1 Rat

Young albino rats having a body weight of 170g to 230g. were used. They were initially aneasthetised with Urethane (Ethyl Carbamate - B.D.H.) in normal saline, 200mg/100g of body weight, injected intraperitoneally (Wells, 1965). This was subsequently supplemented with ether, during the course of the experiment.

The muscle, which was either the soleus or the extensor carpi radialis longus (ECRL), was arranged so that its tendon could be isolated and attached to the stretching apparatus. The fascia and connective tissues were removed to allow freedom of movement (Denny-Brown, 1929), yet the blood supply and proximal insertion remained intact. A length of nerve sufficient to allow tetanic stimulation by external electrodes was left attached to the muscle. All unused branches of the appropriate nerve were cut. The tendon was attached to the stretching apparatus by a length of pre-stretched stainless steel wire.

The animal was placed in a holding frame and rigidly held at the joint appropriate to the muscle under investigation, so that the tensions in the muscle produced no movements in the limb. The initial length of the muscle was set at about resting length so that, upon the application of the mechanical stretch in the unstimulated state, there

was no slack to be taken up before a tension change could be observed. This was in the range 2.6cms - 2.8cms for soleus muscle, and 1.4cms - 1.5cms for ECRL muscle. A rectal temperature of 37°C - 39°C was maintained during each experiment.

Stimuli to the nerve were provided by a Devices Mk.III isolated stimulator which was triggered by a gated pulse generator (Devices, 2521). The frequency of stimulation applied to the soleus muscles was 40 pulses per second and to the ECRL muscles, 65 pulses per second. In both cases an interval of two minutes was allowed between successive cycles of stretch or stimulation.

1.2 Hamster

A non-dystrophic, commercially available line of Syrian hamster (Mesocricetus auratus) was used for the study of normal muscle. These animals were between 80 and 90 days old and the body weight of each was in the range 90-100g. Experiments on dystrophic muscle were performed on animals from a dystrophic in-bred line of hamsters (B 14.6 strain from Bioscience Laboratories, Bar Harbor, U.S.A.), first described by Holmburger (Holmburger et al. 1962a & b). These animals were 80 - 95 days old and the body weight of each was between 65 and 90g.

The gastrocnemius muscles of each animal were set up as in section IV.1.1. Care was taken to separate this muscle from the underlying plantaris and soleus muscles. The muscles were initially set at about resting length. This was 1.3cms in normal muscle and 1.2cms-1.3cms in dystrophic muscle. A rectal temperature of 36°C to 38°C was maintained throughout each experiment.

Stimuli were applied to the sciatic nerve at a frequency of 50 pulses per second for both normal and dystrophic muscle. A two minute rest period was allowed between successive cycles of stretch or stimulation, as for rat muscle.

Each muscle was subsequently sectioned and stained for general structure using Weigert/van Geisen staining technique (Carlton & Drury, 1957). Phosphorylase activity was also checked by the method of Takeuchi & Kuriaki (1955), as modified by Eranko & Palkama (1961).

1.3 Tortoise

Adult tortoises (Tustedo graeca) were pithed and the gastrocnemius muscle removed, together with a length of the sciatic nerve. This preparation was mounted in a muscle bath and immersed in Ringer's solution. The composition of this solution in g. per litre was NaCl, 6.5; KCl, 0.15; $CaCl_2.2H_2O$, 0.16; $NaHCO_3$, 0.5; (Seliskar, 1926). The Ringer's solution was aerated and circulated by a stream of gas consisting of 95% oxygen and 5% carbon dioxide (see Fig.4.1).

The distal tendon of the muscle was clamped firmly to the base of the muscle bath. The proximal end of the muscle, with a short section of the femur, was then attached to the stretching apparatus by a length of previously stretched stainless steel wire.

Fig. 4.1 The temperature control system used in tortoise in-vitro experiments.

A. Gas cylinder, with reduction valve, containing 95% oxygen and 5% carbon dioxide.

B. Perspex muscle bath, containing Ringer's solution.

C. Bubbler mechanism for circulating and aerating the Ringer's solution.

D. Force transducer (attached to spindle of the vibration generator).

E. Thermometer.

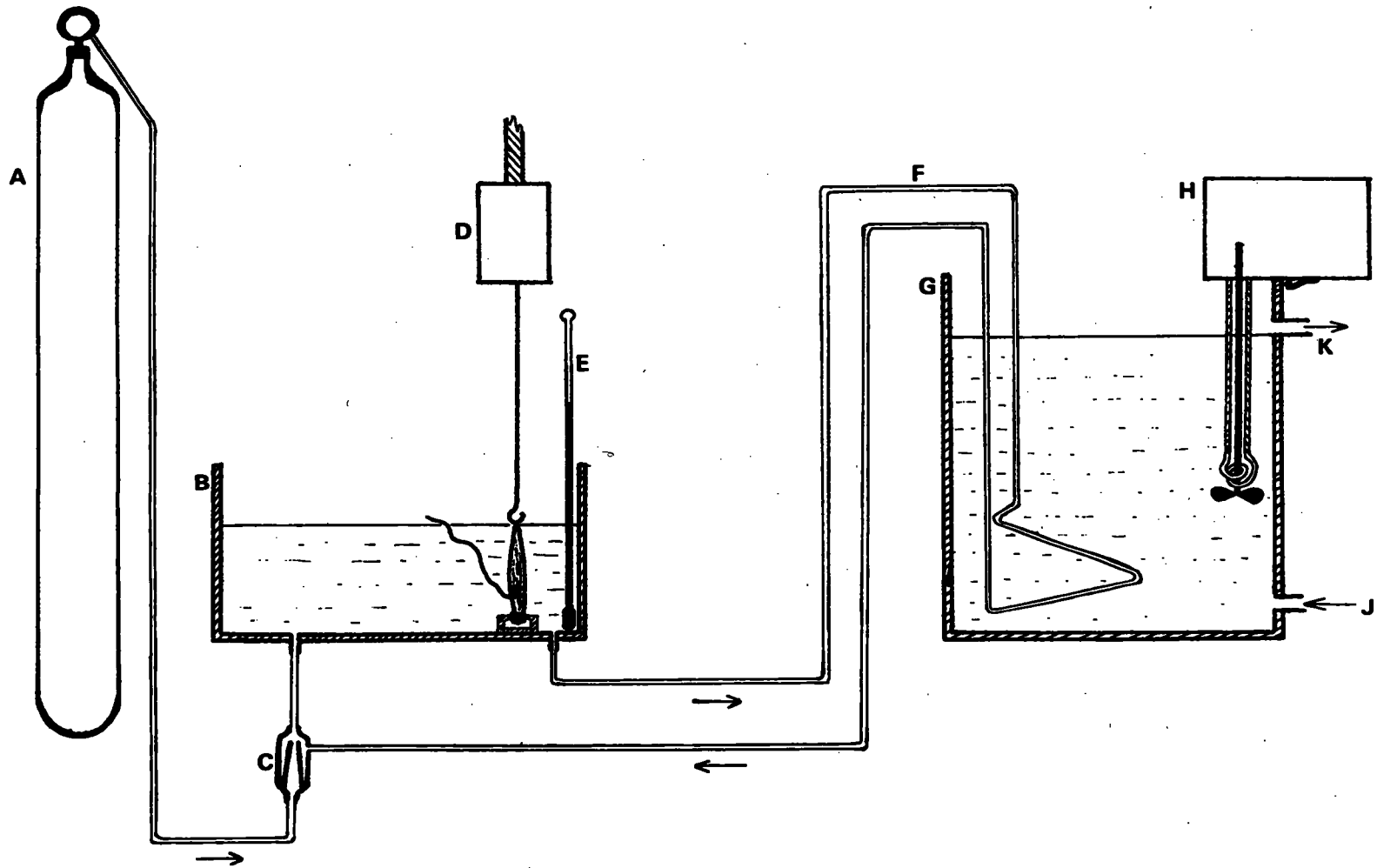
F. P.V.C. tubing for circulation of Ringer's solution in temperature control bath.

G. Temperature control bath.

H. Tempette thermostatically controlled heating unit.

J. Inlet from cold water tap.

K. Outlet to sink.


A
B
C
D
E
F
G
H
J
K

The initial length of the muscle was set at about resting length, which was about 3.4cms in each case.

Stimuli were applied to the nerve at a frequency greater than that required to produce complete tetanus at 30°C. This frequency was 30 pulses per second. The interval between successive cycles of stretch or stimulation was not constant, as for rat and hamster muscles, since the time required to cool the muscle through 5°C was not constant. In no case was this interval of time less than the two minutes allowed for rat and hamster muscle.

2. Apparatus

2.1 The stretching apparatus

The muscle was fixed to the movable spindle of an electromagnetic vibration generator (Goodmans V50 Mk.I). Movement of the spindle was brought about by feeding the voltage signal from a testwave generator (Servomex LF 141) into a current amplifier, the output of which was fed into the coil of the vibration unit. This movement was recorded by a Honeywell LD 11 Linear Displacement Transducer.

The output of the displacement transducer was subtracted from the command signal of the waveform generator in order to achieve a feedback control of the stretch applied to the muscle. A filter was introduced into the loop

to damp out the natural resonant frequency of the system. These techniques allowed the input signal to be accurately followed by the spindle of the vibration generator.

Some problems were encountered with the stability of the system. In particular, oscillations were set up in the vibration unit if the gain of the differential amplifier was too great. This could, to some extent, be prevented by adjustment of the preamplifier gain and the balance of the current amplifier.

At times the testwave generator tended to an instability, which resulted in the vibration unit moving to the stretched position without any external trigger pulse being applied. The circuit of the testwave generator was checked, but no fault found. Triggering, in this case, was probably caused by fluctuations in mains voltage. Experiments carried out at night did not suffer from this particular problem.

2.2 Recording of data

2.2.1 Methods

Tension changes in the muscle were recorded by an Ether UFI Load Transducer placed between the muscle and the spindle of the vibration unit. The outputs of the load transducer and the displacement transducer were simultaneously displayed on a Tektronix 502A dual beam oscilloscope. Records of length and tension changes in the muscle were made by taking single frame 35mm photographs of the oscilloscope display. Measurements were taken from these photographs for subsequent analysis.

Length and tension signals of the in-vitro tortoise experiments were also recorded on an Ampex 3000 FM tape recorder and subsequently analysed on an IBM 1132 computer.

The events of the testwave generator, camera, tape recorder and stimulator were triggered by timed pulses from a Digitimer, type 3290.

A block diagram of the apparatus is given in Fig. 4.2.

2.2.2 Accuracy of length change measurements

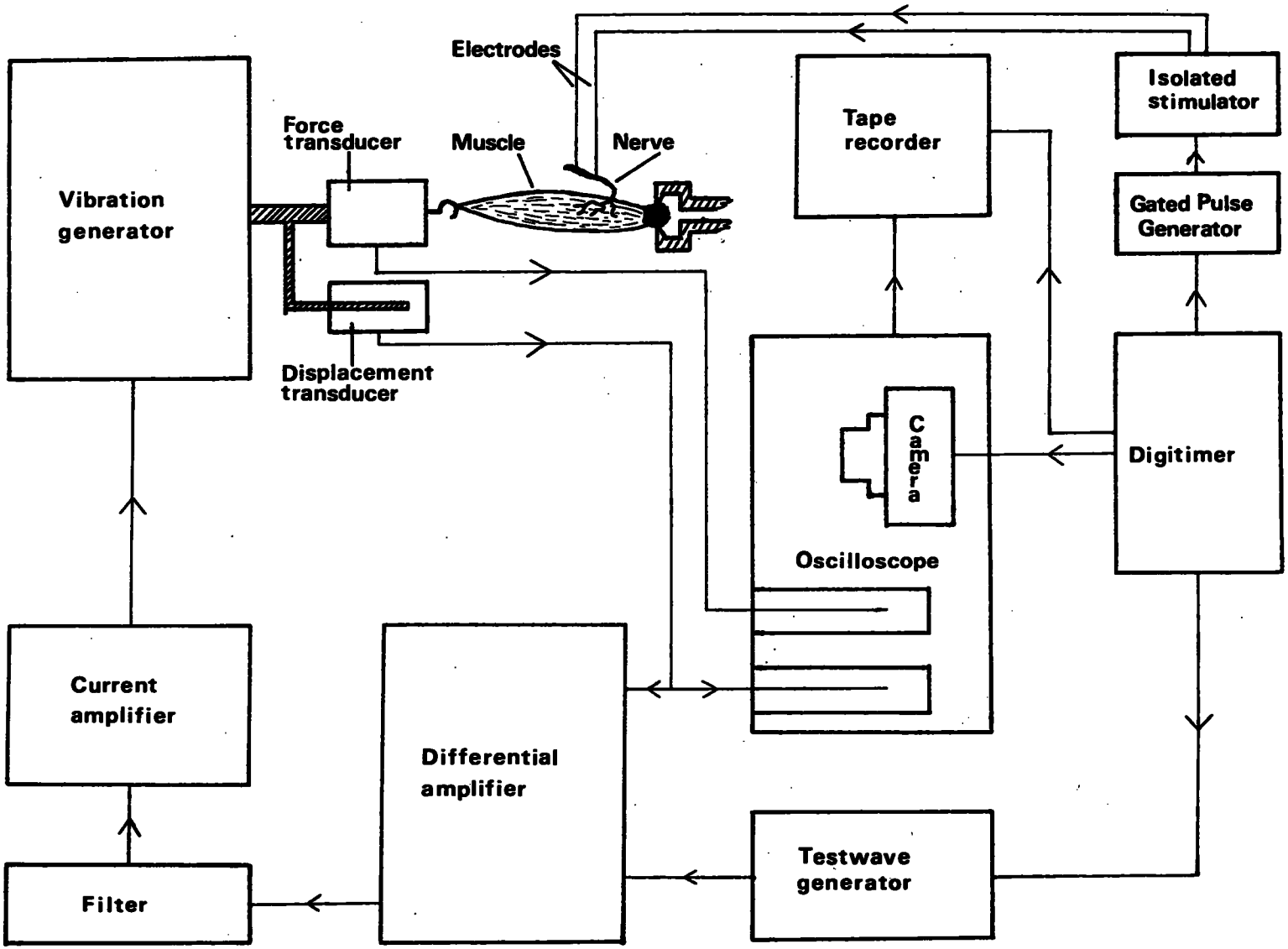
The output of the length transducer was checked against a vernier gauge after each day's experiments and a calibration curve drawn. The output voltage was linear over the range of movement applied to the muscles. The ramp height for any particular input signal could be determined to within ±0.005mm. This represents an error of about 5% for the smallest stretch and about 0.6% for the largest.

2.2.3 Accuracy of tension measurements

Photographs of oscilloscope displays were projected onto graph paper, and measurements of tension changes taken to the nearest 0.5mm. The smallest tension changes recorded gave movements corresponding to a distance of 20mm when projected. Tension could therefore be measured to within ±2.5% of the smallest forces. Large tension changes were accurate to within ±0.15%.

Analogue tension changes recorded on magnetic tape were digitised and stored within a computer. The

Fig. 4.2 Block diagram of stretching and recording apparatus.


Electrodes
Force transducer
Muscle
Nerve
Vibration generator
Tape recorder
Isolated stimulator
Gated Pulse Generator
Displacement transducer
Camera
Digitimer
Oscilloscope
Current amplifier
Differential amplifier
Testwave generator
Filter

tension intervals for data from tetanised muscle were 4.5 x 10^{-3} N, representing an error of 1% of the smallest tension change recorded and 0.15% of the largest. Tension changes in relaxed muscle were digitised with intervals of 4.5 x 10^{-4} N. This represents a possible error in the range 0.5 - 2% of the forces measured.

2.3 Temperature regulation (tortoise)

The Ringer's solution in the muscle bath of the in-vitro tortoise experiments was constantly circulated through a temperature control unit (see Fig. 4.1.). A stream of 95% oxygen and 5% carbon dioxide was used both to circulate and to aerate the Ringer's solution. A Tempette heating unit was used in conjunction with cold tap water to regulate the temperature. Using this method, the temperature of the saline in the muscle bath could be controlled to within plus or minus 0.25°C.

3. Methods of Analysis

3.1 Amplitude and velocity of stretch

It has been shown by Gasser & Hill (1924) and by Abbott & Aubert (1952) that contracting muscle subjected to stretch may be irreversibly damaged, and any tension development subsequent to the stretch is then reduced.

The length of stretch applied to muscle by Gasser & Hill to produce this effect is not clear, but by comparing Figs. 4 and 8 of these authors, an estimate of 10mm can be obtained. The velocity of the applied

stretch was then in the range 8 - 200cms sec^{-1}. Abbott & Aubert applied stretches of 4 - 5mm at a velocity of 0.05 - 3.0cms sec^{-1}.

At the higher velocities of stretch applied by the above authors, a slip was induced, giving a reduction in the tension developed by the muscle. These stretches were probably outwith the elastic limits of the cross-bridges (Sugi, 1972).

In the present study, the maximum amplitude of stretch was 0.85mm and the velocity did not exceed 1.4cms sec^{-1}. These stretches were not great enough to induce the slip phenomenon (a form of which is given in Fig. 4.3a and b) and were probably within the elastic limits of the cross bridges (see Discussion).

Stretching muscle at the amplitude and velocities used in the present experiments did not usually result in any subsequent reduction in isometric tension. The muscles were therefore not being damaged. If any reduction in tension did occur, the results were discarded.

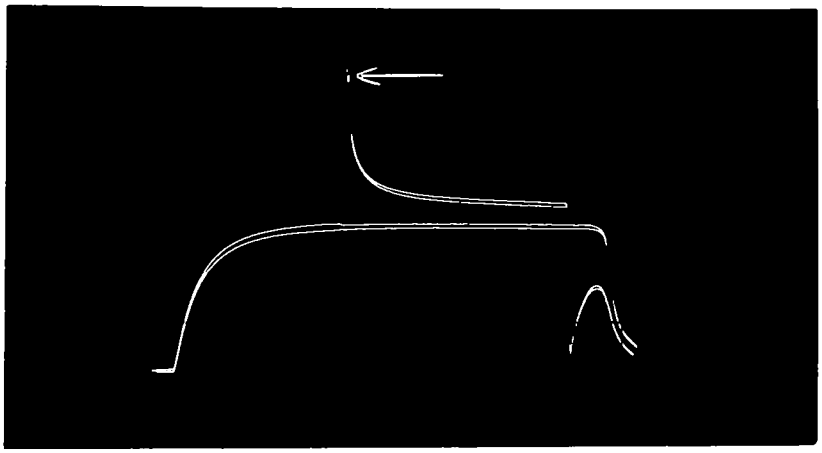
3.2 Linearity

The relaxed muscle was tested for linearity by applying a series of ramp stretches of varying height, H, but of constant dynamic phase duration, T. The resultant tensions were measured at points during the static phase of the ramp. The range of linearity was then determined from the Height/Tension graph.

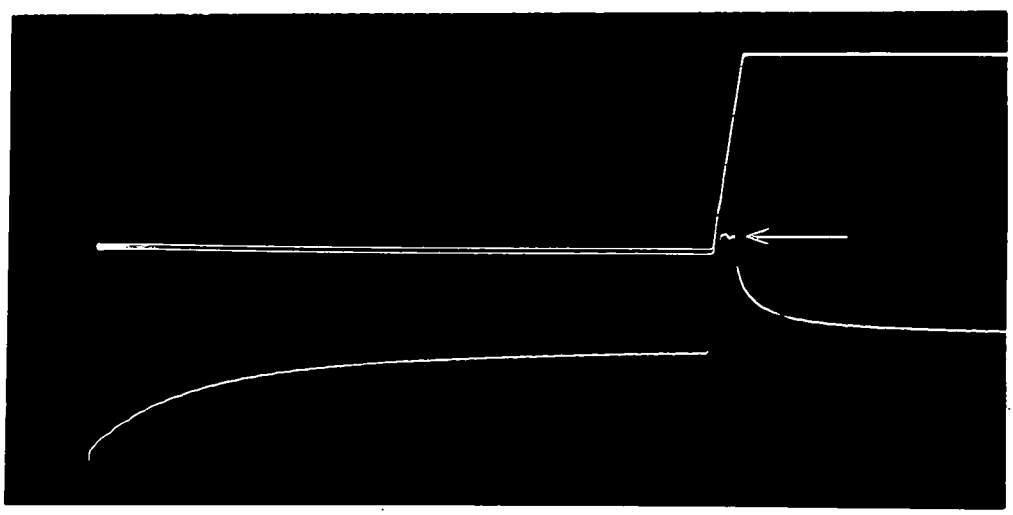
The tests were repeated for muscle in the stimulated state. The muscles were subjected to ramp stretches while undergoing tetanic contraction, and from

Fig. 4.3a Tension produced during a ramp stretch of 0.85mm, applied to tetanised rat soleus muscle, at a velocity of 13.60 mm sec^{-1} . The 'slip' phenomenon, probably similar to that of Sugi (1972), is indicated by an arrow.

Fig. 4.3b Length (upper trace) and tension (lower trace) during a ramp stretch of 0.34 mm applied to tortoise gastrocnemius muscle at 15°C. The stretch velocity of 10.20 mm sec^{-1} has induced a tension slip (indicated by an arrow), which is probably similar to that of Sugi (1972).



a.



b.

these tensions the corresponding values of an unstretched contraction were subtracted. This gave the amount of tension due to the mechanical stretch.

3.3 Static and dynamic elasticity

The difference between tension at the end of the static phase of the ramp, and tension in the absence of a ramp is defined as the static tension change and is equivalent to A of equation 3.13. The amount of static tension change per unit of stretch is the static elasticity, and is given in units of Nm^{-1}.

Similarly, the difference between the peak tension at the end of the dynamic phase of a ramp and the subsequent steady, or near steady, tension at the end of the static phase, is defined as the dynamic tension change. This is equivalent to B of equation 3.13. The amount of dynamic tension per unit of stretch is the dynamic elasticity and is given in units of Nm^{-1}.

These quantities have been used in the discussion to relate the results of this thesis to the model of muscle first put forward by A.F. Huxley (1957).

3.4 $\frac{1}{2}$ and $\frac{3}{4}$ - decay times

The time taken for the tension in a muscle to fall from the peak value at the end of the dynamic phase to the point where the tension is equal to $(A+\frac{1}{2}B)$ is termed the half-decay time of the muscle. The equivalent time for the tension to fall to $(A+\frac{1}{4}B)$ is defined as the $\frac{3}{4}$ - decay time.

3.5 Determination of E_1 E_2 and V_2

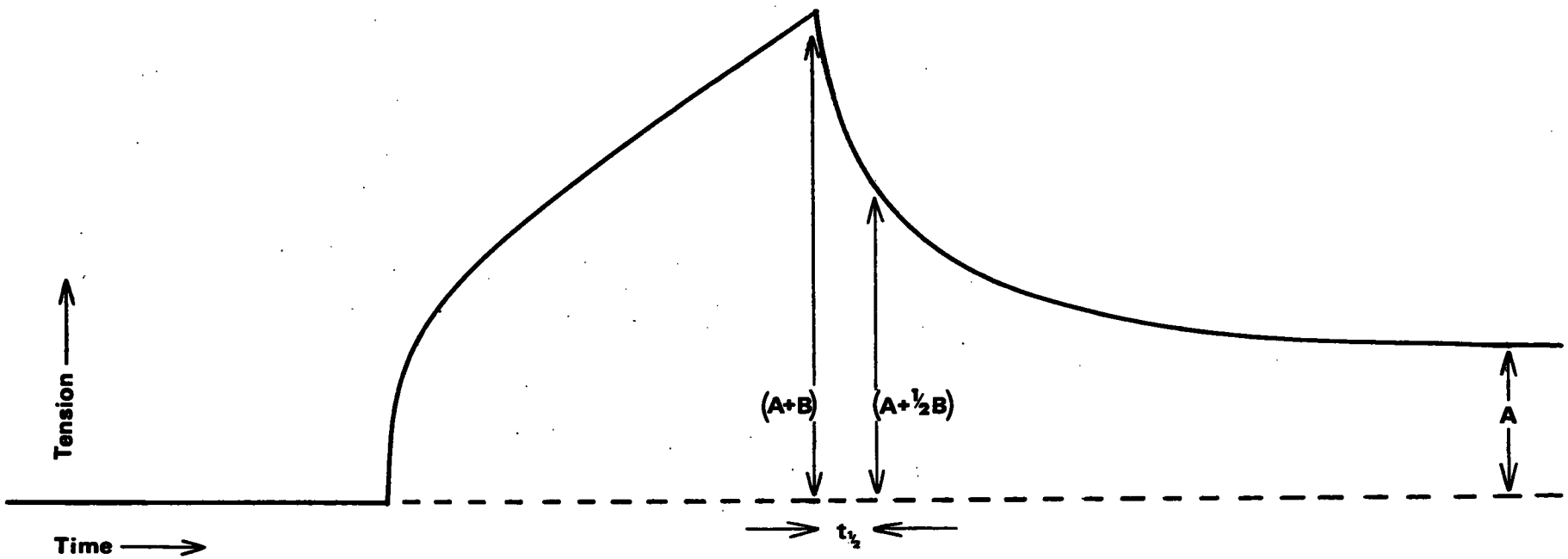
3.5.1 Rat and hamster muscle

In order to determine the values of the viscous and elastic components, and to test the model, the tension records of ramps of a height within the linear range were used. The dynamic phase, T, of stretches applied to the muscles was of 0.0625 seconds duration. Initially it was assumed that the component of the tension due to the ramp stretch could be fitted to the curve of equation 3.13. The plateau tension at the end of the static phase of the ramp was measured to give the value of A. The peak tension (that is, the tension at the end of the dynamic phase) was measured to obtain the value of B. The value of the half-decay time was used to determine C, as in Fig. 4.4. The values of E_1, E_2 and V_2 were then determined according to equation 3.14.

3.5.2 Tortoise muscle

Values of the visco-elastic constants for tortoise muscle were determined by applying ramp stretches with a height, H, within the linear range and of dynamic phase duration, T, of 0.0625 seconds. Analogue tension data from these experiments were stored on magnetic tape. A fortran computer program was written to receive the data from the Ampex tape recorded, digitise it by means of an interface (WDV, Munchen), and store the resultant information on a magnetic disc in the computer. A second fortran program was written to perform a least squares fit

Fig. 4.4 Measurements taken in order to determine the constants A, B and C of equation 3.13. $C = \frac{-\ln \frac{1}{2}}{t_{\frac{1}{2}}}$.


Tension
Time
(A+B)
(A+½B)
A
t½

of the data of the static phase of the ramp, for the curve of equation 3.13 (see section 9.1 on curve fitting). This gave the constants A, B and C of equation 3.13, and hence the values of E_1, E and V for the three component model.

A second least squares fit of the data was found for a curve of the form given by equation 3.20 (see section 9.2 on curve fitting). This gave the five constants of equation 3.20 and hence the values of E_1, E_2, E_3, V_2 and V_3 of the five component model, from equation 3.21.

4. Comparison of results with the model

4.1 Rat and hamster muscle

The fitted curve was compared to the experimental tension records in order to determine how well the experimental curve was an approximation to a single exponential. The fitted curve was obtained by plotting the curve $A + B.\exp(-Ct)$, using derived values of A, B and C.

4.2 Tortoise muscle

The experimental tension was compared to a curve consisting of a single exponential, $A + B.\exp(-Ct)$ and to a curve consisting of the sum of two exponentials, $A + B.\exp(-Ct) + D.\exp(-Et)$.

5. Muscle length and isometric force generation

5.1 The initial length of muscle

The resting length at which each muscle was initially set was near to the maximum in situ length of that muscle. This length corresponds to a position on the

length/tension plot of Gordon et al (1965, fig.12) near to a sarcomere length of about 2.0 μm. At this position, tension changes which were due only to moving the muscle onto a different part of the length/tension plot, would not be expected to be very great in response to a small change in length.

5.2 Consistency and performance of preparations

In order to ensure consistency of performance of the stimulated muscle, each ramp stretch applied to tetanised muscle was preceded and followed by tetanic stimulation without stretch. If these two records were identical and showed no signs of fatigue, it was assumed that the behaviour of the muscle was also acceptable during the intervening contraction subjected to a ramp stretch.

6. Determination of the constants of isometric contraction.

6.1 The maximum tetanic tension

The values of the maximum isometric tetanic tension given in the Results were taken as the maximum tension above resting tension, developed by the muscle during the period of stimulation. In the case of rat soleus and tortoise gastrocnemius muscles, the isometric tension was still increasing slowly at the end of the period of stimulation. The maximum tension given for these muscles is the tension developed just before the removal of the stimulus. The maximum tension given for these two muscles is therefore slightly less than the

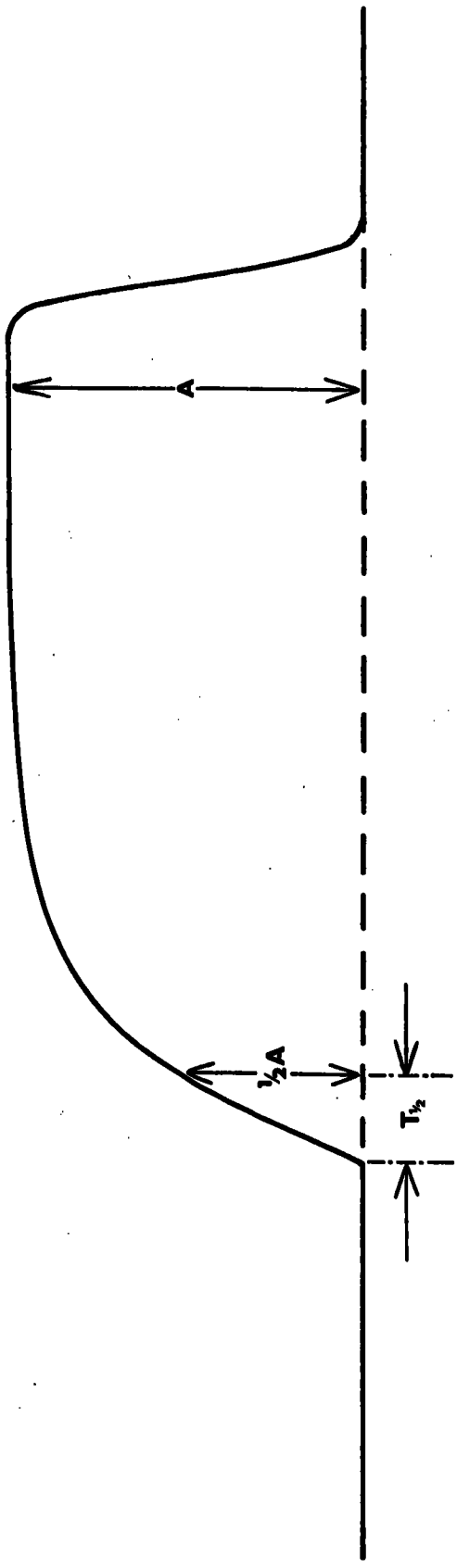
muscle is capable of developing at that particular length.

The tension developed by rat ECRL and by hamster gastrocnemius muscles reached a maximum a few milliseconds after the onset of stimulation, and then declined slightly with continued stimulation. The values of maximum isometric tension given for these muscles are the maximum which could be developed at that particular length of the muscle.

6.2 The half-rise and half-fall times of contraction.

The time, T , from the end of the latent period to the development of one half of the maximum isometric tension is defined as the half-rise time of the muscle. The method of measurement of this parameter is given in Fig. 4.5. The time from the cessation of stimulation to the point where the tension is halfway between resting tension and the tension at the end of stimulation, is defined at the half-fall time of the muscle. These parameters were found to be simpler to measure than the twitch times (Close, 1964) or the maximum rate of tension development (Drachma & Johnston,1973). The half-rise and half-fall times were used as a measure of the relative speed of contraction of the different muscle types, and were used to give relative values of the rate constants f and g of equation 14 of A.F. Huxley (1957).

Fig. 4.5 Measurements taken in order to determine the contraction time, $T_{\frac{1}{2}}$, of a muscle.


A
½A
T½

7. Analysis of pooled data

7.1 Rat and hamster muscle

Variations in the values of the visco-elastic constants of any one muscle were reduced by plotting the constants A and B of equation 3.13 against the height, H, of the ramp stretch. Values of A and B were derived from the slopes of these graphs. The constant, C, of equation 3.13 was taken as the mean value of C of the larger stretches of the muscle, within the linear range of A and B.

The values of E_1 , E_2 and V_2 for rat and hamster muscle, presented in the results section, were all derived from such pooled data.

7.2 Tortoise muscle

Each tortoise muscle was subjected to many tetani over a range of temperatures. To reduce the overall number of tetani, only one ramp (of 0.26mm height) was applied to the muscle at every temperature. Several stretches, with varying height, H, were applied at temperatures of 5°C and 30°C only. Values of the constants A, B and C, using pooled data, were obtained only at these latter temperatures.

8. Units of measurement

Muscle tensions and length changes are expressed in newtons (N) and metres (m) respectively. The elastic constants, E_1 , E_2 and E_3 are expressed in newtons per metre

(Nm^{-1}). Since $\frac{E_2}{V_2}$ and $\frac{E_3}{V_3}$ must have the dimension s^{-1}, the quantities V_2 and V_3 are given in newtons seconds per metre (Nsm^{-1}).

By the techniques used, T and H of equation 3.13 could only be measured to two significant places. Other measurements were accurate to three significant places. The values of A and B of equation 3.13, the maximum isometric tensions and the contraction times are therefore given to three significant places. The values of the constant C of equation 3.13 and the constants E_1, E_2 and V_2 of equation 3.12, the calculation of which involve T and H, are given to two significant places.

9. Curve fitting by computer

9.1 Single exponential

The digitised experimental data were fitted to the curve of equation

$$y = A + B.\exp\left[-Ct\right].$$

If y is sampled at equal intervals of time, h, then

$$y_n = A + B.\exp\left[-Ct_n\right], \qquad 4.1$$

$$y_{n+1} = A + B.\exp\left[-Ct_{n+1}\right], \qquad 4.2$$

$$y_{n+2} = A + B.\exp\left[-Ct_{n+2}\right], \qquad 4.3$$

where

$$t_n = nh,$$

$$t_{n+1} = (n + 1)h$$

and $$t_{n+2} = (n + 2)h$$

B can be eliminated by multiplying equation 4.1 by e^{-ch} and subtracting this from equation 4.2. This gives

$$y_{n+1} - y_n.e^{-ch} = A - A.e^{-ch}. \qquad 4.4$$

Repeating this for equations 4.2 and 4.3 gives

$$y_{n+2} - y_{n+1}.e^{-ch} = A - A.e^{-ch}. \qquad 4.5$$

A can then be eliminated from equations 4.4 and 4.5 to obtain

$$y_{n+1} - y_n.e^{-ch} = y_{n+2} - y_{n+1}.e^{-ch}$$

or $$y_{n+1} - y_{n+2} = e^{-ch}\left[y_n - y_{n+1}\right]. \qquad 4.6$$

Equation 4.6 is equivalent to the line

$$Y = MX, \qquad 4.7$$

where $$Y = y_{n+1} - y_{n+2},$$

$$M = e^{-ch}$$

and $$X = y_n - y_{n+1}.$$

Using the tension data from the static phase, the least squares fit was found for equation 4.7, hence the value of M. The value of C in equation 4.1 can then be derived

from M, since

$$C = \frac{-\ln M}{h}$$

The value of A in equation 4.1 can be derived by inserting C in equation 4.4 and summing both sides, then

$$A = \frac{\sum y_{n+1} - e^{-ch} \sum y_n}{n(1 - e^{-ch})} .$$

The value of B in equation 4.1 can be derived by inserting the values of A and C into this equation and summing both sides to give

$$B = \frac{\sum y_n - nA}{\sum e^{-Ct_n}} .$$

This gives the three constants of equation 4.1.

9.2 Double exponential

In order to fit data to the curve of the equation

$$y = A + B.\exp[-Ct] + D.\exp[-Et],$$

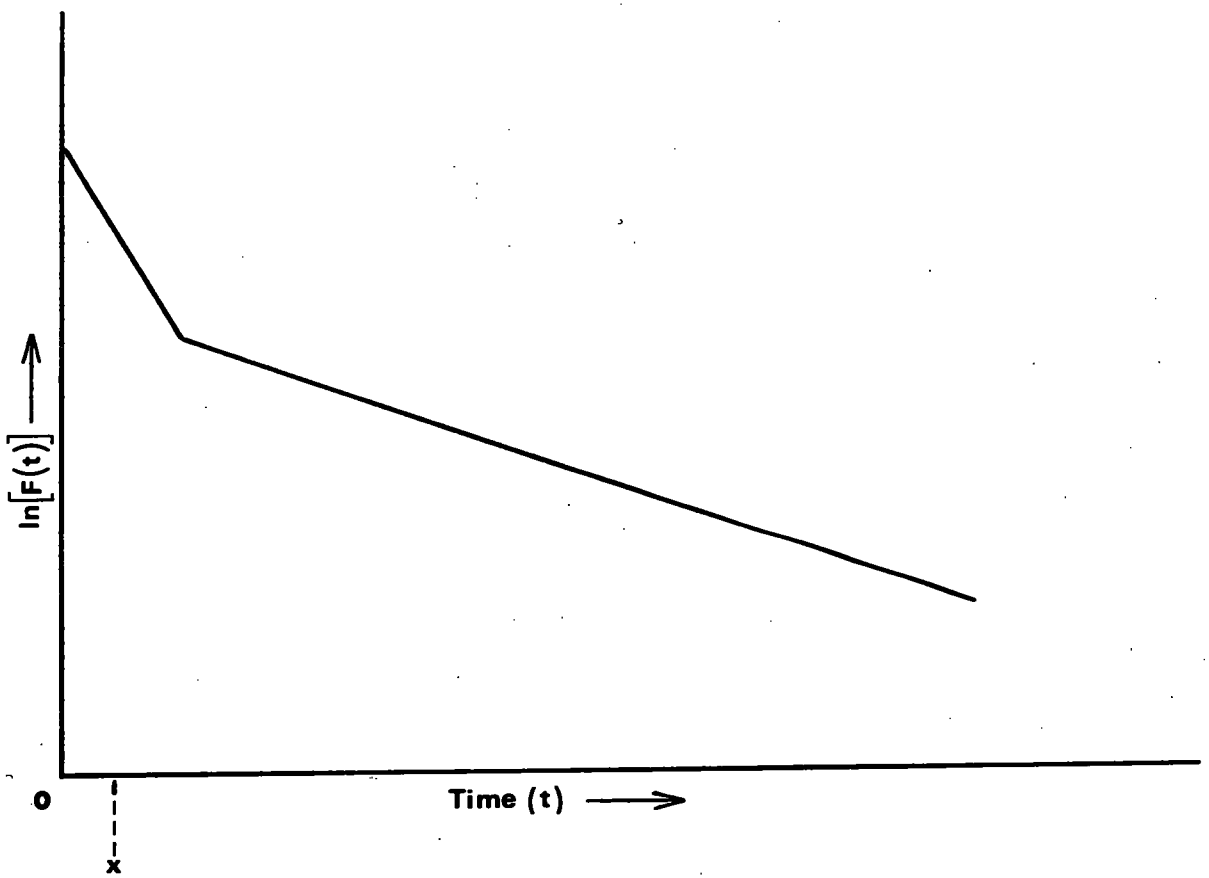
it was initially assumed that one of the exponential terms had a large time constant (C of equation 3.20), relative to the other. This exponential would decay to zero at an early stage of the second, slower exponential decay, as in Fig.4.6.

To derive the slower exponential, the procedure described in section 9.1 was applied, but starting at time

$$t = t_o + X,$$

where t_o is the start of the static phase and x has a value of 10 msecs.

Fig. 4.6 Determination of the two decaying exponentials of the tension during the static phase of a ramp stretch (for five component model only).


ln[F(t)]
0
x
Time (t)

The exponential derived in this way was then extrapolated back to time t_o . The resultant curve was then subtracted from the experimental curve and a second exponential fitted to the remainder. This was repeated in an iterative procedure with increasing values of x, to give the best least squares fit.

10. Statistics

10.1 Mean and standard error

Two methods of presentation of the values of E_1 , E_2 and V_2 have been used. For rat soleus and ECRL, and for hamster normal and dystrophic gastrocnemius muscles, the values of the components are given for each muscle, along with the mean, $\bar{x}$, of these values. In the case of tortoise gastrocnemius muscle, the mean and standard error, S.E., are given for E_1 , E_2 and V_2 at each temperature interval. Bessel's correction for small samples was applied in the calculation of the standard error. The formulae used were

$$\bar{X} = \frac{\sum X}{n}$$

and

$$S.E. = \frac{S.\sqrt{\frac{n}{n-1}}}{\sqrt{n}} ,$$

where

$$S^2 = \frac{\sum X^2 - \frac{(\sum X)^2}{n}}{n - 1} .$$

10.2 t- and d- tests

In sections 3.1, 4.3, 5 and 6 of the results, comparisons have been made between the means of two sets of

values of E_1 , E_2 and V_2 .

A variance ratio test was first applied to determine whether the sample variances were significantly different, or whether it could be assumed that the true population variances were the same. The value of F' was determined from

$$F' = \frac{S_1^2}{S_2^2},$$

where F' is the variance ratio and S^2 is the variance.

The level of significance was then determined from tables of F' (given in Lindley & Miller, 1971). The variances were taken as being significantly different if F' was greater than the value of F' at the 5% level, for $n_1 - 1$, $n_2 - 1$ degrees of freedom.

If the variances were not shown to be significantly different then a t'-test was applied to the data (Bailey, 1959, pp 47-49), where

$$t' = \frac{\bar{X}_1 - \bar{X}_2}{S\sqrt{\frac{1}{n_1} + \frac{1}{n_2}}}$$

and

$$S^2 = \frac{\sum x_1^2 - \frac{(\sum x_1)^2}{n_1} + \sum x_2^2 - \frac{(\sum x_2)^2}{n_2}}{n_1 + n_2 - 2} .$$

The level of significance of the values of t' were then determined for $n_1 + n_2 - 2$ degrees of freedom.

When the variances were taken as being significantly different, the value of d was determined

where

$$d = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}}} .$$

This value was then treated as having a t' distribution and the significance level determined from tables of t', with f degrees of freedom, where

$$f = \frac{1}{\frac{u^2}{n_1 - 1} + \frac{(1-u)^2}{n_2 - 2}}$$

and

$$u = \frac{\frac{S_1^2}{n_1}}{\frac{S_1^2}{n_1} + \frac{S_2^2}{n_2}} .$$

10.3 Coefficient of correlation

In section 7.2 of the results which deals with the effects of temperature on tortoise muscle, a possible linear relationship between the series elasticity and the maximum isometric tension was checked. The correlation coefficient for these two variables was found from

$$r = \frac{\sum(X - \bar{X})(y - \bar{y})}{\sqrt{\sum(x - \bar{x})^2 . \sum(y - \bar{y})^2}} .$$

The significance level, P, of this correlation was then determined from tables of r, for $n-2$ degrees of freedom (Bailey, 1959, pp 78-80).

V RESULTS

1. Linearity

Before proceeding to determine whether muscle can be represented by a three component Maxwell model, the range of linearity of muscle must be established, since the transfer function between length and tension of the model, derived in section III, applies only to linear systems.

If the system is linear, then the constants A and B of equation 3.13 will have a linear relationship to the length of stretch, H. The constant C of the same equation will be independent of H.

The range of values of H within which muscle satisfies these conditions was determined.

1.1. Rat soleus muscle

Relaxed and tetanised muscles were subjected to a number of stretches ranging from 0.09mm to 0.85mm. The tension developed during the static phase of each ramp stretch was fitted to an exponential of the form given in equation 3.13. The values of the constants A, B and C of this equation were calculated by the methods described in section IV.3.3. These values are given for one muscle (muscle number 1 of Table 5.3) in its relaxed and tetanised states. Those for the relaxed muscle are presented in Table 5.1 and for tetanised muscle in Table 5.2.

Ramp height mm	A N.	B N.	C s^{-1}
0.09	0.006	0.006	4.3
0.17	0.014	0.009	10
0.26	0.021	0.024	10
0.34	0.032	0.029	12
0.43	0.043	0.034	14
0.50	0.054	0.047	14
0.59	0.068	0.059	13
0.67	0.084	0.078	14
0.76	0.109	0.094	14
0.85	0.143	0.120	14

Table 5.1 Rat soleus muscle: relaxed

Ramp height mm	A N.	B N.	C s^{-1}
0.09	0.024	0.200	12
0.17	0.042	0.389	12
0.26	0.072	0.538	13
0.34	0.087	0.643	13
0.43	0.102	0.706	13
0.50	0.126	0.753	13
0.59	0.149	0.813	17
0.67	0.167	0.849	14
0.76	0.191	0.873	14
0.85	0.209	0.909	13

Table 5.2 Rat soleus muscle: tetanised

1.1.1 Relaxed muscle

The length/tension curves of the constants A and B of relaxed muscle are given in Fig. 5.1. These constants are linear over the range of stretch from 0.0mm to 0.4mm.

If the muscle behaves as a linear system, then the value of the constant C should be the same for all amounts of stretch. The results of Table 5.1 indicate that for relaxed muscle the value of C is reasonably constant, apart from the value obtained from a stretch of 0.09mm. At this latter level of stretch the tensions generated were small (see Fig.5.2) and the constant C was difficult to measure.

1.1.2 Tetanised muscle

The length/tension curves of the constants A and B of stimulated muscle are given in Fig. 5.3. The constant A can be seen to be linear over the whole range of stretch applied, while B behaves in a linear fashion only within the range of stretch from 0.0mm to 0.26mm.

The values of C, given in Table 5.2, are reasonably constant over the whole range of stretch.

1.2 Hamster, tortoise and rat ECRL muscle

The constants A, B and C (of equation 3.13) calculated for normal and dystrophic hamster gastrocnemius,

Fig. 5.1 Linearity curve of the constants A, (●) and B, (○), of equation 3.13, for relaxed rat soleus muscle (muscle number 1 of Table 5.3).

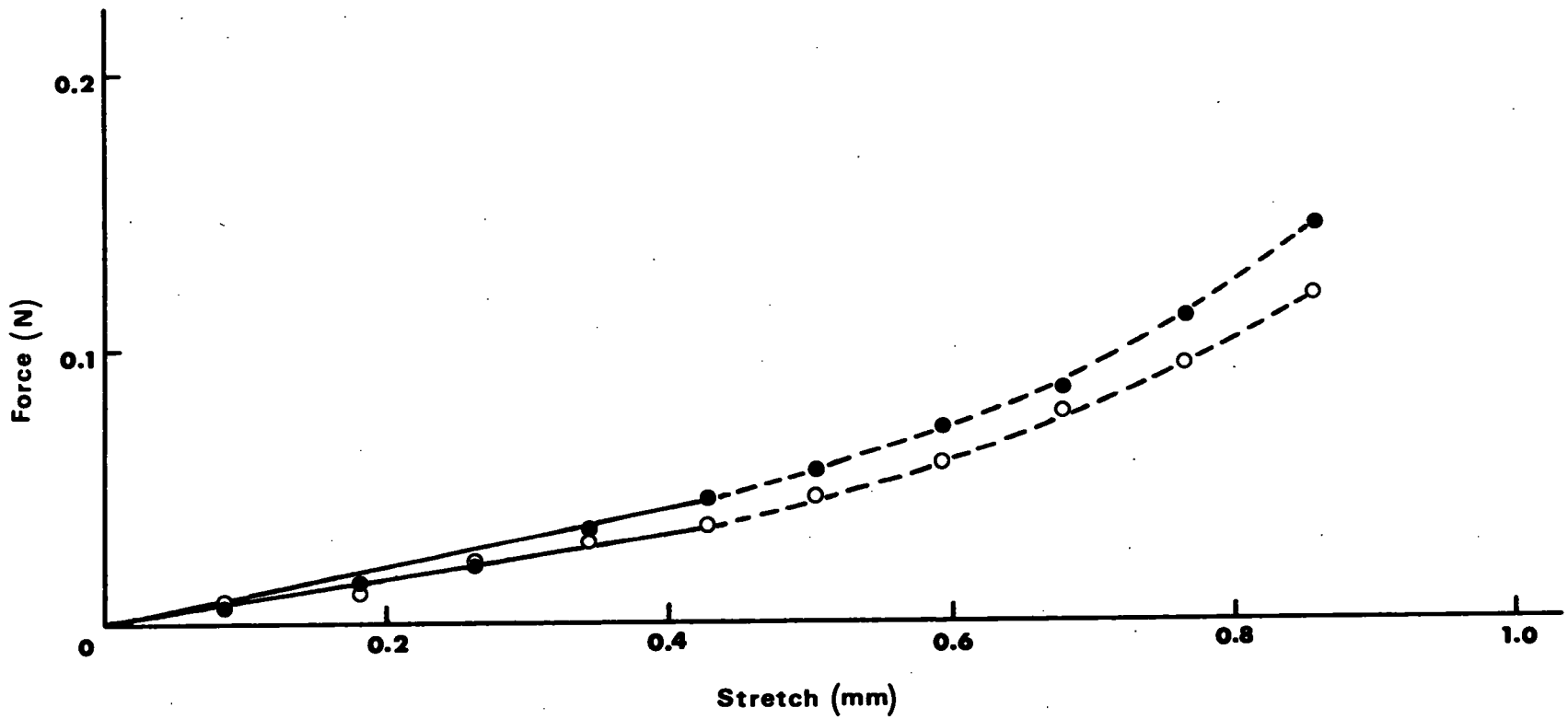

0.2
0.1
Force (N)
0
0.2
0.4
0.6
0.8
1.0
Stretch (mm)

Fig. 5.2 0.09 mm stretch applied to relaxed rat soleus muscle. Upper trace, length record; lower trace, tension record.

The small tensions generated made it difficult to determine the constant as in Fig. 4.4.

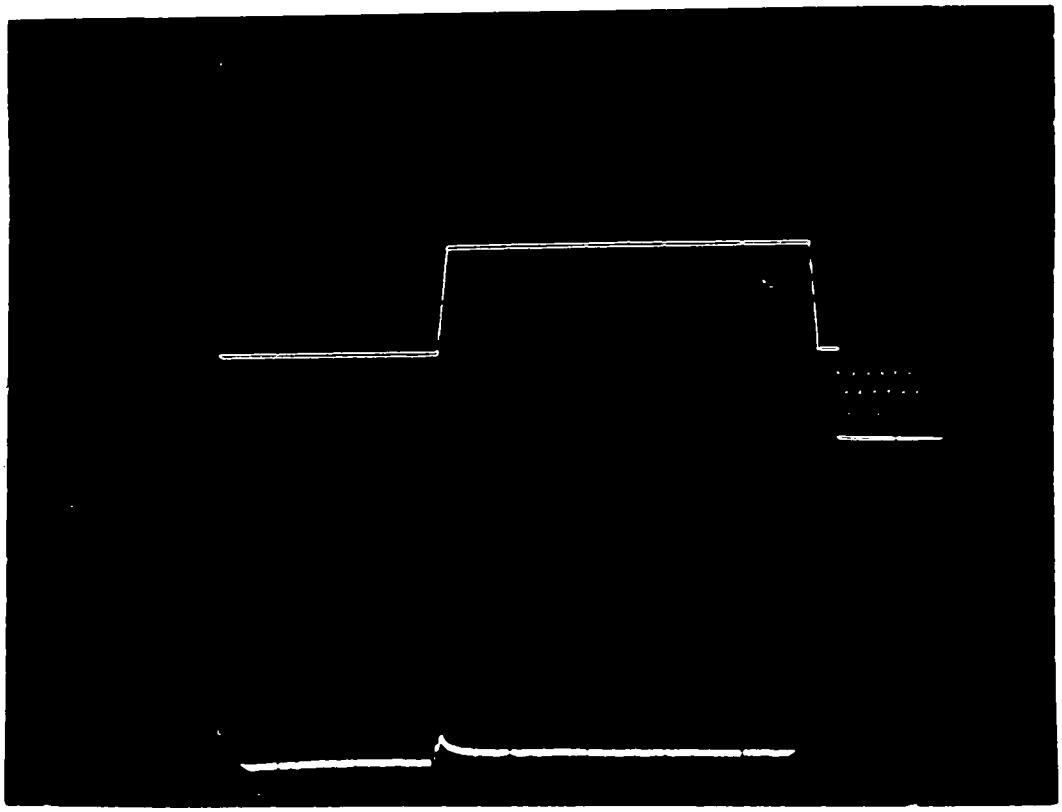
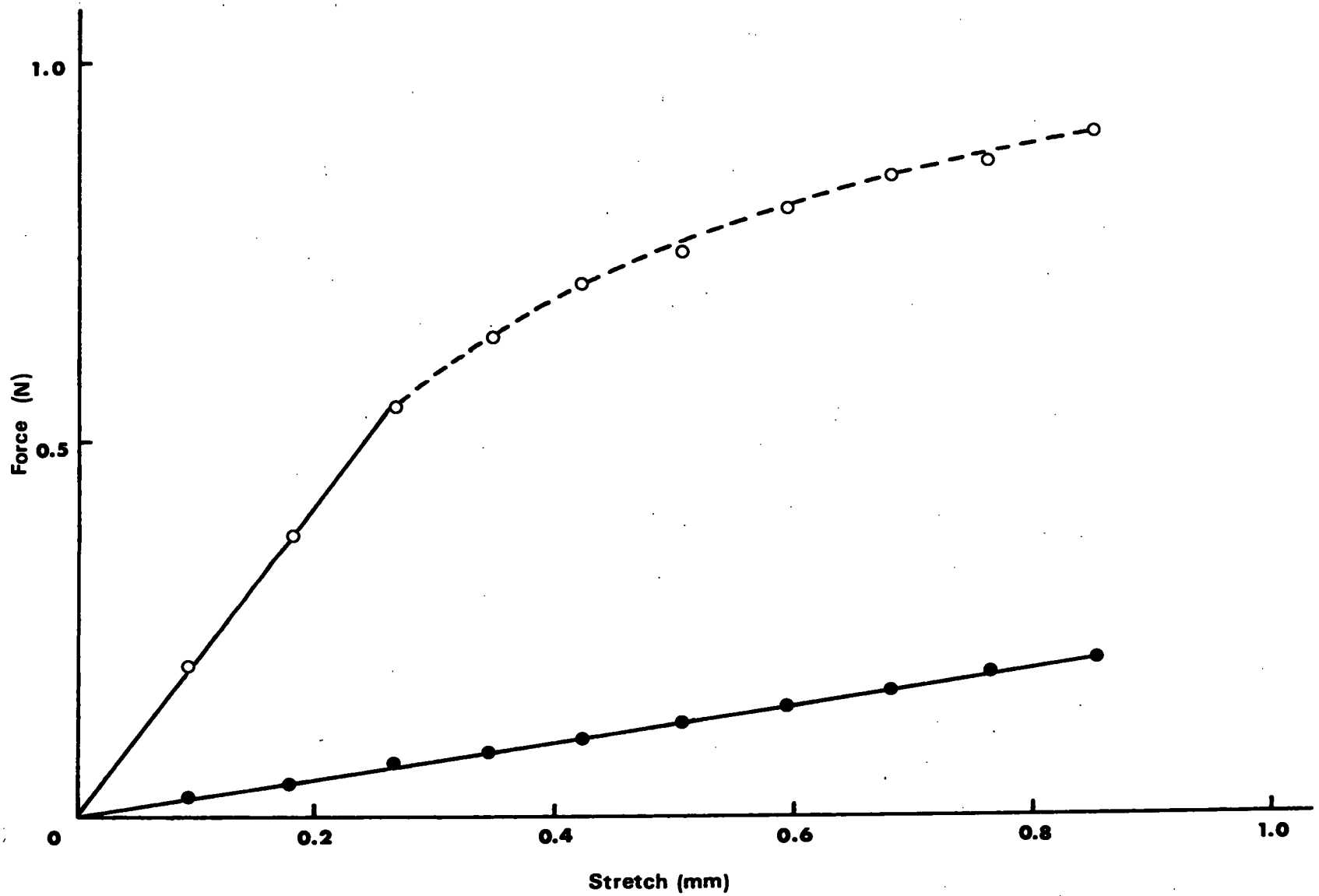


Fig. 5.3 Linearity curves of the constants A, (●), and B, (○), of equation 3.13 for tetanised rat soleus muscle (muscle number 1 of Table 5.3).


1.0
0.5
0
Force (N)
0.2
0.4
0.6
0.8
1.0
Stretch (mm)

tortoise gastrocnemius and rat ECRL muscle were all found to be linear, within the 0.0mm to 0.26mm range of linearity found for rat soleus muscle (see Figs. 5.4, 5.5, 5.6 and 5.7).

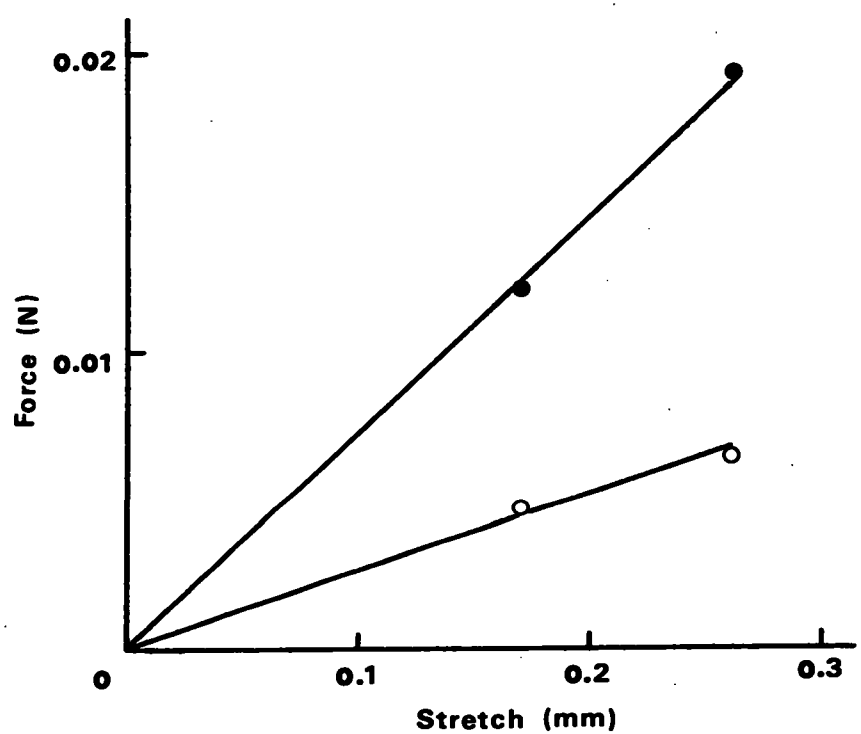
1.3 Determination of E_1 , E_2 and V_2 .

The values of E_1 , E_2 and V_2 of the three component Maxwell model have been determined using values of A, B and C derived from ramp stretches that fall within the linear range of all three constants, in both relaxed and tetanised muscle. The values of E_1 , E_2 and V_2 for tortoise muscle at 15°C, 20°C and 25°C, given in Table 5.12, were calculated from stretches of 0.26mm only. In all other results A and B were plotted against ramp height and the values of A and B, used to determine the visco-elastic constants, were found from the slope of these lines. The value of C was taken as the mean of all C within the linear range.

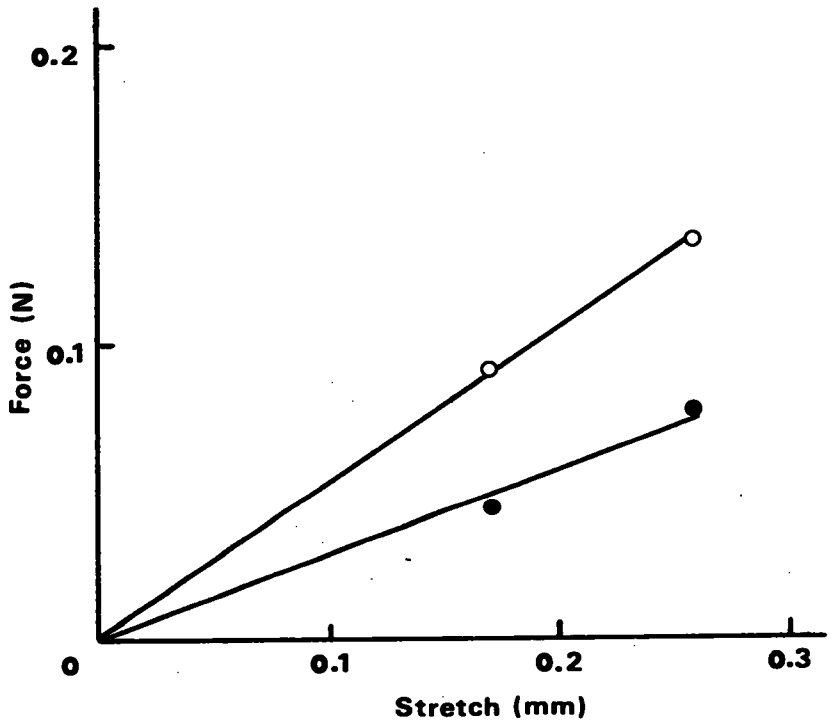
1.4 Comparison with other results

Since A represents the parallel elasticity of the system, E_1 will be linear within the linear range of A. The series elasticity and the viscosity are both directly proportional to B, if C is constant over the range of linearity of B. The series elastic element, E_2 , and the viscous element, V_2 , are therefore linear within the range of stretch of 0.0mm to 0.26mm for contracting muscle. In Fig.3 of Cavagna (1970), the linearity of the series elastic

Fig. 5.4 Linearity curves of the constants A, (●), and B, (○), of equation 3.13 for a rat ECRL muscle. a) relaxed muscle b) tetanised muscle.


0.02
0.01
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

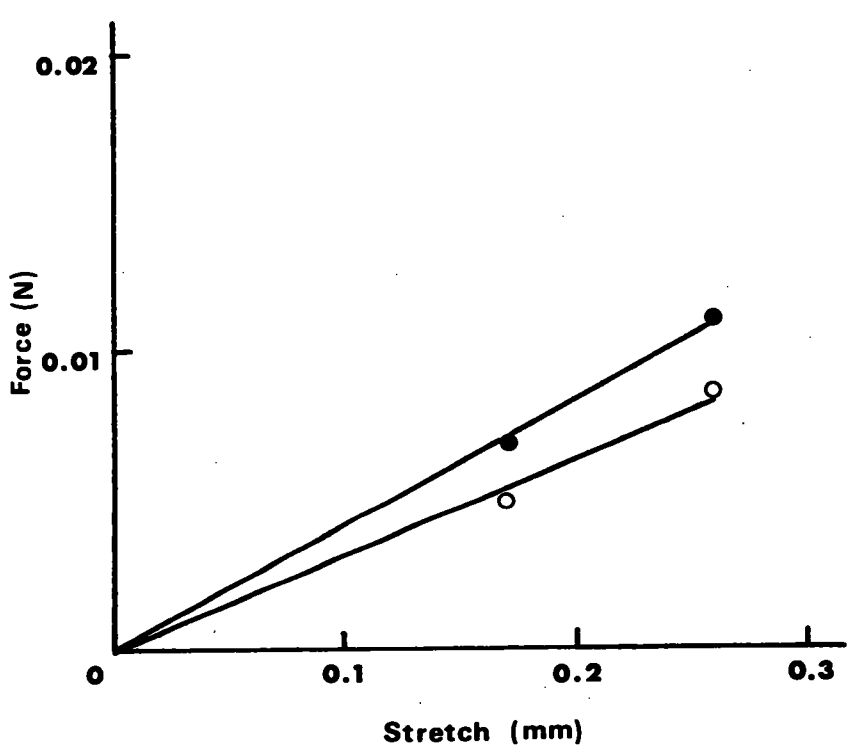
a)


0.2
0.1
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

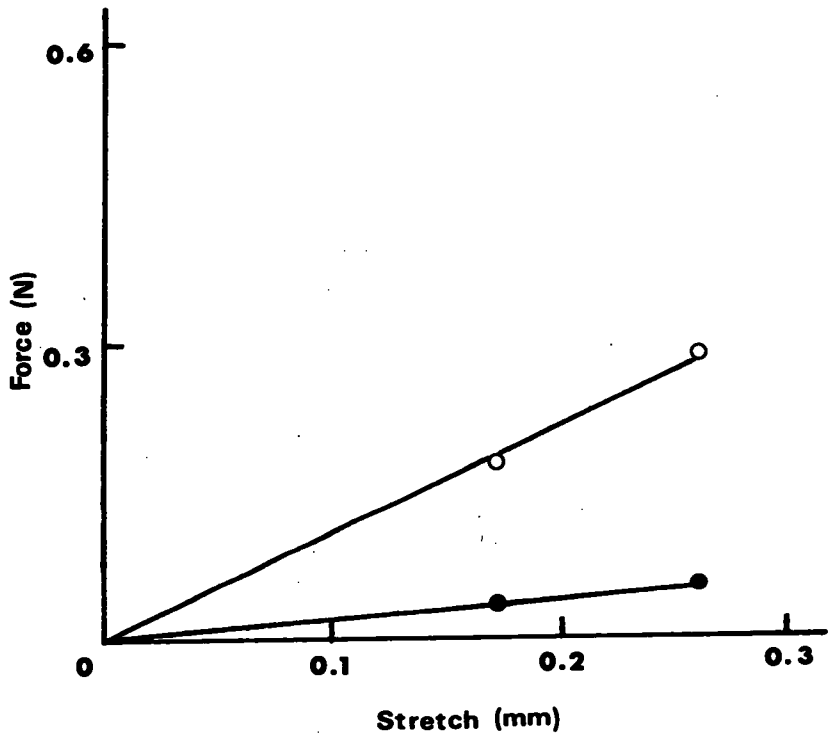
b)

Fig. 5.5 Linearity curves of the constants A, (●), and B, (○), of equation 3.13 for a normal hamster gastrocnemius muscle.

a) relaxed muscle b) tetanised muscle.


0.02
0.01
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

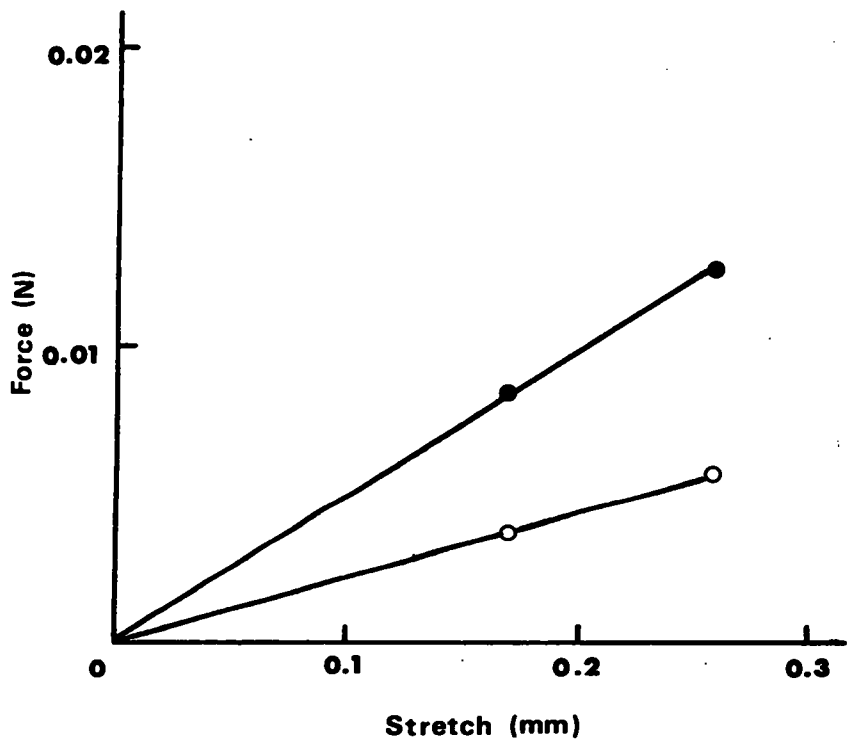
a)


0.6
0.3
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

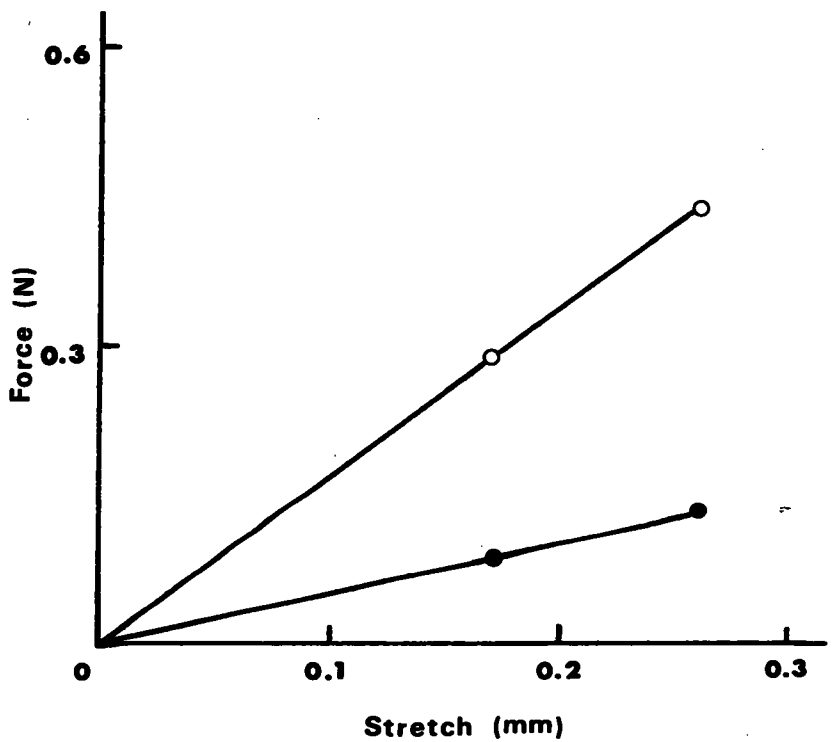
b)

Fig. 5.6 Linearity curves of the constants A, (●), and B (○), of equation 3.13 for a dystrophic hamster gastrocnemius muscle.

a) relaxed muscle b) tetanised muscle.

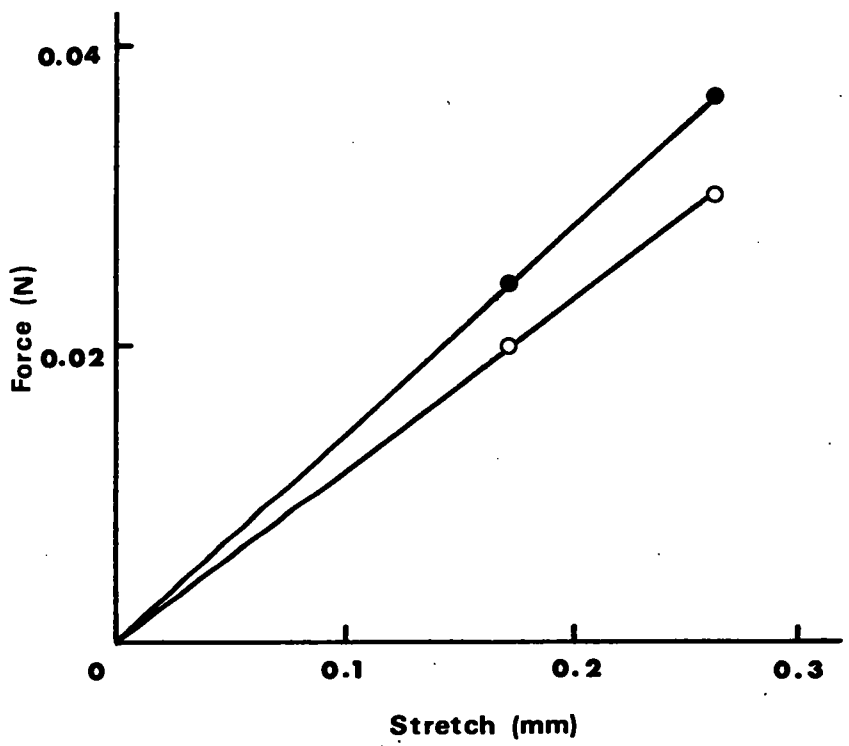

0.02
0.01
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

a)

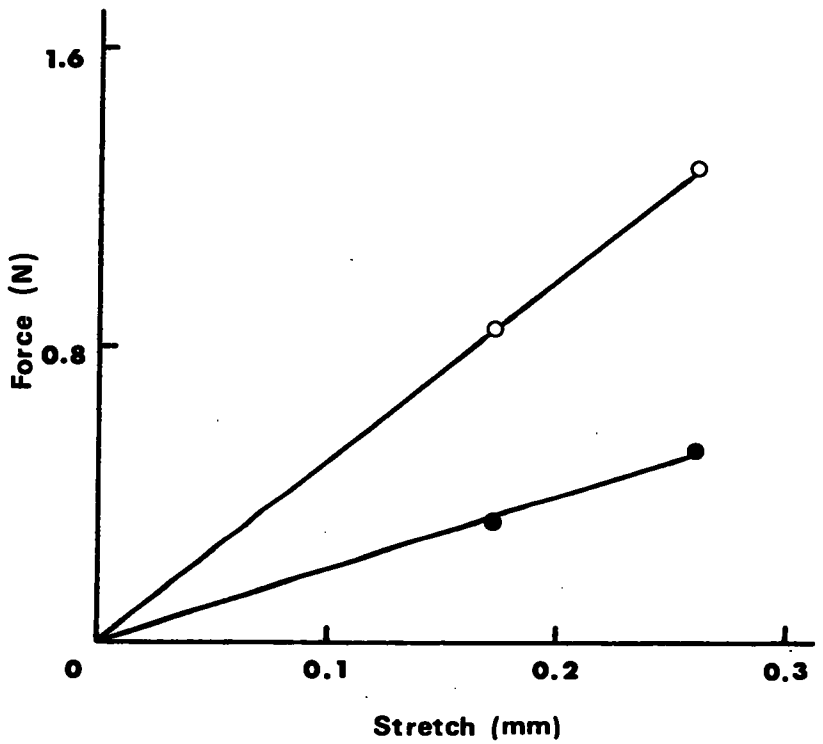

0.6
0.3
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

b)

Fig. 5.7 Linearity curves of the constants A, (●), and B, (○), of equation 3.13 for a tortoise gastrocnemius muscle. a) relaxed muscle b) tetanised muscle.


0.04
0.02
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

a)


1.6
0.8
Force (N)
0
0.1
0.2
0.3
Stretch (mm)

b)

element of frog gastrocnemius muscle is compared with the corresponding results for frog sartorius of Jewell and Wilkie (1958) and rat gracilis anticus of Bahler (1967). Of these three muscles, two of them, frog gastrocnemius and rat gracilis anticus, appear to be linear within the range of stretch used in this study, that is, up to stretches of 1.3% of resting length. However, in all three muscles compared by Cavagna, the stress/strain curve of the series elastic component becomes non-linear and relatively the stress increases at higher strains. This is in contrast to the graph of Fig. 5.3 where relatively the stress of B, which is directly proportional to the series elasticity, decreases with increased strain.

The results of the three authors quoted are all derived using the isotonic quick release method of Wilkie (1956).

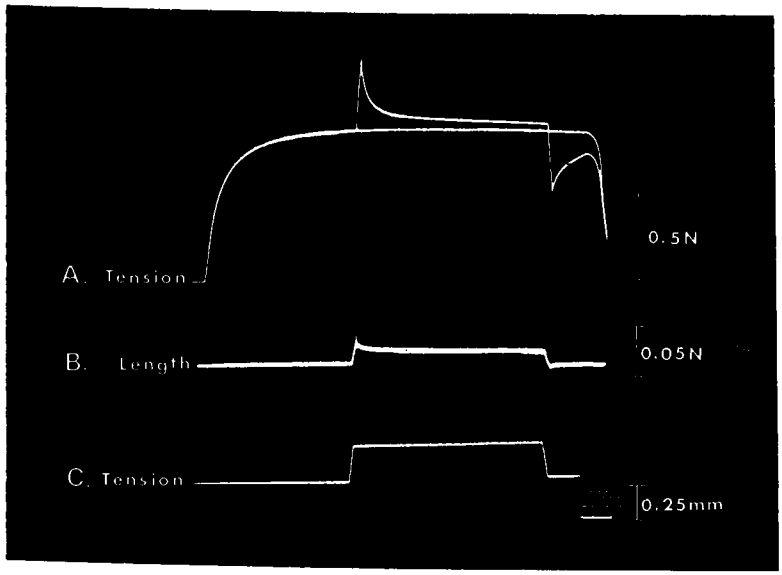
2. Time course of tension changes over the static phase.

Changes in tension during the static phase of ramp stretches were compared with theoretical tension changes predicted by the simple three component model. Only stretches within the range of linearity as determined in section 5.1 were applied. A typical tension record of tetanised rat soleus muscle, from which measurements were taken, is given in Fig. 5.8a.

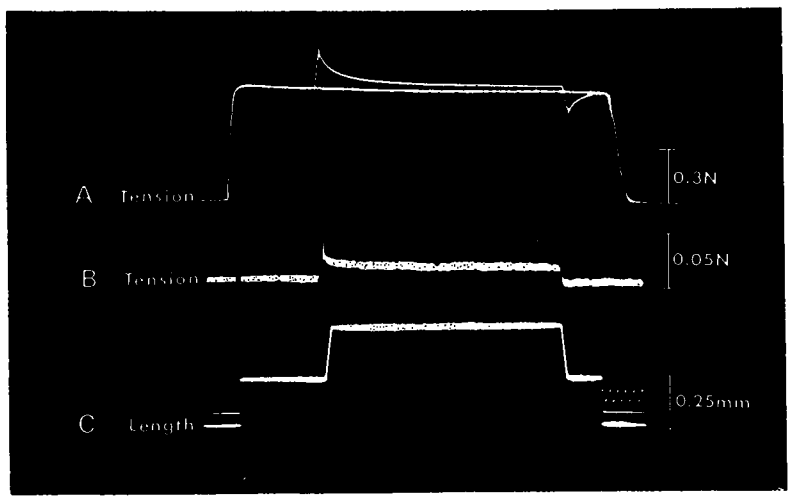
The constants A, B and C of equation 3.13 were derived from tensions produced during the static phase, as in

Fig. 5.8a Length and tension traces of rat soleus muscle. Trace A is that of a ramp stretch applied to stimulated muscle, superimposed on an unstretched tetanus. Trace B is the tension produced when a ramp stretch is applied to relaxed muscle. Trace C is the ramp stretch applied to the muscle. Time markers, derived from the digitimer, seen at the end of the length trace, are spaced at intervals of 0.10 seconds.

Fig. 5.8b Length and tension records of rat ECRL muscle. The sequence of traces is the same as that in Fig. 5.8a. Time markers are at intervals of 0.10 seconds.


A. Tension
B. Length
C. Tension
0.5N
0.05N
0.25mm

a.


A Tension
B Tension
C Length
0.3N
0.05N
0.25mm

b.

section IV.3.3. This derived curve was then plotted over the time course of the static phase and compared with the experimental tension (see Fig. 5.9).

These results indicate that the simple exponential predicted by the three component model is a reasonable, but not perfect, fit to the tension record.

3. Comparison of relaxed and tetanised muscle.

3.1. Rat soleus muscle

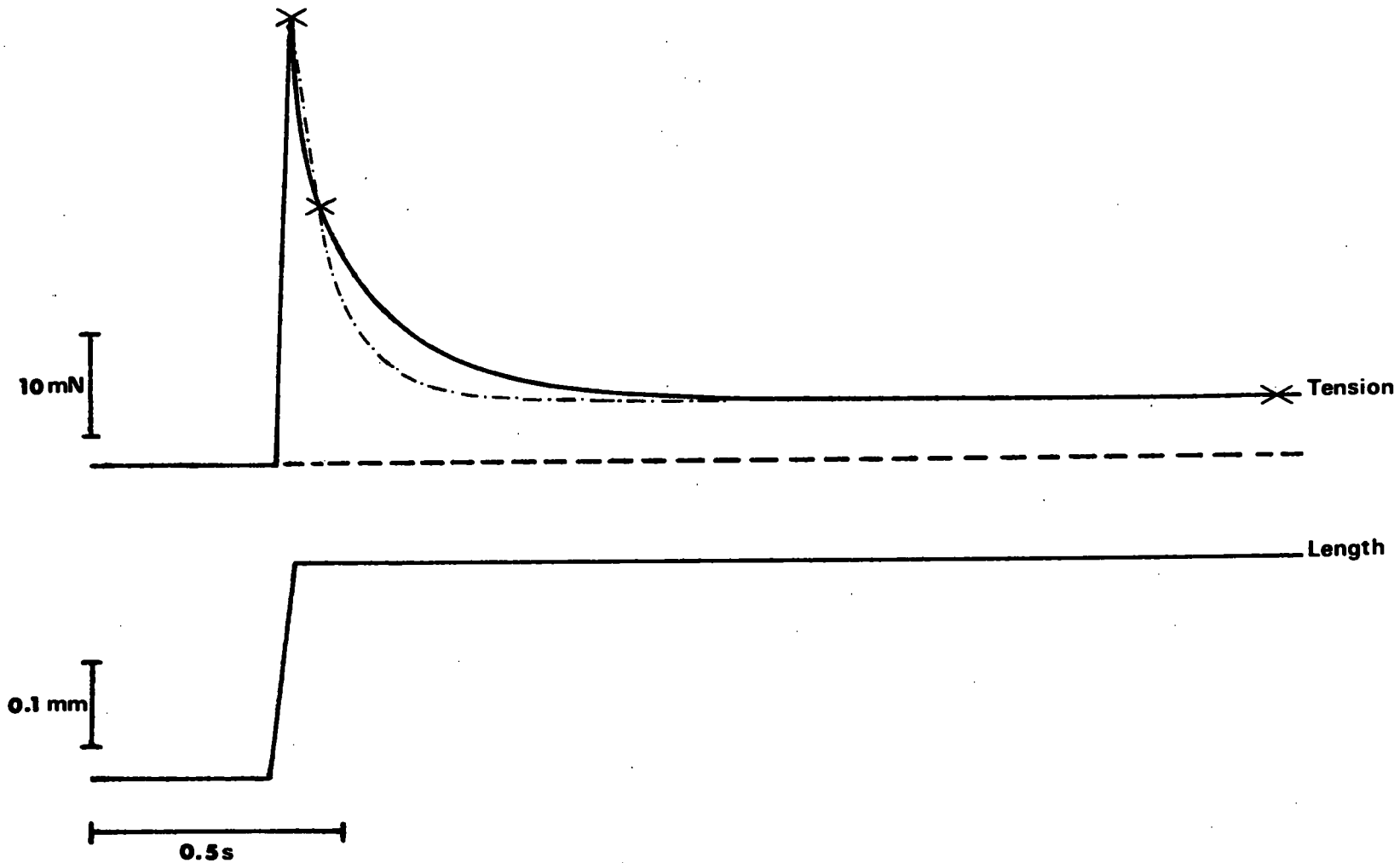
The visco-elastic constants E_1 , E_2 and V_2 were determined for soleus muscles of the rat, in the relaxed and tetanised states. Typical length and tension records of a ramp stretch applied to this muscle are given in Fig. 5.8a.

Data obtained from experiments on nine soleus muscles are given in Table 5.3. The maximum isometric tension generated, the contraction time and the value of the visco-elastic constants are given for each muscle.

The values of E_1 , E_2 and V_2 for each muscle increase upon stimulation. The values of E_2 and V_2 increase by factors of 23 and 25 respectively, while there is a smaller increase, by a factor of 3.1, in the case of E_1 . Similar changes in E_1 and E_2 can be observed in Figs. 2 and 3 of Sugi (1972).

There is considerable variation within the results presented in Table 5.3. Some of this variation may be attributed to variations in the size of the muscle and

Fig. 5.9 Comparison of the tension decay (continuous line) during the static phase of a ramp stretch with the exponential curve (chain line) fitted to the three points indicated. The tension trace is that of a 0.26 mm stretch applied to a rat soleus muscle.


10 mN
Tension
Length
0.1 mm
0.5 s

Muscle number	Isometric tension N.	Contraction time s.	E_1 Nm^{-1}		E_2 Nm^{-1}		V_2 Nsm^{-1}		$\frac{E_1 \text{ Stim.}}{E_1 \text{ Relax.}}$	$\frac{E_2 \text{ Stim.}}{E_2 \text{ Relax.}}$	$\frac{V_2 \text{ Stim.}}{V_2 \text{ Relax.}}$
			Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated			
1	0.939	0.303	80	250	110	3100	10.0	240	2.9	∞ 28	26
2	0.758	0.281	115	190	140	2500	11.0	210	1.6	18	19
3	0.810	0.400	85	290	110	2500	10.0	220	3.4	23	22
4	0.610	0.359	60	210	120	2100	8.7	170	3.5	17	19
5	0.660	0.440	70	220	120	2000	8.6	220	3.1	17	26
6	0.948	0.279	80	170	110	2700	7.9	240	2.1	24	30
7	0.939	0.276	40	240	92	2800	6.3	220	6.0	30	35
8	0.958	0.282	110	220	190	2600	16.0	230	2.0	14	14
9	0.939	0.241	55	200	80	2900	6.3	240	3.6	36	38
Mean values	0.840	0.318	78	220	120	2600	9.4	220	3.1	23	25

Table 5.3 Rat soleus muscle

variations in the maximum isometric tensions generated.

It can be seen from the results in Table 5.4 that the changes in each component upon stimulation are highly significant.

3.2 Hamster, tortoise and rat ECRL muscle

Changes similar to those seen in rat soleus muscle can also be observed in the other four types of muscle examined. In every muscle the value of E_1 , E_2 and V_2 increases upon stimulation (see Tables 5.5, 5.7, 5.8 and 5.13). The increase in the value of E_2 and V_2 is always greater than the increase in the value of E_1 , except in the case of one dystrophic hamster gastrocnemius muscle.

4. Comparison of fast and slow muscle of rat.

4.1. Rat ECRL muscle

The visco-elastic constants were determined for ECRL muscles of the rat. Typical length and tension records of this muscle are given in Fig. 5.8b. Data from experiments carried out on nine muscles are given in Table 5.5.

4.2 Dimensionless ratios

If a muscle behaves according to the simple three component system, then it should be possible to determine values of E_1 , E_2 and V_2 . These values are presented in Tables 5.3 and 5.5 for rat soleus and ECRL muscles. However,

	Rat soleus: relaxed (mean values) n = 9	Rat soleus: stimulated (mean values) n = 9	F′ N.S. = not significant	t′ or d	P
E_1	78	220	2.07 (N.S.)	$t' = 8.69$ $^{o}f = 16$	< 0.001
E_2	120	2600	127.3	$d = 20.8$ $^{o}f = 8.13$	< 0.001
V_2	9.4	220	72.01	$d = 25.1$ $^{o}f = 8.22$	< 0.001

Table 5.4 Comparison of rat soleus muscle in relaxed and tetanised states.

Muscle number	Isometric tension N.	Contraction time s.	E_1 Nm^{-1}		E_2 Nm^{-1}		V_2 Nsm^{-1}		$\frac{E_1 \text{ Stim.}}{E_1 \text{ Relax.}}$	$\frac{E_2 \text{ Stim.}}{E_2 \text{ Relax.}}$	$\frac{V_2 \text{ Stim.}}{V_2 \text{ Relax.}}$
			Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated			
1	0.270	0.061	73	290	94	1000	13	130	4.0	11	10
2	0.329	0.073	110	270	27	1200	30	170	2.4	44	5.7
3	0.325	0.080	170	280	44	950	15	180	1.6	22	12
4	0.242	0.061	130	330	86	720	17	87	2.5	8.4	5.1
5	0.311	0.061	53	170	32	490	8.3	114	3.2	15	14
6	0.488	0.080	88	300	74	880	13	76	3.4	12	5.8
7	0.868	0.080	65	180	63	1300	13	360	2.8	21	30
8	0.576	0.080	40	240	22	1200	6.8	410	6.0	54	60
9	0.384	0.080	61	290	48	2100	10	350	4.7	44	35
Mean values	0.421	0.073	88	260	54	1100	14	210	1.8	26	20

Table 5.5 Rat ECRL muscle

these values take no account of the dimensions of the muscles. The muscles themselves do not conform to a regular geometrical shape and it is therefore not easy to express these constants in terms of a sample of unit size.

If it is assumed that there is little variation between the dimensions of a relaxed muscle and a tetanised muscle, then the dimensionless ratios

$$\frac{E_1 \text{ tetanised}}{E_1 \text{ relaxed}}, \quad \frac{E_2 \text{ tetanised}}{E_2 \text{ relaxed}} \text{ and } \frac{V_2 \text{ tetanised}}{V_2 \text{ relaxed}},$$

may perhaps be used as a basis for comparing muscles of one type with those of another.

4.3 Comparison of rat soleus and ECRL muscle

It can be seen from Table 5.5 that there is considerable variation in the values of the visco-elastic constants derived for each ECRL muscle and that this variation is greater than that observed in soleus muscles. This can be illustrated by comparing the range of the parameter E_1 for each type of muscle. The value of E_1 for tetanised soleus muscle varies from 170 Nm^{-1} to 250 Nm^{-1}, while E_1 of tetanised ECRL muscle lies within the range of 170 Nm^{-1} to 330 Nm^{-1}.

In Table 5.6, the means of the ratios of each constant, the means of the isometric tensions generated and the means of the contraction times for soleus and ECRL muscles are compared. From this table it can be seen that the isometric tensions of the two muscles are significantly

	Rat soleus (mean values) n = 9	Rat ECRL (mean values) n = 9	F′ N.S. = not significant	t′ or d	P N.S. = not significant
$\frac{E_1 \text{ Stim.}}{E_1 \text{ Relax.}}$	3.1	1.8	1.06 (N.S.)	t′ = 0.203 °f = 16	N.S.
$\frac{E_2 \text{ Stim.}}{E_2 \text{ Relax.}}$	23	26	5.51	d = 0.439 °f = 11	N.S.
$\frac{V_2 \text{ Stim.}}{V_2 \text{ Relax.}}$	25	20	5.55	d = 0.745 °f = 11	N.S.
Isometric tension N.	0.840	0.421	2.08 (N.S.)	t′ = 5.30 °f = 16	< 0.001
Contraction time s.	0.269	0.073	18.06	d = 13.6 °f = 9	< 0.001

Table 5.6 Comparison of rat soleus and ECRL muscle

different, as are the contraction times. However, no significant differences can be shown between the three components, E_1 , E_2 and V_2 , by this method.

5. Normal and dystrophic muscle

Experiments were performed on fourteen normal and on thirteen dystrophic hamster gastrocnemius muscles. Transverse sections of the dystrophic and normal muscles are reproduced in Figs. 5.10 and 5.11. The dystrophic muscle fibres shown in Fig. 5.10a vary in diameter from 30 μm to 80 μm and almost all of them have centrally placed nuclei. Several of the dystrophic muscle fibres (those indicated by arrows in Fig. 5.10a) have lost their angular outline and are stained darker than the others. These darker fibres showed no evidence of phosphorylase activity, whilst all other fibres showed moderate (see two fibres in the top left of Fig. 5.10c) or intense activity. This is illustrated in Fig. 5.10b, where abnormal muscle fibres are shown enlarged and stained for general structure, and in Fig. 5.10c where the same fibre is shown stained for phosphorylase activity.

In Fig. 5.11 a section of a gastrocnemius muscle taken from a hamster from a non-dystrophic line is shown. The fibres of this normal muscle vary in diameter from 40 μm to 110 μm. No rounded, dark-staining fibres were present in any of the muscles from non-dystrophic hamsters. However, many of the fibres had the centrally placed nuclei, which

Fig. 5.10a A section of a gastrocnemius muscle from a dystrophic hamster. Magnification is x192. The abnormal, rounded, dark staining fibres are indicated by arrows. Note the centrally placed nuclei in many of the fibres.

Fig. 5.10b One of the abnormal fibres of Fig. 5.11a at increased magnification (x 325).

Fig. 5.10c The same section as shown in Fig.5.11b but stained for phosphorylase activity. Note the total lack of activity in the abnormal fibre.

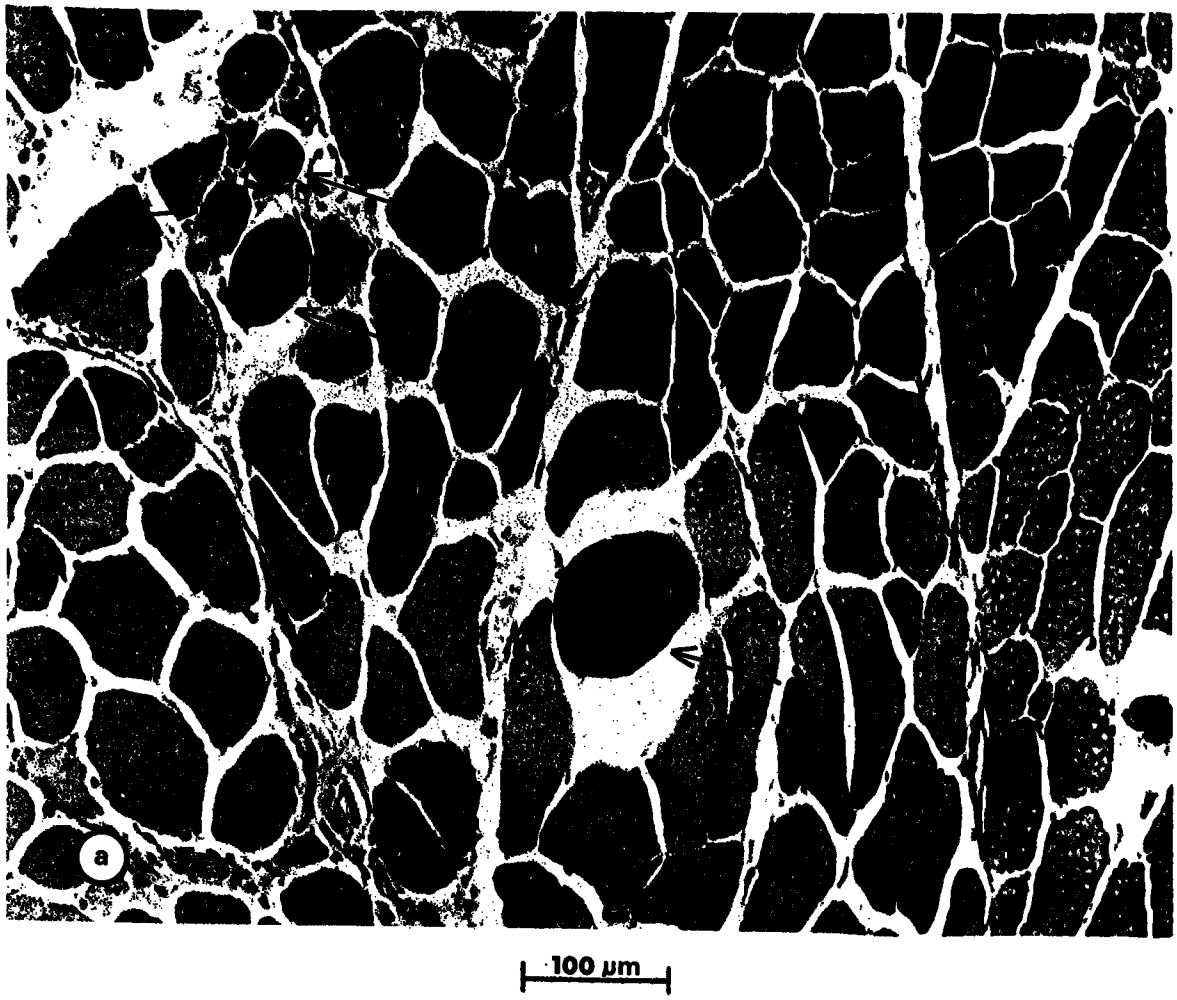

a
100 µm
b
60 µm
c
60 µm

Fig. 5.11 A section of gastrocnemius muscle from a normal hamster. Magnification x 368. Note the absence of any abnormal fibres.


50 µm

would not be expected for normal muscle (Holmburger, 1966).

Typical length and tension records of normal and dystrophic muscles are given in Fig. 5.12a and Fig. 5.12b respectively. Data from the experiments on these muscles are presented in Tables 5.7 and 5.8. Muscle number 11. in Table 5.8 is the only muscle in which the increase in E_1 upon stimulation is greater than the corresponding increase in E_2 .

The normal and dystrophic muscles are compared in Table 5.9. As the same anatomical muscle is examined in both normal and dystrophic animals, it should be possible to make a direct comparison between the values of the visco-elastic constants, without the necessity of comparing dimensionless ratios.

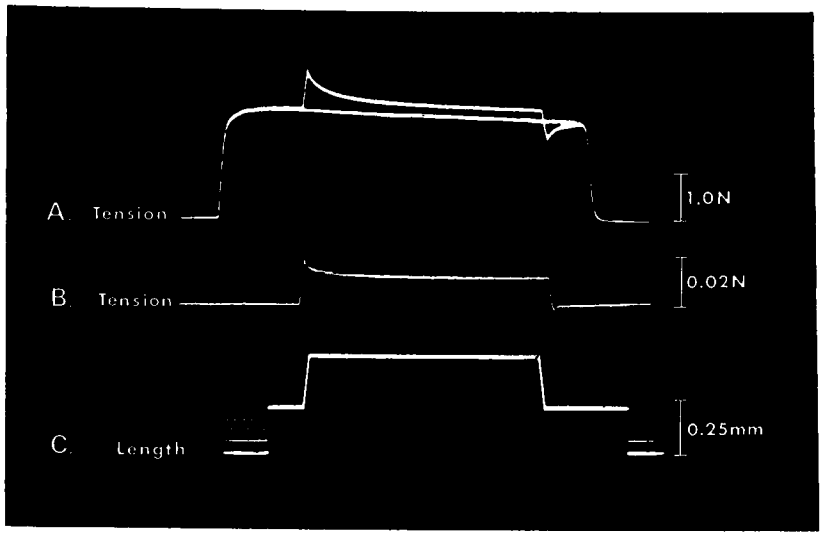
These two types of muscle are compared in both the relaxed and tetanised states. As may be seen from Table 5.9, in both the relaxed and tetanised state, there is a significant difference between the values of E_1 derived from normal muscle and those derived from dystrophic muscle. The values of E_2 also show these significant differences, while no differences are shown between the values of V_2 . In addition the isometric tensions and the contraction times of the two groups of muscles are shown to be significantly different.

6. Comparison of other pairs of muscles

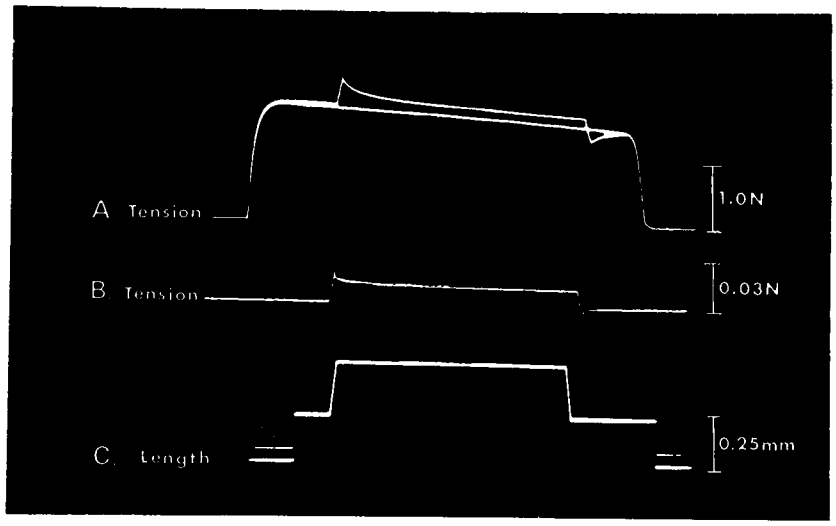
Of the five muscle types which have been examined, tortoise gastrocnemius muscle at 15°C and normal hamster

Fig. 5.12a Length and tension traces of a ramp stretch applied to normal hamster gastrocnemius muscle. Trace A is the tension produced by a ramp stretch of 0.26 mm applied to tetanised muscle, superimposed on an unstretched tetanic contraction. Trace B is the tension produced when the same stretch is applied to relaxed muscle. Trace C is the ramp stretch applied to the muscle. The time markers at the start of the length trace are spaced at intervals of 0.10 seconds.

Fig. 5.12b Length and tension traces of a ramp applied to dystrophic hamster gastrocnemius muscle. The sequence of traces is the same as for normal hamster muscle. Time markers are at intervals of 0.10 seconds.


A. Tension
1.0N
B. Tension
0.02N
C. Length
0.25mm

a.


A Tension
1.0N
B Tension
0.03N
C. Length
0.25mm

b.

Muscle number	Isometric tension N.	Contraction time s.	E_1 Nm^{-1}		E_2 Nm^{-1}		V_2 Nsm^{-1}		$\frac{E_1 \text{ Stim.}}{E_1 \text{ Relax.}}$	$\frac{E_2 \text{ Stim.}}{E_2 \text{ Relax.}}$	$\frac{V_2 \text{ Stim.}}{V_2 \text{ Relax.}}$
			Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated			
1	2.70	0.056	48	710	42	3600	1.5	220	15	86	150
2	3.21	0.056	36	860	43	2400	1.3	180	24	56	140
3	4.55	0.056	52	690	63	4800	2.3	300	13	76	130
4	2.94	0.064	80	730	83	3500	5.3	270	9.1	42	51
5	4.00	0.064	99	940	65	4100	3.6	330	9.5	63	92
6	2.94	0.081	100	630	106	3500	5.4	310	6.3	33	57
7	2.31	0.048	82	850	53	2700	2.6	150	10	51	58
8	3.90	0.065	95	850	76	4000	3.2	180	8.9	52	56
9	3.94	0.065	38	940	51	4100	1.4	210	25	80	150
10	2.70	0.065	48	790	54	4000	2.5	400	16	74	160
11	4.30	0.065	85	730	73	4100	3.8	280	8.6	56	74
12	4.74	0.065	26	1100	45	3800	2.2	320	42	84	140
13	3.21	0.065	58	920	47	4700	2.8	310	16	100	110
14	4.60	0.069	76	990	76	4100	1.7	220	13	54	130
Mean values	3.57	0.063	66	840	63	3800	2.8	260	15	65	107

Table 5.7 Normal hamster gastrocnemius muscle

Muscle number	Isometric tension N.	Contraction time s.	E_1 Nm^{-1}		E_2 Nm^{-1}		V_2 Nsm^{-1}		$\frac{E_1 \text{ Stim.}}{E_1 \text{ Relax.}}$	$\frac{E_2 \text{ Stim.}}{E_2 \text{ Relax.}}$	$\frac{V_2 \text{ Stim.}}{V_2 \text{ Relax.}}$
			Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated			
1	3.87	0.110	42	310	41	1700	4.9	220	7.4	41	45
2	2.60	0.110	18	500	29	1900	3.1	210	28	65	68
3	3.24	0.110	18	510	31	2200	4.8	400	28	71	83
4	2.35	0.098	28	530	42	2200	4.1	460	19	52	110
5	2.73	0.110	24	540	27	1800	3.6	250	22	67	69
6	1.67	0.167	11	250	22	1200	1.3	110	23	54	85
7	1.96	0.110	44	540	37	1400	2.6	310	12	38	119
8	2.14	0.119	24	310	40	1600	5.0	490	13	40	98
9	2.21	0.119	21	770	48	1800	2.4	330	37	37	140
10	1.79	0.119	13	730	20	1200	3.0	340	56	60	110
11	2.84	0.119	12	540	38	1500	2.3	320	45	39	140
12	2.19	0.104	25	790	29	1500	2.0	280	32	52	140
13	3.01	0.110	39	690	49	2000	5.9	400	18	41	68
Mean values	2.51	0.116	24	540	35	1700	3.5	320	26	50	98

Table 5.8 Dystrophic hamster gastrocnemius muscle

		Normal hamster gastrocnemius (mean values) n = 14	Dystrophic hamster gastrocnemius (mean values) n = 13	F′ N.S. = not significant	t′ or d	P N.S. = not significant
Relaxed muscle	E_1	66	24	5.11	d = 5.96 ^{o}f = 18.2	<0.001
	E_2	63	35	3.96	d = 5.03 ^{o}f = 19.5	<0.001
	V_2	2.8	3.5	1.10 (N.S.)	t′ = 1.36 ^{o}f = 25	N.S.
Stimulated muscle	E_1	840	540	1.75 (N.S.)	t′ = 5.51 ^{o}f = 25	<0.001
	E_2	3800	1700	3.91	t′ = 10.6 ^{o}f = 19.5	<0.001
	V_2	260	320	2.22 (N.S.)	t′ = 0.68 ^{o}f = 25	N.S.
Isometric tension	N.	3.57	2.51	1.69 (N.S.)	t′ = 4.84 ^{o}f = 25	<0.001
Contraction time	s.	0.126	0.116	1.02 (N.S.)	t′ = 3.47 ^{o}f = 25	<0.002

Table 5.9 Comparison of gastrocnemii muscles from normal and dystrophic hamsters

gastrocnemius muscle develop maximum isometric tensions which are similar, with values of 3.25N and 3.57N respectively. These two muscles are very different with regard to their contraction times, with values of 1.51 seconds and 0.126 seconds respectively. The isometric tensions, contraction times and the visco-elastic constants of the stimulated muscle are compared in Table 5.10. It can be seen from this table that both the parallel elasticity, E_1 , and the series elasticity, E_2 , are significantly different, but there is no significant difference between the viscosities of the two muscles.

In Table 5.11 normal hamster muscle is compared with rat soleus muscle. In this pair of muscles the contraction times and the maximum isometric tensions are both highly significantly different. The parallel elasticity and the series elasticity are also highly significantly different for each muscle, but as in the comparisons made in Table 5.10, no difference is shown between the viscosities of the muscles.

7. Effect of temperature on components of tortoise muscle

Tortoise gastrocnemius muscle was stretched in the relaxed and tetanised state at temperatures within the range of 30°C to 5°C (which was the lower limit of the cooling apparatus). Stretches were applied at temperature intervals of 5°C. Typical length and tension records for tortoise muscle at 30°C and 5°C are given in Figs. 5.13a and 5.13b.

	Tortoise (15°C) gastrocnemius mean values n = 9	Normal hamster gastrocnemius mean values n = 14	F′ N.S.= not significant	t′ or d	P N.S. = not significant
E_1 stim.	1200	840	9.84	d = 2.54 ^{o}f = 9	<0.05
E_2 stim.	5400	3800	6.91	d = 2.60 ^{o}f = 9	<0.05
V_2 stim.	360	260	3.53	d = 2.03 ^{o}f = 11	N.S.
Isometric tension N.	3.25	3.57	3.17	t′ = 0.68 ^{o}f = 21	N.S.
Contraction time s.	1.51	0.13	2232	d = 5.85 ^{o}f = 8	<0.001

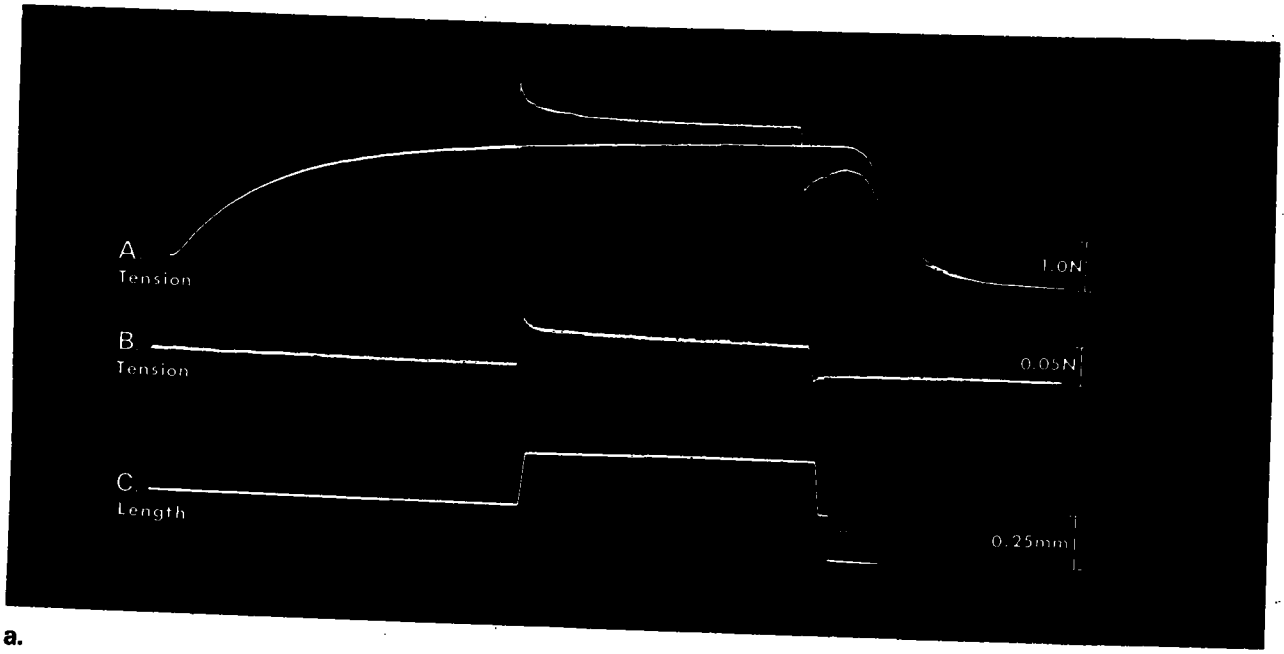
Table 5.10 Comparison of normal hamster gastrocnemius muscle with tortoise gastrocnemius muscle at 15°C.

	Normal hamster gastrocnemius mean values n = 14	Rat soleus mean values n = 9	F N.S. = not significant	t or d	P N.S. = not significant
E_1 stim.	840	220	13.9	d = 16.5 ^{o}f = 16	<0.001
E_2 stim.	3800	2600	3.42	d = 5.75 ^{o}f = 21	<0.001
V_2 stim.	260	220	8.08	d = 2.06 ^{o}f = 17	N.S.
Isometric tension N.	3.57	0.84	34.7	d = 12.3 ^{o}f = 14	<0.001
Contraction time s.	0.13	0.32	12.5	d = 10.5 ^{o}f = 9	<0.001

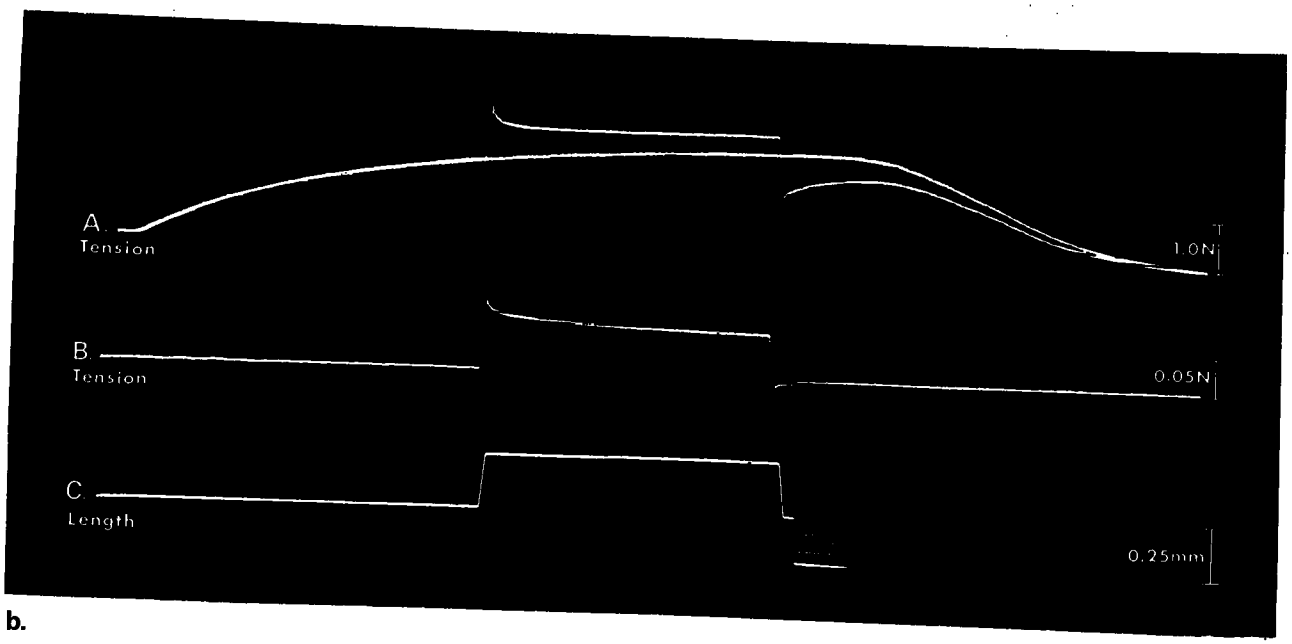
Table 5.11 Comparison of normal hamster gastrocnemius muscle with rat soleus muscle

Fig. 5.13a Length and tension traces from a 0.26 mm ramp stretch applied to tortoise gastrocnemius muscle at 30°C. Trace A is the tension produced by the application of a ramp stretch to tetanised muscle, superimposed over an unstretched tetanus. Trace B is the tension produced in relaxed muscle when a ramp stretch is applied, and trace C is the applied ramp stretch. The time markers at the end of the length record are spaced at intervals of 0.10 seconds.

Fig. 5.13b Traces similar to those of Fig. 5.13a, but at a temperature of 5°C. The time markers are spaced at intervals of 0.10 seconds.


A
Tension
1.0N
B
Tension
0.05N
C
Length
0.25mm

a.


A
Tension
1.0N
B
Tension
0.05N
C
Length
0.25mm

b.

Results were obtained at 30, 25, 20 and 15°C. Thereafter the apparatus was left to cool to 5°C and further stretches applied to the muscle at this temperature. The apparatus required a considerable time to cool at the lower temperature and it was therefore not possible to equilibrate the temperature at 10°C. Mean values of the constants E_1 , E_2 and V_2 of seven muscles in the relaxed and tetanised states are given, for each temperature interval, in Table 5.12.

7.1 Relaxed muscle

The values of E_1 , E_2 and V_2 for a single muscle are displayed graphically in Fig. 5.14 and the equivalent graphs for the mean values of these constants are given in Fig. 5.15. It can be seen that the visco-elastic constants all decrease in value with increasing temperature, with the largest decrease taking place in E_2 .

7.2 Tetanised muscle

The values of E_1 , E_2 and V_2 for a single muscle are displayed graphically in Fig. 5.16 and the equivalent graphs for the mean values of these constants are given in Fig. 5.17. In the stimulated state, the parallel elasticity, E_1 , increases with increasing temperature. The viscosity does not vary a great deal but has its highest value at the lowest temperature. The value of the series elastic element, E_2 , is low at 5°C, increases to a maximum at 20°C and then decreases once more above this temperature. The maximum

Temperature °C	Isometric tension (mean values) N.	Contraction time (mean values) s.	E_1 (mean values) Nm^{-1}		E_2 (mean values)		V_2 (mean values) Nsm^{-1}	
			Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated
30	4.13 S.E.= 0.63	0.87 S.E.= 0.13	150 S.E.= 22	1900 S.E.= 160	180 S.E.= 17	5600 S.E.= 600	23 S.E.= 4.2	410 S.E.= 54
25	4.21 S.E.= 0.79	1.36 S.E.= 0.14	140 S.E.= 21	1500 S.E.= 180	160 S.E.= 24	5900 S.E.= 520	24 S.E.= 6.5	470 S.E.= 94
20	4.02 S.E.= 0.59	1.39 S.E.= 0.18	190 S.E.= 68	1100 S.E.= 91	195.4 S.E.= 33	6000 S.E.= 650	38 S.E.= 9.2	420 S.E.= 65
15	3.25 S.E.= 0.50	1.51 S.E.= 0.23	240 S.E.= 76	1200 S.E.= 150	310 S.E.= 90	5400 S.E.= 610	39 S.E.= 16	360 S.E.= 47
5	2.06 S.E.= 0.39	2.77 S.E.= 0.36	240 S.E.= 41	1000 S.E.= 63	330 S.E.= 24	4700 S.E.= 320	35 S.E.= 3.5	590 S.E.= 71

Table 5.12 Effect of temperature on the visco-elastic constants of tortoise gastrocnemius muscle

Fig. 5.14 The effect of temperature on the values of the elastic constants, E_1 (□) and E_2 (▲), and on the viscous constant, V_2 (●), for a single tortoise gastrocnemius muscle (muscle number 1 of Table 5.13) in the relaxed state.

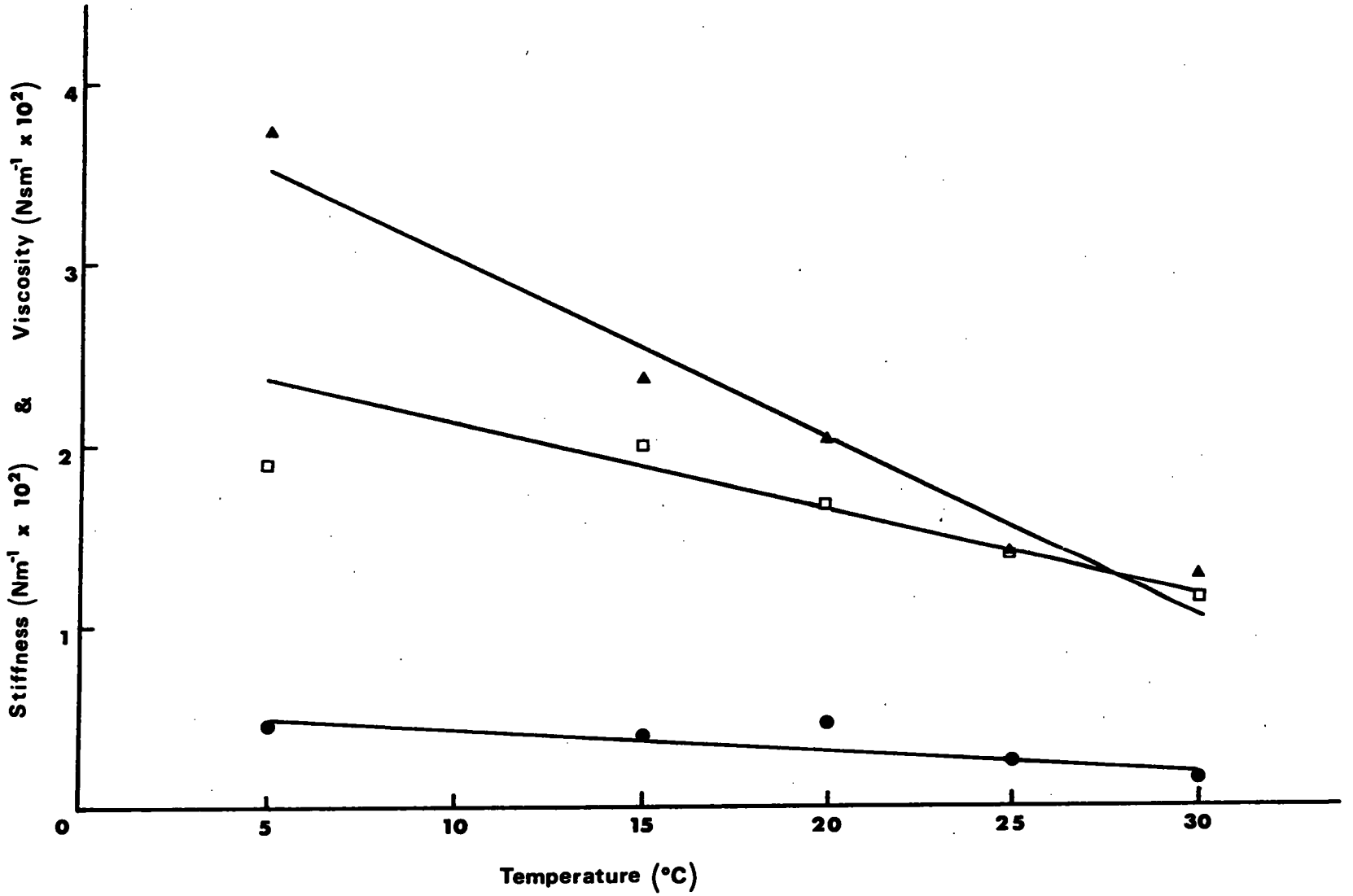

Stiffness (Nm^{-1} x 10^2) & Viscosity (Nsm^{-1} x 10^2)
4
3
2
1
0
5
10
15
20
25
30
Temperature (°C)

Fig. 5.15 The effects of temperature on the mean values of the elastic constants, E_1 (□) and E_2 (▲), and on the viscous constant, V_2 (●), for tortoise gastrocnemius muscle in the relaxed state.

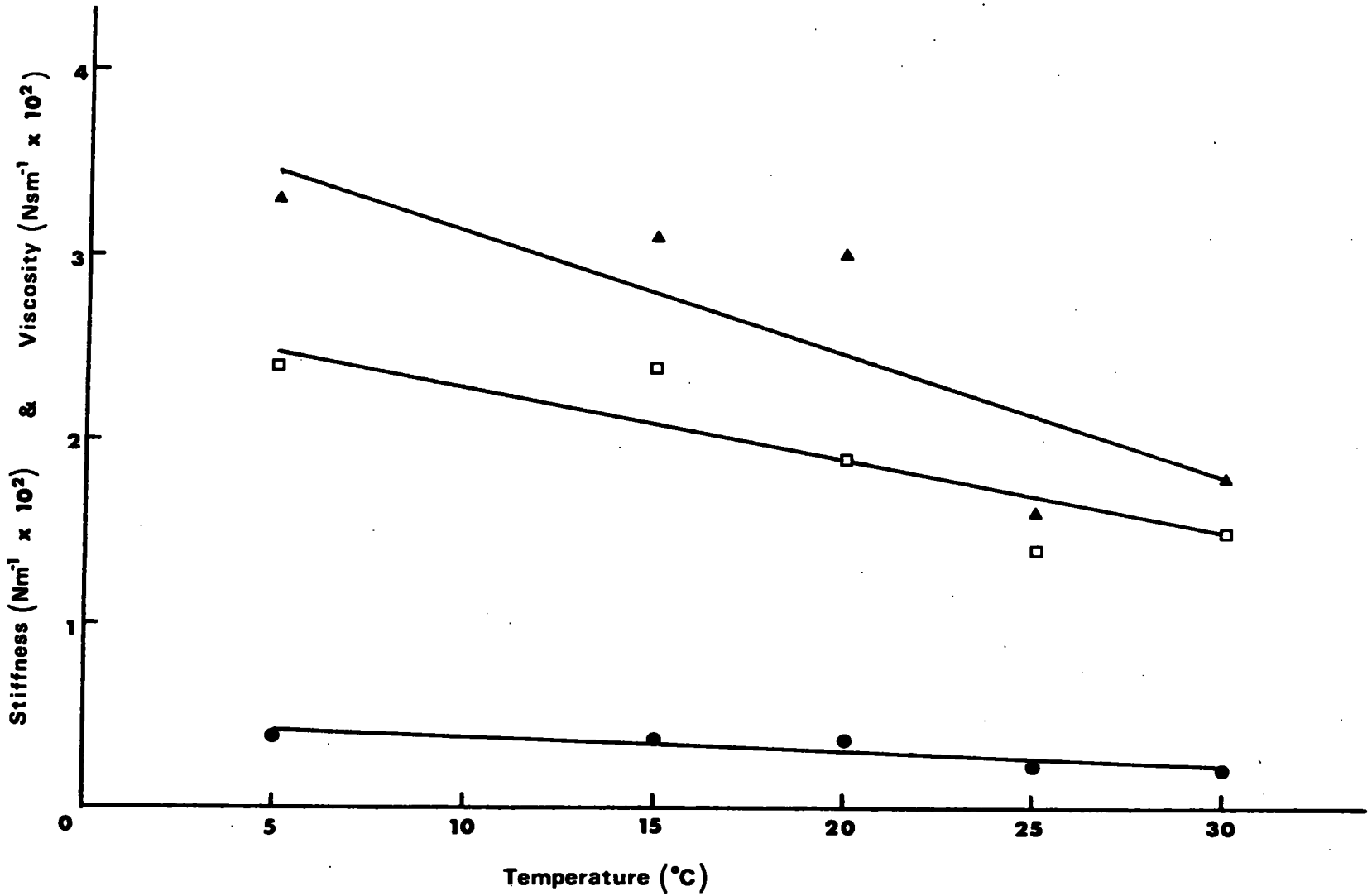

Stiffness (Nm^{-1} x 10^2) & Viscosity (Nsm^{-1} x 10^2)
0
1
2
3
4
5
10
15
20
25
30
Temperature (°C)

Fig. 5.16 The effects of temperature on the elastic constants, E_1 (□) and E_2 (▲), and on the viscous constant, V_2 (●), for a single tortoise gastrocnemius muscle (muscle number 1 of Table 5.13) in the tetanised state. The rise in viscosity between 15°C and 5°C is indicated by a dotted line.

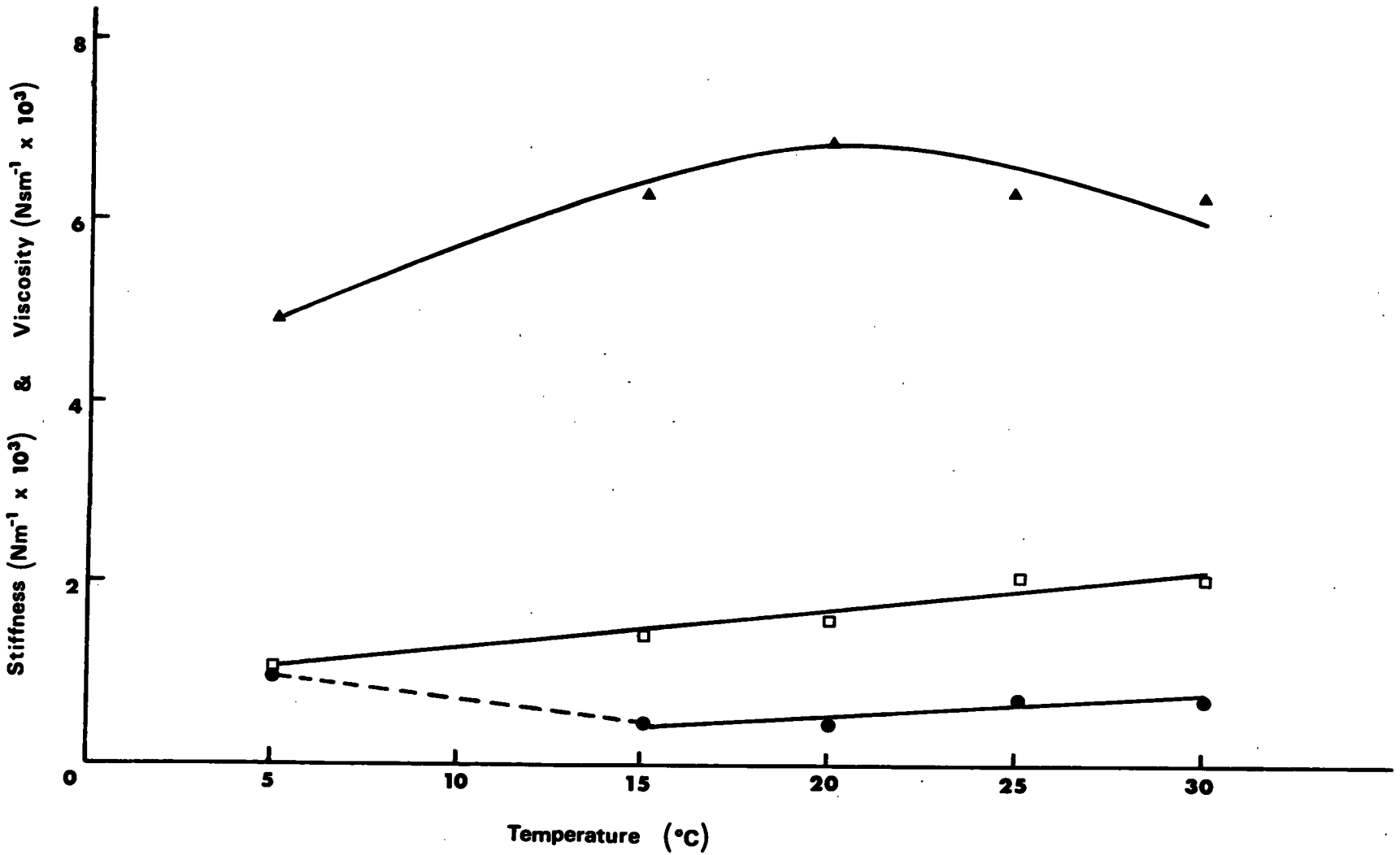
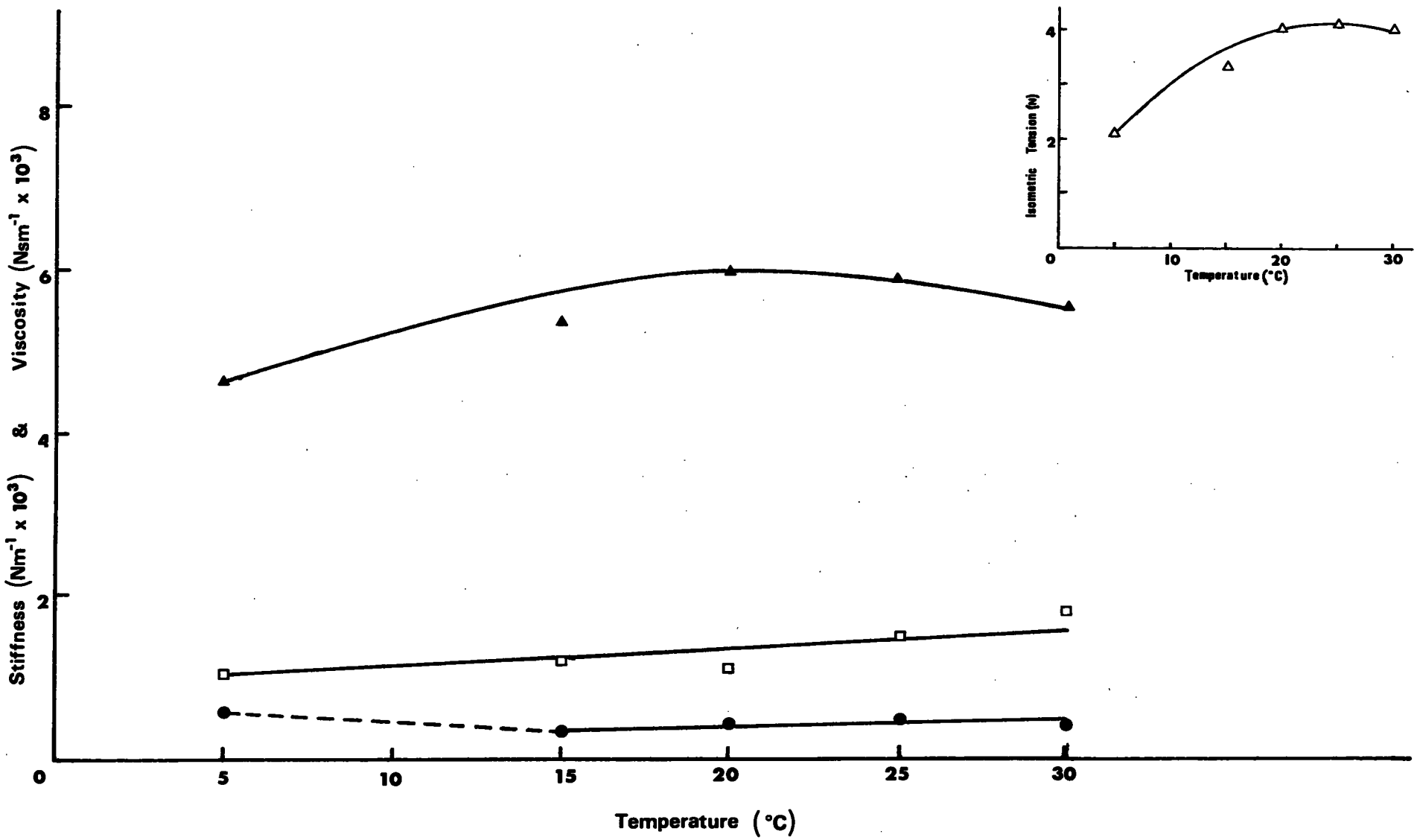

Stiffness (Nm^{-1} x 10^3) & Viscosity (Nsm^{-1} x 10^3)
8
6
4
2
0
5
10
15
20
25
30
Temperature (°C)

Fig. 5.17 The effects of temperature on the mean values of the elastic constants, E_1 (□) and E_2 (▲), and on the viscous constant, V_2 (●), for tortoise gastrocnemius muscle. Inset is the effect of temperature on the mean maximum isometric tension, for comparison with the series elasticity, E_2 . The rise in viscosity between 15°C and 5°C is indicated by a dotted line.


Stiffness (Nm^{-1} x 10^3) & Viscosity (Nsm^{-1} x 10^3)
0
2
4
6
8
5
10
15
20
25
30
Temperature (°C)
Isometric Tension (N)
0
2
4
10
20
30
Temperature (°C)

isometric tension increases with temperature with a Q_{10} of 1.4 between 5°C and 20°C, reaching its highest value at 25°C then decreasing slightly at 30°C. There is a similarity between the relationship to temperature of both the series elasticity and the isometric tension. The relationship between E_2 and the isometric tension is linear ($r = 0.949$, $P < 0.02$) over the range of temperature from 5°C to 30°C.

8. The five component model

Values of the visco-elastic constants of the three component Maxwell model are given in Table 5.13 for seven tortoise gastrocnemius muscles at 25°C. Values of the visco-elastic constants of the five component model were derived for the same muscles (Materials and Methods, section 3.3.2). These values are presented in Table 5.14.

The value of the parallel elastic component of each muscle is the same for both models. The value of the series elastic element, E_2, is smaller in every case, in the five component model than in the three component model. In most muscles the viscous element, V_2, remains unaltered.

In Fig. 5.18 the experimental curve is compared with curves derived from each model. The experimental tension decay can be seen to be best fitted by a curve which can be predicted by the five component Maxwell model. This curve consists of the sum of two exponential terms, one of which has a large decay constant and the other a relatively small one.

Muscle number	Isometric tension N.	Contraction time s.	E_1 Nm^{-1}		E_2 Nm^{-1}		V_2 Nsm^{-1}		$\frac{E_1 \text{ Stim.}}{E_1 \text{ Relax.}}$	$\frac{E_2 \text{ Stim.}}{E_2 \text{ Relax.}}$	$\frac{V_2 \text{ Stim.}}{V_2 \text{ Relax.}}$
			Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated			
1	6.64	1.51	140	2100	140	6300	22	740	15	45	34
2	3.79	1.15	100	1400	130	5500	31	390	14	42	13
3	6.11	1.36	150	1700	190	6900	12	680	11	36	57
4	3.56	1.27	180	2000	190	7000	22	380	11	37	17
5	1.95	1.62	65	1000	85	4300	9.1	210	15	51	23
6	1.86	1.86	220	1000	260	4100	59	200	4.5	16	3.4
7	5.60	0.70	100	1400	110	7100	16	690	14	64	43
Mean values	4.21	1.35	140	1500	160	5900	24	470	12	41	27

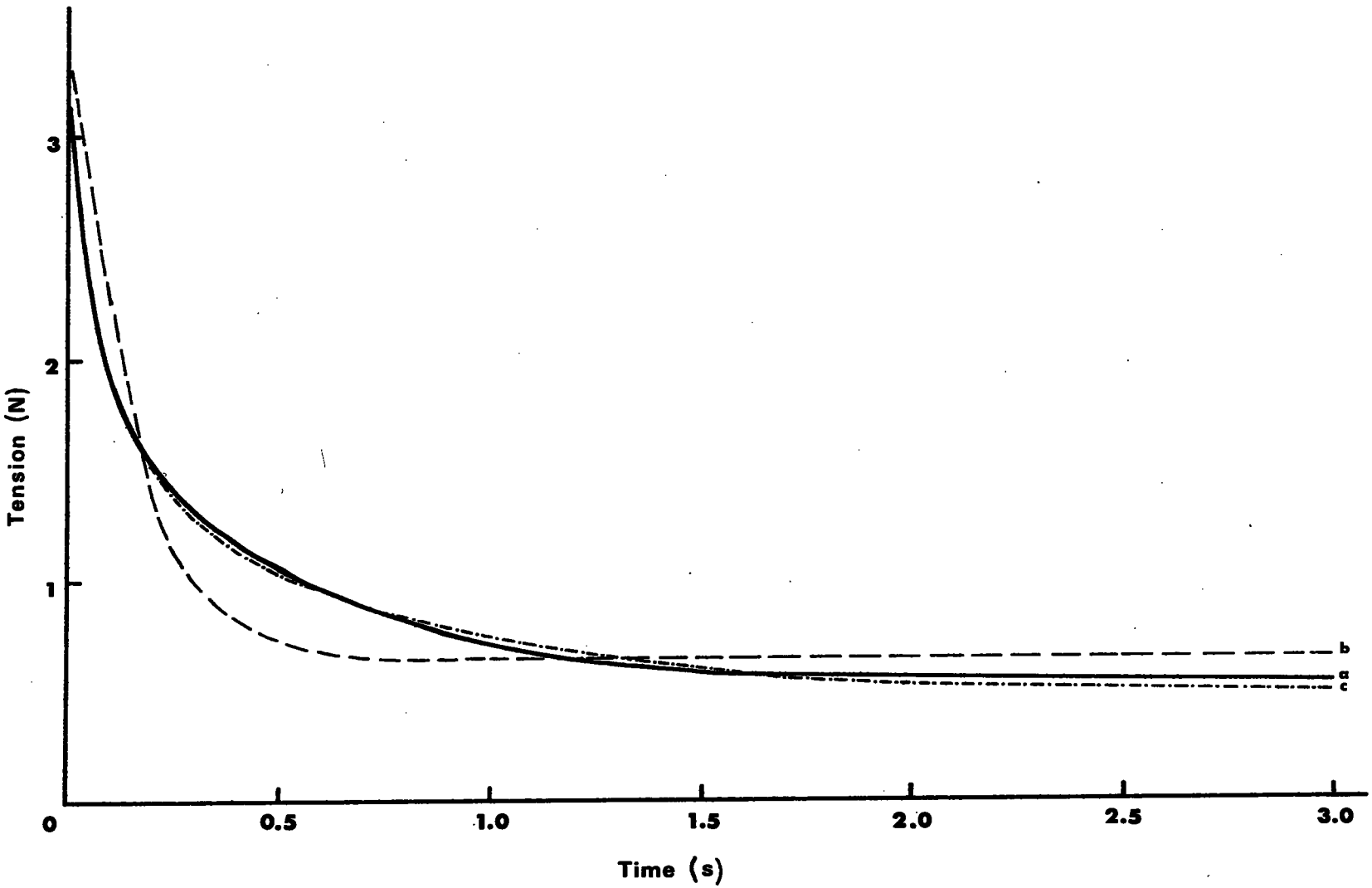
Table 5.13 Tortoise gastrocnemius muscle: three component Maxwell model
Temperature, 25°C.

Muscle number	Isometric tension N.	Contraction time s.	E_1 $N\,m^{-1}$		E_2 $N\,m^{-1}$		V_2 $N\,s\,m^{-1}$		E_3 $N\,m^{-1}$		V_3 $N\,s\,m^{-1}$	
			Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated	Relaxed	Stimulated
1	6.64	1.51	160	2000	140	4800	74	800	240	1400	2.9	35
2	3.79	1.15	100	1400	100	4300	32	380	8.6	740	0.33	13
3	6.11	1.36	150	1700	120	5600	12	710	37	300	0.81	12
4	3.56	1.27	170	1900	110	5800	27	480	37	1000	1.2	24
5	1.95	1.62	65	810	59	4000	7.4	310	71	400	1.5	20
6	1.86	1.86	210	1000	170	3800	70	200	220	44	4.6	0.70
7	5.60	0.7	99	1400	87	5600	16	710	50	67	1.7	19
Mean values	4.21	1.35	140	1500	110	4800	34	510	95	560	1.9	18

Table 5.14 Tortoise gastrocnemius muscle: five component Maxwell model
Temperature, 25°C.

Fig. 5.18 Curves fitted to the tension decay (continuous line, a) of the static phase of a ramp stretch applied to tetanised tortoise gastrocnemius muscle at 5°C. Curve b, (dotted line), is the fit of the three component model, and curve c, (chain line), is the fit of the five component model.

The root mean square difference (of 30 equidistant points) for the three component fit is 0.52N and for the five component fit is 0.14N.


Tension (N)
3
2
1
0
0.5
1.0
1.5
2.0
2.5
3.0
Time (s)
b
a
c

9. Tension changes used in discussion of the Huxley model of muscle.

In the discussion, tension changes in relaxed and tetanised muscle are related to the model of muscle derived by A.F. Huxley (1957). Differences between rat soleus and ECRL muscles, and between the gastrocnemius muscles of normal and dystrophic hamsters, are discussed in terms of this model. Changes in the responses of tortoise gastrocnemius muscles with temperature are also examined.

The tension changes related to the Huxley model are given in Table 5.15, for rat muscles, Table 5.16 for hamster muscles, and Table 5.17 for the muscles of the tortoise.

	SOLEUS		ECRL	
	Mean	S.E.	Mean	S.E.
TETANISED MUSCLE				
Half-rise time (s)	0.16	±0.018	0.043	±0.0031
Half-fall time (s)	0.24	±0.021	0.043	±0.0045
Maximum isometric tension (N)	0.84	±0.048	0.42	±0.070
Dynamic elasticity (Nm^{-1})	1800	±73	910	±140
Static elasticity (Nm^{-1})	220	±13	260	±7.3
$\frac{1}{2}$-decay time (s)	0.061	±0.0025	0.13	±0.020
$\frac{3}{4}$-decay time (s)	0.19	±0.011	0.29	±0.032
RELAXED MUSCLE				
Dynamic elasticity (Nm^{-1})	82	±8.1	47	±7.3
Static elasticity (Nm^{-1})	78	±9.0	88	±15
$\frac{1}{2}$-decay time (s)	0.056	±0.0022	0.23	±0.073

TABLE 5.15 Rat soleus and ECRL muscle

	NORMAL		DYSTROPHIC	
	Mean	S.E.	Mean	S.E.
TETANISED MUSCLE				
Half-rise time (s)	0.063	±0.0022	0.058	±0.0024
Half-fall time (s)	0.078	±0.0037	0.090	±0.0052
Maximum isometric tension (N)	3.56	±0.22	2.51	±0.18
Dynamic elasticity (Nm^{-1})	2500	±130	1400	±82
Static elasticity (Nm^{-1})	840	±36	540	±50
$\frac{1}{2}$-decay time (s)	0.048	±0.0029	0.13	±0.012
RELAXED MUSCLE				
Static elasticity (Nm^{-1})	64	±6.9	24	±3.1
Dynamic elasticity (Nm^{-1})	33	±3.5	25	±2.0
$\frac{1}{2}$-decay time (s)	0.031	±0.0024	0.070	±0.0069

TABLE 5.16 Normal and dystrophic gastrocnemius muscles

	30°C		25°C		20°C		15°C		5°C	
STIMULATED MUSCLE	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S.E.
half-rise time (secs)	0.44	±0.064	0.65	±0.093	0.70	± 0.093	0.75	±0.12	1.3	±0.17
half-fall time (secs)	0.48	±0.083	0.61	±0.10	0.91	± 0.19	1.43	±0.32	4.2	±1.0
max.isometric tension (N)	4.1	±0.064	4.1	±0.67	4.0	± 0.58	3.2	±0.50	2.1	±0.34
dynamic elasticity (Nm^{-1})	3600	±288	3400	±460	3700	± 350	3400	±380	3300	±350
static elasticity (Nm^{-1})	1800	±204	1400	±156	1100	± 92	1200	±145	1000	± 75
half-decay time (secs)	0.059	±0.017	0.053	±0.0077	0.049	± 0.0043	0.047	±0.0039	0.089	±0.0081
$\frac{3}{4}$ decay time (secs)	0.25	±0.043	0.28	±0.073	0.15	± 0.014	0.14	±0.012	0.54	± 0.091
RELAXED MUSCLE										
dynamic elasticity (Nm^{-1})	130	±12	120	±20	160	± 28	240	±79	260	± 21
static elasticity (Nm^{-1})	150	±15	140	±21	190	± 67	240	±75	250	±37
half-decay time (secs)	0.095	±0.014	0.10	±0.017	0.13	± 0.015	0.082	±0.012	0.08	±0.01

TABLE 5.17 Tortoise gastrocnemius muscle

VI DISCUSSION

In section V of the thesis results are given which are derived from length changes applied to muscles of the rat, hamster and tortoise, both in the relaxed and contracted states. The static and dynamic tension changes, and the half-decay times were determined for each stretch. These figures were used to compute the dynamic and static elasticity for each muscle and were also used to compare the behaviour of each muscle with a linear Maxwell model consisting of two elastic elements, E_1 and E_2, and a viscous element, V_2 .

These results will now be considered in relation to the sliding filament model of muscle which was proposed by A.F. Huxley (1957) and extended by A.F. Huxley and Simmons (1970, 1971), and A.F. Huxley (1974). A summary of the Huxley model is given before correlating the results of this thesis with that model.

The Sliding Filament Model of Muscle

Structure of muscle fibres

Muscle fibres contain the two filamentous proteins, actin and myosin (Straub, 1943; Szent-Gyorgyi, 1943). These filaments interdigitate between one another (H.E. Huxley, 1953). The myosin is confined to the A bands of a muscle fibre (Hanson and H.E. Huxley, 1953) and the A bands maintain a constant length when the muscle is lengthened and shortened (H.E. Huxley and

Hanson, 1954; A.F. Huxley and Niedergerke, 1954). This has led to the structure of the contractile proteins of muscle being represented as in fig.1 of A.F. Huxley (1957).

The length of the myosin filament is about 1.60/um (Page and H.E. Huxley, 1963) and the actin is about 1.95/um long (Page 1968). It is probable that the lengths of these two filaments remain fairly constant during activation of the muscle (H.E. Huxley and Brown, 1967). Length changes in muscle are brought about by the two sets of filaments sliding over one another.

The repeating unit of these interdigitating proteins in a muscle fibre, the sarcomere, varies in length as the filaments slide over one another. The maximum force is generated by a muscle when the length of the sarcomere is 2.0 - 2.2/um (Gorden et al., 1966). It is likely that this sarcomere length is constant within the vertebrates (A.F. Huxley, 1974).

In addition to the proteins actin and myosin, it seems probable that there is another series of filaments, which are continuous throughout the myofibril (Carlsen et al., 1961; Sjostrand, 1962; McNeill and Hoyle, 1967; Walcott and Ridgeway, 1967).

Generation of force by a muscle

It has been shown by Ramsey and Street (1940), and by A.F. Huxley and Niedergerke (1954), that the tension which can be generated by a muscle during isometric contraction depends upon the length at which the muscle is held. Alteration of muscle length and hence

a change in the area of overlap of the filaments alters the force generated, but has no effect on the rate of shortening of the muscle, under conditions of zero load (A.F. Huxley and Julian, 1964; Gordon et al., 1966). This would indicate that force is generated by structures acting in parallel within the sarcomeres, and that these structures have an intrinsic maximum speed of shortening. The rate of shortening of a muscle would then be the sum of the speed of shortening of each sarcomere in series within a muscle fibre.

One aspect of this shows up in muscles with a different sarcomere length from that found in the vertebrates. Muscles with more numerous but shorter sarcomeres should be capable of more rapid rates of shortening while those with longer sarcomere lengths should be slower. A relationship of this kind has been shown in arthropods, where sarcomere length varies greatly from one muscle to another (Jasper and Pezard, 1934; Atwood et al, 1965).

Cain et al. (1962) have shown that during muscle contraction adenosine triphosphate (ATP) is converted to adenosine diphosphate (ADP) and inorganic phosphate (Pi).

These relationships indicate that force is generated by some mechanism in the overlap zone of actin and myosin. These force generators act in parallel to generate a force, and the rate of shortening is the same for each. The generation of force within the sarcomeres is coupled to the supply of energy from the breakdown of ATP.

It is probable that force generation is initiated in response to the release of calcium ions from the transverse membrane system which invaginates throughout each muscle fibre (see Ebashi and Endo, 1968, and Ebashi et al, 1969).

Action of the force generators

The force generators, or cross-bridges, can probably be identified with projections from the myosin molecules observed by H.E. Huxley (1957, 1963).

Since the total length of a myosin molecule is about 0.2/um (Rice, 1961; Zobal and Carolson, 1963; Rowe, 1964) and the amount of sliding in a single contraction may be as much as 1.0/um, it is unlikely that any one cross-bridge remains intact throughout a contraction. It is likely that there is a cycle of forming, breaking and reforming of each cross-bridge. According to the hypothesis for the mechanism of muscle contraction put forward by A.F. Huxley (1957), the tension, P, generated during an isometric contraction is:

$$P = \left(\frac{f}{f+g}\right) \cdot \frac{msw}{2l}$$

where m = the number of binding sites on the myosin, per cubic metre.

s = sarcomere length

w = the maximum work done in a cycle at one site

l = separation of binding sites along the actin filament

f = rate constant governing formation of cross-bridges

g = rate constant governing breakdown of cross-bridges.

It has been suggested (H.E. Huxley, 1969) that each of these cross-bridges consists of two components: an element which can rotate and an inelastic element which links the rotating head to the actin molecule. This proposed structure of the cross-bridge has been modified (A.F. Huxley and Simmons, 1971; A.F. Huxley, 1974) so that the link between the rotating head and the myosin molecule is elastic instead of inelastic, and further that the rotating head can exist in two, three or four stable positions, each with a lower potential energy than the one before. The cross-bridge head would be able to switch, reversibly, from one stable state to another.

To stretch the cross-bridge link, and hence generate a force, the cross-bridge head must rotate to a position of lower potential energy. This would require activation energy only.

The energy necessary to drive such a system could come from the final detachment of the cross-bridge head from the actin molecule, after the cross-bridge head has reached the position of lowest potential energy. This final splitting of the acto-myosin link would be coupled to the hydrolysis of a molecule of ATP (Taylor, 1972). Once detached, the myosin head would then become available for binding with another site on the actin molecule.

Repetition of this process would bring about the sliding of actin and myosin molecules between one another, with the subsequent shortening of the sacromeres and shortening of the muscle during isotonic contraction. The cycles of shortening of cross-bridges during an isometric contraction of the muscle would result in the generation of force.

Some variations on this theory of contraction of muscle are discussed by A.F. Huxley (1974).

The Response of Muscle to Alteration of Length

Stress relaxation following stretch

The application of controlled stretches to muscle has been used to study the properties of muscle and to help elucidate the mechanism of muscle contraction.

Gasser and Hill (1924) applied ramp stretches of about 4.5mm to the sartorius muscle of frog and found that at moderate velocities of stretch, probably in the range of 1.0 - 10cms sec^{-1}, the tension rose to a peak during the dynamic phase of the ramp and then decayed roughly exponentially to some new level of tension. At slow rates of stretch, probably $<$1.0cms sec^{-1}, the change in tension had the same form as the extension curve, and was reversible. At faster rates of stretch, up to 80cms sec^{-1}, Gasser and Hill found that the tension rose to a peak and then fell to a level below that of isometric tension, before redeveloping to some new level approaching that of isometric.

These findings were related by Gasser and Hill to a model of muscle consisting of a protein matrix in a fluid medium, with the proteins having different viscous and elastic coefficients in the passive and active states.

The results of Sugi (1972) are similar to those of Gasser and Hill (1924). Moderate velocities of ramp stretches applied to the semitendinosus muscle of frog produced tension responses similar to those of the moderate velocity stretches of Gasser and Hill, and similar to those shown in fig.5.8 trace A. Ramp stretches greater than 1% of the length of the muscle, and at velocities >30cms sec^{-1} produced tension responses similar to those of the rapid stretches of Gasser and Hill. However, the rapid stretches of Sugi for less than 1% of muscle length did not show the decay below isometric tension, but continued to develop tension until the end of the ramp stretch, and then decayed to some plateau value. Sugi has explained his results in terms of the A.F. Huxley (1957) sliding filament model of muscle.

Stress relaxation curves, similar to those for moderate velocity stretches applied to active muscle by Gasser and Hill, by Sugi and as seen in fig.5.8 trace A of this thesis, are also shown by Abbott and Aubert (1951). Stress relaxation following ramp stretches applied to passive muscle, similar to those of fig. 5.8 trace B, can be seen in the publications of Abbott and Lowy (1956), D.K. Hill (1968) and Ullrich (1970).

Stress recovery following release

When tension redevelopment following a constant velocity reduction in length is examined, two phases can be seen (A.F. Huxley and Simmons, 1970, 1971; A.F. Huxley, 1974). In the example of Huxley and Simmons, the tension recovery of frog semitendinosus muscle at 0°C - 4°C has a fast phase with a half time of about 0.5msecs - 1.0msecs, and a slow phase with a half time of about 25msecs.

Similar results to those of Huxley and Simmons have been shown in response to rapid constant velocity shortening or lengthening of glycerinated frog sartorius and tortoise ileo fibularis muscles by Heinl et al.(1974).

Length transients in response to step changes in load, consisting of two phases, which may be equivalent to the tension transients of Huxley and Simmons, have been demonstrated by Podolsky (1960), Civan and Podolsky (1966) and by Joyce and Rack (1969).

A stress relaxation curve with two decay constants is discussed in relation to the results of this thesis.

Linearity of Tension Responses

Soleus muscles of the rat were subjected to stretches of up to 0.85mm. The static tension changes in active muscle, as a result of ramp stretches, were linear up to stretches of 0.5mm in every muscle, and in some muscles were linear over the range of stretch from 0 - 0.85mm. The dynamic tension changes of active muscle were found to

be linear for length changes up to 0.26mm.

A ramp of 0.26mm is equivalent to a length of change of about 1% of total muscle length. However, soleus muscle of the rat has fibres which are approximately 50% of the length of the muscle (Close, 1964). A stretch of 1% of the muscle length would thus represent an increase in sarcomere length of 2%, if all of the stretch extended the fibres. If some of the length change is taken up by passive elements of the muscle in series with the muscle fibres (e.g. tendon - see Pennycuick, 1964; D.K. Hill, 1969; Blangé et al 1972) then the linearity of the tension response is likely to be less than 2% of the sarcomere.

The range of linearity of the elastic components of rat soleus muscle would appear to lie within the range of 1 - 2% extension of the muscle fibres.

The value of the dynamic tension may depend on purely passive parts of the muscle or may also be due to elasticity within the cross-bridges of the muscle. The non-linearity of the dynamic tension change could be due to the mechanical breakdown of these cross-bridges, when the muscle fibres are extended by more than 1 - 2% of their length. If this is the case, the non linearity would be such that the dynamic tension change should become relatively smaller at longer extensions of the muscle. This is what is found in active muscle (see fig. 5.3). This non linearity of the dynamic tension may be still further increased, since the rise time of each stretch was constant, thus increasing the velocity of stretch with any

increase in amplitude of stretch. According to the A.F. Huxley theory of muscle contraction, increasing the rate of stretch will increase the rate of breakdown of cross-bridges.

Since the non linearity of the tension responses of passive muscle is the result of the tension becoming relatively greater at longer lengths of stretch (see fig. 5.1), it is unlikely that, at these longer lengths of stretch, the static and dynamic tensions of passive muscle are a measure of the same parameter as in active muscle.

The ECRL muscles of rat, and the gastrocnemius muscles of normal and dystrophic hamster, and of tortoise, were found to give linear tension responses within the linear limits of stretch found for rat muscle. When discussing these muscles, tension changes have been used which correspond either to a 0.26mm extension of the muscle or to a stretch which would give approximately a 1% extension of the sarcomeres. In either case, the amount of stretch applied to the muscle is within the linear range of the tension responses.

Rat Muscle

Dynamic tension changes and stress relaxation

The values for dynamic tension changes in passive and active muscles of the rat, and decay times of the stress relaxation curves are given in Table 5.15.

The dynamic tension changes observed in relaxed muscle may be due to the extension of inert parts of the

muscle (Guth, 1947; A.V. Hill, 1952; McNeill and Hoyle, 1967; Rapoport, 1973), or they may be the result of the extension of cross-bridges within the passive muscle (D.K. Hill, 1968, 1970). Alternatively, the dynamic tension changes of passive muscle may be due to a combination of these two possible processes.

If the dynamic elasticity of passive muscle is due to cross-bridge activity, then this activity could be expected to increase upon stimulation. If the dynamic elasticity of passive muscle is due to passive elastic elements in parallel with the contractile mechanism, then, when the sarcomeres of the muscle shorten at the expense of series elastic elements, during isometric contraction, the parallel structures would also shorten, giving a reduction in the dynamic tension. It can be seen from Table 5.15 that the dynamic elasticity increases upon stimulation of the muscle. The dynamic tension change in passive muscle is only about 5% of the dynamic tension change in active muscle, for the same stretch. It is therefore likely that dynamic tension changes in contracting muscle are due to extension of some part of the contractile mechanism of the muscle.

It is not possible to say whether the dynamic elasticity of passive muscle is due to passive or active elements of the muscle, nor is it possible to say whether the dynamic elasticity is measuring the same part of the muscle in its passive and active states.

If the dynamic elasticity is a function of the contractile mechanism of the muscle, it might be expected that some correlation should be found between the dynamic tension changes and the isometric force generated by the muscle. In fact a high degree of correlation was found for soleus muscle ($r \simeq 0.923$ giving a P value < 0.001), but this correlation was not shown for ECRL muscles.

The value of the dynamic tension of active muscle can be calculated for a 1% increase in the length of the sarcomeres. Some cursory measurements of fibres of ECRL muscles indicate that they run for approximately 50% of the length of the muscle. A 1% in the length of the soleus muscles would correspond to an extension of 0.135mm and the equivalent extension of ECRL muscle would be 0.0725mm. These extensions presume a rigid tension, but for the purposes of comparison, may be legitimate.

The increase in the dynamic tension in response to a 1% extension of the sarcomeres, when calculated as a percentage increase over the isometric tension, is approximately 29% for soleus muscles and 16% for ECRL.

The value of the dynamic tension changes for soleus muscle is about the same order of magnitude given by Rack and Westbury (1974) for cat soleus muscle (35% - 50% for a 1% extension of the sarcomeres), though the values in this thesis are slightly lower. The figures quoted from Rack and Westbury refer to their "short range stiffness" within the muscle. The values of the dynamic elasticity quoted for rat muscle in this thesis are also

comparable in magnitude to the slower elasticity of A.F. Huxley and Simmons (1971), though it can be seen from their fig.3 that the increase in tension in response to a 1% increase in the length of the sarcomeres is somewhat greater than those given here for soleus muscle.

In soleus muscles, the extensions of more that 1 - 2% of the sarcomeres produce non-linear dynamic tension responses. It has been shown by Sugi (1972) that at stretches greater than 1% extension of the sarcomeres, and at rapid rates of stretch, the tension response shows a 'slip' phenomenon, attributed to the mechanical breaking of cross-bridges.

From these observations, it seems likely that the dynamic elasticity, as measured for contracting muscle of the rat, is a measure of elasticity within the cross-bridges. The non-linear tension responses of longer stretches are probably the result of the breakdown of cross-bridges.

It has already been noted that the value of the dynamic elasticity of ECRL muscles is low, compared with that of soleus muscles. From fig. 5.8 it can also be seen that in soleus muscle there is a sudden decrease in tension at the end of the dynamic phase, and that stress relaxation during the static phase of the ramp is therefore biphasic in nature. The equivalent stress relaxation in ECRL is not biphasic (see also values of $t_{\frac{1}{2}}$ and $t_{\frac{3}{4}}$ in Table 5.15). The overall decay of tension is more rapid in ECRL than in soleus. If fig. 5.8 is examined, it can

be seen that the decay of tension in ECRL muscle is completed after 1.5 seconds, but in soleus muscle the tension is still decaying at the end of the static phase, after 2.5 seconds.

If the dynamic tension changes are the result of distortion of cross-bridges, then subsequent decay in tension during the static phase of the ramp may be due to the breaking and reforming of these bridges. Some tension decrease at the end of a stretch has also been attributed by A.F. Huxley and Simmons (1971) and A.F. Huxley (1974) to visco-elastic changes within the cross-bridges. However, these tension changes are on a very short time scale (1 - 2 msecs) and probably cannot be observed in the results of this thesis.

When the muscle is stretched, it is possible that some of the cross-bridges are extended into the region where none is made, but only breakdown can take place (c.f. A.F. Huxley, 1957). In the region where cross-bridges can be both made and broken, some cross-bridges will be broken and more will be made, but since the new cross-bridges are not in an extended state, they will not have so great a tension as those which have just been broken. Interaction of cross-bridges in these two regions may explain the initial rapid decline in tension followed by a slower one, which is seen in soleus muscle.

If the above explanation of the tension changes is valid, then the events during the static phase of the ramp should depend on the rate of biochemical activity for

the making and breaking of cross-bridges (rate constants f and g respectively, of A.F. Huxley, 1957). The rate of breaking of cross-bridges should then also be related to the decay of tension after a tetanus, and the rates of making and breaking of cross-bridges should be related to the rise in tension at the start of a tetanus. From Table 5.15 it can be seen that the half-rise and half-fall times of tetanus are longer in soleus than in ECRL, indicating slower rates of reaction, f and g, in soleus muscle.

The faster rates of reaction of ECRL are seen to give a faster decay of tension to the final plateau level than is the case in soleus muscle (compare figs.5.8a and 5.8b).

The duration of the dynamic phase of the ramp stretch was the same for both soleus and ECRL muscles (0.0625s). If the enzymes of ECRL are relatively fast, then the readjustment of making and breaking of cross-bridges would take place during the dynamic phase, as well as the static phase of a ramp stretch. This would mean that the static phase would start on a less steep part of the tension decay, and would also account for the reduction in dynamic elasticity seen in ECRL muscle.

For this explanation to be valid it must be possible for the cross-bridges to be detached by the action of enzymes at relatively long lengths of the cross-bridges. It may be possible for the cross-bridges to be acted on in this way (see A.F. Huxley, 1974 and Julian et al, 1973).

Static Tension Changes

The static tension changes in response to a ramp stretch can be seen to be greater in stimulated muscle than in passive muscle. This is similar to the findings for dynamic elasticity. The increase in static elasticity upon stimulation is not so great as the increase in dynamic elasticity, the static elasticity of passive muscle being about 30% of that found in active muscle, both in soleus and ECRL.

In order to explain the change in the static elasticity, it is necessary first to try to correlate the static tension changes with particular structures of the muscle.

In passive muscle, it may be that static elasticity is a measure of some cross-bridge activity. This may be the case at short lengths of the muscle (D.K. Hill, 1968, 1970) but it is likely that passive elements also contribute to this tension. The passive elements which may play a part are the sarcolemma, at longer muscle lengths (Fields and Faber, 1970; Podolsky, 1964; Rapoport, 1973) or the postulated s-protein filaments linking actin molecules in a sarcomere (Guba and Harsanyi, 1966); Hanson and H.E. Huxley, 1960; McNeill and Hoyle, 1967).

In active muscle, the static elasticity is always greater than in passive muscle. In this case, it is unlikely that the static elasticity is a measure of elasticity of passive structures in the muscle, since any

of these passive structures would be expected to decrease in length and hence exert less tension when the muscle fibres shorten during contraction. It is likely that the static elasticity of active muscle is situated in the contractile mechanism of the muscle.

Since the static elasticity of passive and active muscle is not a measure of the same parameter in each case, and since elements which give rise to static tension in passive muscle no longer do so in active muscle, then it is not surprising that no significant correlation is found between the static elasticity and the isometric tension generated by the muscle, in either soleus or ECRL.

The application of a stretch which would give a 1% increase in the length of the sarcomeres gives corresponding increases in the static tension. When expressed as a percentage increase in tension over the corresponding isometric tension, this increase in tension in the muscle would be 3.21% for soleus and 4.27% for ECRL. The increase in the static tension is slightly greater in ECRL muscle than in soleus and this difference may be further increased, since the tension in soleus has not fully decayed by the end of the static phase. This excess of tension over the isometric can also be seen in the results of Sugi (1972), who showed that for bundles of stimulated frog muscle fibres, a stretch of 5% gave an excess of tension, after a hold phase lasting 0.2 seconds.

One possible explanation for the increase in the value of the static tension over the isometric tension is that the muscle is being stretched on to a different part of the length/tension plot of the muscle. In this case, the increase in the tension would be the same whether the muscle was stretched prior to stimulation or after stimulation. However, for this hypothesis to be true, the muscle would have to be stimulated at an initial length corresponding to the rising limb of fig.12 of Gordon et al., (1966). This is the only part of that curve where an increase in length of the sarcomeres by 1% would give a corresponding increase in force of 4%. However, for this to be the case, the initial striation spacing of the muscle would be less than one half that required to give optimum tension in the muscle. In the present experiments, this would mean that the muscle should have undergone a large initial shortening before the stimulation and stretch were applied. This is not likely to be the case, since the muscles were each fixed at about resting length prior to stimulation and stretch. It would have been useful to have measured the tension generated isometrically by the muscle, at the new longer length, so that tensions which were arrived at by different means could have been compared; unfortunately this was not done. However, it is unlikely that the passive tension increase is due to increased overlap of actin and myosin and subsequent increased numbers of cross-bridges.

In his discussion, Sugi (1972) has suggested that cross-bridges may behave as though they are "locked-on" at small displacements, though rapidly broken down when the stress exceeds a critical limit at larger displacements. The extra tension of the static tension over the isometric tension would then be seen as the result of increased force in some of the cross-bridges. The mechanism of locking on is not given by Sugi, but one possibility is presented here.

Cross-bridges within the muscles can be broken by the action of enzymes, but this may only be possible if the cross-bridge head is at the position of lowest potential energy. It is also possible to snap the cross-bridges mechanically if they are stretched above some critical limit. If the stretch applied to a muscle is sufficient to prevent the cross-bridges reaching this lowest potential energy position, but not long enough to bring about the mechanical breakdown of cross-bridges, then it is possible that after the redistribution of tension in the muscle, during the static phase, some of the cross-bridges will remain in an extended position, unable to be broken by enzymes. It seems likely, as was discussed in the section on dynamic elasticity, that the degree of stretch applied to the muscle would satisfy the conditions necessary for an excess of static tension over isometric tension to be set up.

Another possible explanation for the excess of tension over isometric is that when the muscle is stretched,

some sites on the actin and myosin which were not quite close enough to form cross-bridges, are now able to do so (Podolsky and Nolan, 1973; Podolsky, Nolan and Zaveler, 1969). Existing cross-bridges, since the stretches are within the elastic limits, will tend not to be broken down. The excess tension would then be due to the additional cross-bridges. On the evidence derived from rapid release of muscle, this theory has been largely discounted by A.F. Huxley (1974) and Ford et al., (1974).

Hamster Muscle

The gastrocnemius muscles of normal and dystrophic hamsters were found to give linear tension responses for ramp extensions similar to those which gave linear responses in rat soleus and ECRL muscles. The linear responses of hamster muscle are therefore in the range up to approximately 2 - 4% extension of the sarcomeres. All subsequent discussion of hamster muscle refers to tension responses as a result of stretches within this linear range.

Dynamic tension changes and stress relaxation

The values of the dynamic tension changes and $t_{\frac{1}{2}}$ and $t_{\frac{3}{4}}$ values for the stress relaxation of the static phase of ramp stretches are given in Table 5.16 for normal and dystrophic hamster gastrocnemius muscles in both the passive and active states. These

results can be considered in relation to the discussion on rat muscles, and can also be used to compare normal gastrocnemius muscles with the equivalent muscles from dystrophic hamsters.

In the gastrocnemius muscles of normal hamsters, the static elasticity of passive muscle was found to be 1.33% of the static elasticity of active muscle. In the muscles from dystrophic hamsters, the equivalent figure is 1.75%. Since it was concluded from rat muscle results that the dynamic elasticity is not a function of the same anatomical part of the muscle in active and passive states, the absolute value of this increase is probably not important. However, the increase in dynamic elasticity upon stimulation of rat muscle is confirmed by these results, and it may be possible to use them in order to compare the two types of hamster gastrocnemius muscles.

The larger increase in dynamic elasticity observed in normal muscle is not due to differences in the dynamic tension of the muscles in the passive state, but to differences in the active muscles (see Table 5.16). The dynamic elasticity is much greater in normal muscle than in dystrophic muscle. From Table 5.16 it can be seen that the value of the maximum isometric tension is also greater in the normal muscle.

When the dynamic tension is expressed as a percentage increase in tension over isometric tension, for a 1% extension of the sarcomeres, a figure of 8.1% is

derived for normal muscle and 6.61% for dystrophic muscle. It can now be seen that the difference between the two types of muscle is small, and any dissimilarities between normal and dystrophic muscle do not show as differences between dynamic elasticities.

From fig.5.12 it can be seen that in hamster gastrocnemius muscle, there is no sudden drop in tension at the end of the dynamic phase. In this respect, the response of the hamster muscles is more like that of rat ECRL muscle than rat soleus muscle. In the discussion of rat muscle, it was postulated that differences between muscles in the rapid tension decay following the dynamic phase could be the result of different rates of enzyme controlled breakdown of cross-bridges during the dynamic phase.

If the half-rise time and half-fall time of isometric tension are once more used as indicators of the rate constants f and g of A.F. Huxley (1957), then tension responses more like those of ECRL muscles than of soleus muscles could be expected since f and g of hamster muscles more closely resemble those of ECRL than those of soleus.

The gastrocnemius muscles are also similar to ECRL in that the plateau level of tension (the static tension) is reached about half way through the static phase of the ramp, after about 1.5 seconds, once more indicating faster acting enzymes than are found in rat soleus muscles.

On these grounds, it is reasonable to say that the lack of a sharp drop in tension at the end of the

dynamic phase may be due to relatively quick acting enzymes controlling the making and breaking of cross-bridges.

Static tension changes

The static tension change in response to a ramp stretch was found to be greater in stimulated muscle than in relaxed muscle. This was the case in muscles from both normal and dystrophic hamsters and in this respect, the results are similar to those of rat muscle. In normal hamster gastrocnemius muscle, the static elasticity of passive muscle was 7.69% of the elasticity of active muscle. In dystrophic hamster muscle, the static elasticity of passive muscle was 4.54% of the elasticity of active muscle. Because of the number of variables involved in the calculation of these increases in static tension changes, it is not possible to correlate them with values determined for rat muscles. However, it may be possible to look for differences between the two types of hamster muscle in order to explain the different increases in static elasticity.

The static tension changes in response to a ramp stretch are greater in normal muscle than in dystrophic muscle, and this is the case for both active and passive muscle. The difference is not so great in active muscle, thus giving the larger increase in static elasticity seen in dystrophic muscle. The reduced static elasticity of passive dystrophic muscle may be the result of the presence

of the apparently inactive muscle fibres seen in fig.5.10. The low static elasticity of the muscle would probably be due either to reduced cross-bridge activity, or possibly to the absence of thin s-filaments. Fewer active muscle fibres would also account for the reduction in isometric tension (see also Montgomery, 1975), while the overall size of the muscle remains about the same.

The static tension change for active muscle can be expressed as a percentage increase in tension over isometric tension in response to a 1% increase in the length of the sarcomeres. When this is done, it is found that the increase in normal muscle is 2.7%, while in dystrophic muscle it is about 2.5%. No correlation was found between static elasticity and the maximum isometric force generated by the muscle. In this respect, the result is the same as for rat muscles.

It can be seen that there is no great difference between the values of the static elasticities of normal and dystrophic hamster muscles, and the similarity of the two types of muscles in their dynamic tensions has already been noted.

These results verify those of Montgomery (1975) and Harris and Ward (1975) who found that there is a decrease in force generated in the muscles of dystrophic hamsters, when compared with the muscles of normal hamsters. These authors could show no differences in the mechanical, biophysical or pharmacological properties of the muscles.

Montgomery and Harris and Ward concluded that though some changes are apparent in the muscles of dystrophic hamsters, the myopathy is secondary to the cardiomyopathy present in the dystrophic animals.

It is clear that by means of the techniques employed in the present study, no differences can be shown between the visco-elastic properties of normal and dystrophic hamster gastrocnemius muscles. However, the results obtained from hamster muscles are consistent with those from rat soleus and ECRL muscles.

Tortoise Muscle

The mechanical properties of rat and hamster muscles were studied at the body temperature of these mammals, as given in sections 1.1 and 1.2 of Materials and Methods. These properties have been discussed in terms of the sliding filament model of muscle contraction. In this model, tension changes within a muscle are explained in terms of alterations in number and configuration of cross-bridges linking actin and myosin filaments within the sarcomeres. Much of the discussion of rat and hamster muscle involved the making and breaking of cross-bridges. However, the rate of making and breaking of these cross-bridges should be temperature dependent, since biochemical reactions are involved. At lower temperatures, the rate of the reactions should be slowed down, and this slowing down should not only affect the behaviour of the muscle under isometric stimulation, but should also have

consequences for the behaviour of muscle under controlled stretches, if such behaviour is to be explained in terms of cross-bridge activity.

Tension during isometric tetanic stimulation

Muscles were stimulated over a period of 8.0 seconds. Although the isometric tension was still rising slightly at the end of this period, the trend of the records does indicate that the tension values reached were near maximum (see fig.5.13).

In spite of this inaccuracy, it can be seen that the temperature has a marked effect on the isometric tension. The tension at 20°C is two to three times the value at 5°C. There is usually a slight increase over the range 20°C - 30°C. The pattern of isometric tension change over the temperature range from 5°C to 30°C was a comparatively rapid increase in tension up to 20°C, then a levelling off up to 30°C (see inset of fig.5.17 and Table 5.12).

The half-rise time, as previously defined, decreased markedly with increase in temperature. Most of the decrease was over the range 5°C to 15°C, with a further steady fall up to 30°C. The value at 30°C was about one third of that at 5°C (see Table 5.17). It is noticeable that while the half-rise times are reasonably consistent from muscle to muscle at 30°C, there is quite a variation between them at 5°C. A similar pattern of variation with temperature was seen when the half-rise times were measured.

However, when the values at 30°C and 5°C are compared (see Table 5.17), the proportional amount of decrease of the half-fall time as the temperature is raised is greater than that of the half-rise time.

Several workers have studied the contractile behaviour of mammalian skeletal muscle when subjected to temperature changes (Isaacson et al., 1970; Truong et al., 1964; Close and Hoh, 1968; Cullingham et al., 1960; Duodoumopoulos, 1959).

The present findings are in agreement with previous work in that the isometric tension decreases observed as the temperature is lowered, are accompanied by increases in the half-rise time and the half-fall time of the tension.

In the model of muscle contraction of A.F. Huxley (1957), the formation of cross-bridges has a rate constant, f, and the breakdown of cross-bridges has a rate constant, g. In terms of the Huxley model, one would expect the rate of formation and breakdown of cross-bridges to be slowed down at lower temperatures, and therefore the half-rise times and half-fall times to increase. However, the observed changes in these quantities could also be dependent upon the time taken for the chemical reactions to become fully active or inactive with respect to the onset and cessation or stimulation. This point could be of importance in interpreting the increase in isometric tension with temperature, in terms of the Huxley model. According to the model, the isometric tension, P, is given by the equation on

page 54 of this discussion. If it is assumed that only f and g of this equation vary substantially with temperature, then, as the temperature is lowered, f should decrease more than g to obtain a smaller value of P. The present results, where the half-fall time increases by a greater proportion than the half-rise time, would suggest the opposite, if the half-fall time is considered only as a function of g and the half-rise time only as a function of f and g. However, such an argument is based upon the absence of relative movement between the actin and myosin as the tension changes take place. Possible movements due to extension of the tendon connection cannot be excluded, and such extensions could alter the relative positions of the actin and myosin and therefore affect the cross-bridge activity.

In spite of the possibilities of other factors influencing the half-rise and half-fall times, it does seem reasonable to suppose that the observed changes are compatible with decreases in the values of f and g with temperature.

Temperature effects on dynamic tension changes.

Changes in the dynamic elasticity of tortoise gastrocnemius muscle similar to those of rat and hamster muscle were observed. The dynamic elasticity was always greater in stimulated muscle than in relaxed muscle.

The dynamic stiffness, as a percentage of the isometric tension, showed a considerable decrease as the temperature was raised. In most muscles, the value at 5 C

was about twice that at 30°C. If no correction is made for variations in isometric tension, the change in dynamic stiffness over the range of temperature is not substantially altered. The greater part of the decrease took place over the range 5°C - 15°C with a slight decay to level off over the range 20°C - 30°C.

When considered as a percentage of isometric tension generated in response to a 1% increase in the length of the sarcomeres, the dynamic tension change is 15% at 30°C and 27% at 5°C. These figures are comparable to those found for rat muscle.

It is reasonable to suppose that, as in rat and hamster muscles, the dynamic elasticity in active muscle is a function of the contractile mechanism. In the case of tortoise muscle, this is also confirmed by the correlation between the value of the dynamic tension change and the maximum isometric tension generated by the muscle. At all temperatures except 25°C, there is a high degree of correlation between these two variables, and if the dynamic tension changes calculated at each temperature interval for each muscle are considered together, a linear correlation of $r = 0.695$ giving a P value < 0.001 is found. The regression line of this correlation does not pass through the origin, thus giving the proportionally higher dynamic elasticity at lower temperatures.

In the discussion of dynamic elasticity of rat muscles, a hypothesis was put forward to account for increase

in tension during the dynamic phase, and to account for any differences in the size of the dynamic tension change. In this hypothesis, it was suggested that the tension increase is due to deformation of cross-bridges and that these cross-bridges may be broken either mechanically or by the action of enzymes. It was further suggested that the amplitude and rate of stretches which have been applied to the muscles would make the mechanical breakdown of the cross-bridges unlikely. Some breakdown by the action of enzymes would take place and the greater the rate of action of these enzymes, the more cross-bridges there would be broken. This is consistent with the results from tortoise muscle. As mentioned in the discussion of isometric tension, the rate of formation of bonds, f, and the rate of breakdown of the bonds, g, both decrease with temperature, especially in the region 5°C - 20°C. This is also the temperature range where one would expect to find, and where there is, a relative increase in the dynamic stiffness.

This increase in dynamic stiffness with decreasing temperature can be explained by the hypothesis put forward to account for differences in dynamic elasticity between fast and slow muscles of the rat. However, this is not the only possible explanation of the temperature related changes in elasticity.

If the tortoise gastrocnemius muscle is a mixed fibre muscle, consisting of both slow and fast fibres (Crowe and Ragab, 1972) then the rates of reaction, f and g,

of each fibre type may not change with temperature at the same rate. If the amount of dynamic tension, as a percentage of the isometric tension, is not the same for each fibre type, then the overall dynamic elasticity could be different at different temperatures.

In this case, if the fast fibres stop contracting at a higher temperature than the slow fibres, as is likely, then the slow fibres must have a greater dynamic stiffness than the fast fibres.

Decay of tension during the static phase of the ramp

The mean values of $t_{\frac{1}{2}}$ and $t_{\frac{3}{4}}$ for tortoise muscle are given in Table 5.17. The half-decay times are very much shorter than the corresponding half-fall times for the same muscle at the same temperature. At 5°C, the half-decay time is 3 - 4% of the half-fall time. At 30°C, it is about 8 - 10% of the half-fall time. For any particular muscle, the half-decay time at 5°C is about twice the value at 30°C (see Table 5.17). Even with these discrepancies, it can be seen that the increases in half-fall times with decreasing temperature show concomitant increases in half-decay times, possibly both being determined, in part at least, by g, the rate of breakdown of cross-bridges.

In the discussion of stress relaxation in rat muscles, it was suggested that the reduction in tension is likely to be the result of the enzyme controlled breakdown and formation of cross-bridges. Further, it was suggested that if the rate of reaction, g, were slow, then

a sudden decrease in tension might be seen at the end of the dynamic phase, giving a biphasic aspect to the stress relaxation of the static phase. If the traces for active muscle in fig.5.13 are examined, it can be seen that this sudden drop in tension takes place in tortoise muscle both at 30°C and 5°C. This biphasic aspect of the tension decay is also seen in Table 5.17, where it can be seen that the difference between $t_{\frac{1}{2}}$ and $t_{\frac{3}{4}}$ is larger than the value of $t_{\frac{1}{2}}$, indicating that the initial decay in tension is more rapid than the subsequent decay. It can also be seen that the difference is greater at 5°C than at 30°C. This tends to substantiate the hypothesis put forward for rat muscle to explain the decay of tension during the static phase of a ramp.

The effects of temperature on the static tension changes

The effects of temperature on static stiffness are not so pronounced as the effects upon the isometric tension and the dynamic tension changes. The minimum value for this quantity was usually seen at about 20°C. The value at 30°C was about the same as, or, in some cases less than the value at 5°C. The value at 5°C was usually about twice the value at 20°C (see Table 5.17).

The static tension changes, given as percentages of isometric tension for a 1% lengthening of the sarcomeres is 8.25% at 30°C, 4.7% at 20°C and 7.8% at 5°C.

The excess tension over isometric tension can be explained as for rat and hamster muscles in terms of the

"locked-on" cross-bridges of Sugi (1972). The relationship between the static tension and the cross-bridges is further established here, since it is found that there is a positive linear correlation between the static tension changes and the maximum isometric tension generated by the muscles, when all muscle stretches at every temperature interval are considered together ($r = 0.435$ giving a P value < 0.01).

There may be relatively more "locked-on" cross-bridges at 5°C than at 20°C and two possible explanations can be given which may account for this. The slower biochemical breakdown of bridges could allow a relatively high proportion of cross-bridges to remain in the "locked-on" state at the lower temperature, or, the slow muscle fibres of a mixed muscle contribute relatively more static tension at lower temperatures than do fast fibres.

The relationship whereby there is relatively more static tension at 30°C than at 20°C is more difficult to explain. Since the enzyme catalysed breakdown of cross-bridges would decrease with decreasing temperature, the relative increase in the proportion of cross-bridges remaining unbroken when the temperature is lowered from 20°C to 5°C is as expected. On this basis, the static tension relative to the isometric tension would be expected to further decrease when the temperature is raised from 20°C to 30°C, but in fact the reverse of this is found. Clearly the interaction between rates of making and breaking of cross-bridges, and the distortion of the cross-

bridges is not so simple as is suggested in this thesis.

It is, however, interesting to note from Table 5.17 that the value of $t_{\frac{1}{2}}$ has a minimum at 20°C indicating the fastest rearrangement of cross-bridges at this temperature. The isometric tension also has its greatest value at 20°C - 25°C before decreasing slightly at 30°C. It could well be that the proportionally lowest static tension at 20°C is in fact the expected result.

The Visco-Elastic Model

The three component model

Initially tension responses of active and passive muscle were examined to determine whether or not the muscle could be represented by a Maxwell visco-elastic model consisting of three components, as given in fig.3.1a.

Discussion of the results referred mainly to static and dynamic elasticities and to half-decay times of muscle. When these quantities are related to the three component model it can be seen that the static elasticity, the dynamic elasticity and the half-decay time correspond respectively to the parallel elasticity, the series elasticity and the viscosity.

Some aspects of muscle length/tension relationships can be modelled by the three component Maxwell system. It is possible to make the model predict changes in static and dynamic tension in both passive and

contracted muscle, within the linear range of the model. However, it is not possible to predict the responses of one type of muscle using constants for the model derived from a different muscle type, since the static and dynamic elasticities vary from muscle to muscle. In addition to this, it has been shown for tortoise that the static elasticity is temperature dependent. In the three component model, the static elasticity would be represented by E_1. This, being a purely elastic element, would not be expected to vary with temperature.

Tension transients during the static phase of a ramp stretch can be monophasic, and approximately exponential, for example in rat ECRL and hamster gastrocnemius muscles. Such transients could be represented by the three component Maxwell model. The equivalent tension transients in rat soleus and tortoise gastrocnemius muscles are biphasic and could not be represented by such a simple model. Even in ECRL and hamster gastrocnemius muscles, the representation, though acceptable, would be superficial and would give no clue to the underlying mechanisms of muscle contraction.

Another aspect of the muscle tension responses which could be represented by the three component model is the difference in tension between resting muscle and contracted muscle. The tension difference would be represented in the model as a decrease in the unstretched length of the parallel elastic element, or an increase in its elastic coefficient, or both. Tension changes during

the change from the active to the contracted state, and vice versa, would not be so easily predicted by the model.

The three component Maxwell model shows many deficiencies in the representation of muscle.

The five component model

A Maxwell model consisting of five components, as given in fig.3.1b would give a better representation of muscle, since tension transients could be more accurately predicted.

Such a model would give a better representation of tension changes during isometric contraction and relaxation of the muscle and would also give a reasonable fit to the biphasic tension decay found in rat soleus and tortoise gastrocnemius muscles, during the static phase of the ramp stretch. Both of these tension responses are difficult to predict using the three component model.

It was previously suggested that static and dynamic tensions are not due to the same structure in passive and active muscle. The transition from passive to the contracted states may be modelled by the five component model, but it is not possible to correlate components of the model with structures of the muscle during such changes. However, the properties of passive and active muscle may be correlated separately with the five component model.

In previous sections of the discussion, a hypothesis was put forward which would account for differences in dynamic elasticity and differences in stress relaxation during the static phase of a ramp applied to

different muscle types. This hypothesis can be related to a five component Maxwell model, and the properties of the muscle represented by the components of this model.

E_1, the parallel elasticity of the model, would represent the force generated by the cross-bridges, and, on elongation, would also represent the additional tension due to stretched cross-bridges, giving the static elasticity.

E_2 and V_2, one visco-elastic element, would represent the rate of breakdown of cross-bridges in the part of the muscle where cross-bridges can be broken down, but no new ones made.

E_3 and V_3, the other visco-elastic element, would represent the forces due to the interaction of rate constants f and g, controlling the making and breaking of cross-bridges in the area of overlap of actin and myosin, where cross-bridges can be both broken and made.

Using these relationships for active muscle, it may even be possible, by the choice of suitable conditions, to represent the "slip" phenomenon of Sugi (1972), as well as the other visco-elastic properties of muscle. The model would still not be able to represent changes in static elasticity with temperature. Further refinement of the model would be necessary to achieve this.

In conclusion it is possible to say that the results of this thesis can be explained in terms of the Huxley model of muscle and, in a limited way, the visco-elastic properties can be represented by a five component Maxwell model.

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APPENDIX I

Abbreviations used in the text, figures and tables

d = the ratio $\frac{\bar{x}_1 - \bar{x}_2}{S.E.}$, where the means are not assumed to be of samples from one population.

e = base of natural logarithms $\simeq 2.718$

E.C.R.L. = extensor carpi radialis longus

E_1 = parallel elastic element of the Maxwell model

E_2 = series elastic element of the Maxwell model

E_3 = lightly damped series elastic element of the five component Maxwell model

f = degrees of freedom

F = force

F′ = variance ratio

g = grams

G = the transfer function between length and tension of the Maxwell model

H = height of ramp stretch

L = length

m = metres

N = newtons

N.S. = not significant

P = probability

r = correlation coefficient

s = seconds

S.E. = standard error

t = time

t' = the ratio $\frac{\bar{x}_1 - \bar{x}_2}{S.E.}$, where the means are assumed to be of samples from one population.

t_o = the time at the start of the static phase of a ramp stretch.

$t_{\frac{1}{2}}$ = the time taken from the start of the static phase to where the tension generated is equal to half the tension at the end of the dynamic phase.

T = duration of the dynamic phase of a ramp

$T_{\frac{1}{2}}$ = the time from the end of the latent period of a muscle contraction to where the tension is half maximum.

V_2 = viscous element of the Maxwell model

V_3 = smaller of the two viscous elements of the five component Maxwell model

$\bar{x}$ = the mean of a number of samples.